MOLECULAR CHARACTERISATION OF ANTIBIOTIC-RESISTANT BACTERIA FROM SELECTED WATER DISTRIBUTION SYSTEMS IN SOUTHWESTERN NIGERIA

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Abstract

The presence of Antibiotic-Resistant Bacteria (ARB) in water sources and treated drinking water is an emerging public health issue. Antibiotic resistance genes and mobile genetic elements (integron and gene cassettes) have been reported as bases of resistance in ARB. Available data on ARB in southwestern Nigeria are based on phenotypic studies. Information on molecular basis of resistance in ARB is necessary to determine the mode of resistance transfer among bacteria. This research was therefore aimed at molecular characterisation of ARB in water distribution systems of dams in Southwestern Nigeria.

Ninety-six water samples were purposively collected aseptically into sterile screw cap bottles from six selected water distribution systems of dams in Ife, Ede, Asejire, Eleyele, Owena-Ondo and Owena-Idanre in Southwestern Nigeria. Samples were collected four times between December 2010 and July 2011 from raw, treated and two randomly selected municipal distribution taps. Bacteria were isolated from water samples and characterised using 16S rDNA sequencing. Antibiotic susceptibility of isolates was determined using point inoculation method. Multi-Drug Resistant (MDR) bacteria were selected based on resistance to over three classes of antibiotics and resistance genes were characterised by PCR and microarray analysis. Class 1 integron was detected by PCR while variable regions of integrase positive isolates were sequenced to identify inserted gene cassettes. Data were analysed using descriptive statistics and ANOVA at p=0.05.

A total of 292 bacteria isolates were obtained. The highest (11.0%) bacteria occurrence was from Owena-Idanre raw water while the lowest (0.3%) was obtained from Eleyele treated water. They were identified as α-proteobacteria (4), β-proteobacteria (39), γ-proteobacteria (131), bacteroidetes (4), actinobacteria (2), firmicutes (105) and uncultured bacteria (7). Resistance to tetracycline, sulfamethoxazole, ampicillin and streptomycin among the 191 found to be MDR bacteria were 62.8, 93.7, 90.0 and 52.8 % respectively. Significant variation was observed between percentage of MDR bacteria in Owena-Idanre dam (12.6%) and those from Ife dam (4.2%). The most frequent resistance genes detected among MDR bacteria were *ant(3')*^{b} (56/101), *bla*$_{TEM}$ (59/172), *sul 2* (51/179) and *tetA* (23/120). The highest incidence of *ant(3')*^{b} (12.5%) was found among bacteria obtained from Eleyele raw water while *tetA* (13.0%) was from Owena-Ondo treated water. Other antibiotic resistance genes such as *floR*, *tetJ*, *tetH*, *StrB*, *qnrA1*, *dfrA21* and *aadE* from bacteria isolated in Asejire treated water and Owena-Idanre municipal taps were detected with Microarray. Class 1 integron was found in 42 bacteria with the highest frequency (14.3%) in Eleyele raw water

while 2.4% each were obtained from Ede, Asejire and Eleyele municipal taps. Variable region was detected from 69.0% of bacteria carrying class 1 integron. Gene cassettes identified in the variable region of class 1 integron include *aadA1, bla CTX-M, dfrA, Sul 1* and *qnr A1* encoding resistance to aminoglycoside, extended β-lactams, trimethoprim, sulfamethoxazole and quinolones respectively.

The presence of multi-drug resistant bacteria carrying different antibiotic resistance genes, integrons and gene cassettes as revealed by molecular characterisation, in the water distribution systems could facilitate the transfer of antibiotic resistance among bacteria of public health significance, hence the need for surveillance.

Keywords: Water distribution systems, Multi-drug resistant bacteria, Integrons and gene cass ettes, Antibiotic resistance genes, Microarray gene detection

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To God be the glory.

Certification

I certify that this research work was carried out under my supervision by Ayodele Timilehin ADESOJI in the Department of Microbiology, Faculty of Science, University of Ibadan, Nigeria.

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Dedication

To God Almighty and the entire family of Prof. and Mrs. F. A. Adesoji who God used to nurture me from childhood to who I am at present

Abbreviations

BLAST	Basic Local Alignment Search Tool
BOD	Biochemical Oxygen Demand
BSA	Bovine Serum Albumin
CLSI	Clinical and Laboratory Standard Institute
COD	Chemical Oxygen Demand
CS	Conserved Segment
DNA	Deoxyribonucleic Acid
EMB	Eosine Methylene Blue
FES	Fetal Equine Serum
LB	Luria Bertani
MDR	Multi-drug Resistance
PCR	Polymerase Chain Reaction
SA-HRP	Streptavidin-Horseradish Peroxidase
SDS	Sodium dodecyl Sulphate
SSC	Saline Sodium Citrate
TAE	Tris-Acetic EDTA
TBE	Tris-Borate EDTA
TDS	Total Dissolved solid
TNT	Tris-Nacl Tween
TOC	Total Organic Carbon
TS	Total Solid
TSA	Tyramide Signal Amplification
TSS	Total Suspended Solid

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CHAPTER ONE

Introduction

The demand for portable water for drinking and other purposes is in no doubt exceeding its supply especially in some regions of developing countries where drought has claimed thousands of lives and inflicted economic and social damage (Frederiksen, 1996). According to World Health Organization (WHO), two thirds of diseases affecting people worldwide are of water-origin. Polluted water may contain pathogenic bacteria, protozoan, viruses or eggs of helminthes, which are known to cause serious health hazards in humans (Enujiugha *et al.*, 1994 and WHO, 1996). However, for water to be potable it must be microbiologically safe and in order to achieve this, an approach that will eliminate pathogenic organisms from water supply must be ensured.

Water is an essential natural resource for sustainability of life on earth. Humans may survive for several weeks without food, but barely few days without water because constant supply of water is needed to replenish the fluids lost through normal physiological activities, such as respiration, perspiration, urination (Murray *et al.*, 2003). Though the hydrosphere is estimated to contain about 1.36 billion Km^3, only about 0.3% of the water, existing as fresh water in rivers, streams springs and aquifers, are available for human use; the remaining 99.7% is locked up in seas and oceans (Wilson, 1978).

In most developing countries particularly Nigeria, the various state governments are responsible for monitoring and determining the quality of water supplied. Water treatment plants are the most essential and direct means of controlling the microbial quality of drinking water. However, many water treatment plants designed for and constructed in many third world countries have inherent operational problems such as sedimentation tanks which cannot be drained, sand filters which are prone to flooding, non-functional chlorinators, and inadequate provision of laboratory facilities (Adiyan, 1999). The implication of these problems is that treated water drawn from taps, even within the premises of most treatment plants, is not entirely devoid of pathogenic organisms (Akinyemi *et al.*, 2006).

The geological constraints limit accessibility of many human communities to water that is adequate in terms of quantity, quality and sustainability. Lack of adequate supply of potable water is a critical challenge in developing countries such as Nigeria. Potable water, also called

drinking water in reference to its intended use, is defined as water which is fit for consumption by humans and other animals (Tchobanoglous *et al.*, 2003).

In the last two decades, there has been an increase in the number of reported cases of water-borne diseases such as typhoid fever, cholera and dysentery. These cases are reported in both print and electronic media without any known fact on the predisposing factors that might be responsible for such disease upsurge in some southwestern part of Nigeria. Availability of safe potable water is considered to be a luxury and far from reach by over 80% of the total population belonging to the low socio-economic class in Nigeria where many still depend on untreated waters for their survival (Akinyemi *et al.*, 2006).

The microbial quality of potable water is determined in part by the number of culturable microorganisms present. United State Environmental Protection Agency (USEPA) surface water treatment rules state that heterotrophic plate counts (HPC) should be no more than 500 CFU/ml (USEPA, 1989). Prokaryotic and eukaryotic microorganisms that are present in water leaving the treatment plant, though probably inactivated by the disinfection process, may recover and grow in water distribution system (WDS) biofilms (LeChevallier *et al.*, 1987).

According to WHO (2011), antibiotics were defined as substances of natural, synthetic or semisynthetic origin which at low concentrations kills or inhibits the growth of micro-organisms but causes little or no damage to the host. Resistance to antibiotics which has many definitions by various authors is a property of bacteria that confers the capacity to inactivate or exclude antibiotics, or a mechanism that blocks the inhibitory or killing effects of antibiotics (Harrison and Lederberg, 1998). Ministry of Agriculture, Fisheries and Food (1998) defined resistance to antibiotics as a relative term which provides an interpretation of the clinical significance of concentrations of an antimicrobial that inhibit the growth of an organism or kill it in laboratory systems (*in vitro*). In the definition of Her Majesty's Stationery Office (HMSO) (1999) resistance to antibiotics is defined as the ability of a microorganism to withstand an antibiotic.

The spread of antibiotic-resistant pathogens is a growing problem all around the world (Amy *et al.*, 2006). WHO (2000) report on overcoming antimicrobial resistance among infectious diseases focuses on antibiotics resistance as one of the most critical human health challenges of the next century and heralded the need for "a global strategy to contain resistance". According to the report, more than two million Americans are infected each year with resistant pathogens and

14 000 died as a result. Amy *et al.* (2006) avows that the rapid growth of the problem emphasizes the need for intervention.

The increase in antibiotics resistance is considered to be closely linked with the widespread use of antibiotics in humans and animals therapy. In particular, more than one-half of the antibiotics used in the U. S. are administered to livestock for purposes of growth promotion or infection treatment (Levy, 1998 and Gaskins *et al.,* 2002). There is an uncontrolled availability of antibiotics and other drugs in several developing countries, Nigeria included (Chigbu and Ezeronye 2003; Torimiro *et al.*, 2005). Of greater significance is the use of antimicrobials in agriculture, especially as growth promoters, chemotherapeutic and prophylactic agents in food for animals (Witte, 1998 and Bedford, 2000). In Nigeria, there is a widespread use of antimicrobial agents as additive in animal feeds (Smith *et al.*, 2003) and for prophylaxis, and disease control.

In both animals and humans, up to 95% of antibiotics can be excreted in an unaltered state. About 50–90% of drugs administered to farm animals are excreted into the environment either un-metabolized or as metabolic intermediates which even though inactive, may undergo transformation to the active form in the environment. Likewise, they can persist as residue in waste, soil, food and water with a number of consequences (Kummerer, 2003).

Wastewater treatment plants (WWTPs) have been observed to remove some pollutant. However, as is true with the larger problem of pharmaceutical compounds, WWTPs are not designed for the removal of micro pollutants (Suidan *et al*., 2005 and Sumpter and Johnson, 2005). Residual antibiotics thus are released into the environment where they may exert selection pressure on microorganisms. While overprescribing as well as other improper use/disposal of antibiotics in humans is generally considered to contribute to the problem, several studies have also linked the use of antibiotics in agricultural with antibiotic-resistant infections in humans (Smith *et al.*, 2003; Sørum and L'Abe´e-Lund, 2002 and Shea, 2003).

Common antibiotics reportedly used in the poultry industry in southwestern and northern Nigeria include furazolidone, streptomycin, erythromycin, tetracycline, ampicilin, oxytetracycline, chlortetracycline, penicillin, sulphonamides, colistin, tylosin, neomycin and nitrofurantoin (Dipeolu *et al.* 2004 and Kabir *et al*. 2004). Observations worldwide suggest that antibiotics use in animal husbandry is a major driving force behind the development and dissemination of

antibiotic resistance in certain pathogenic bacteria species (Smith *et al.,* 2003; Hamscher *et al.*, 2002, and Chapin *et al.*, 2005).

Antibiotic resistance bacteria has been detected in various aquatic environments including rivers, sewage, ocean water and drinking water (Ash *et al.*, 2002; Reinthaler *et al.*, 2003; Schwartz *et al.*, 2003). Acquisition and transfer of antibiotic resistance and virulence genes by the bacteria via horizontal transfer of the resistance (R) plasmids, transposons and integrons are increasing problems in infectious diseases (Leverstein-van Hall *et al.*, 2001). Integrons are capable of mobilizing or integrating gene cassettes encoding antibiotic resistance determinants such as resistance to trimethoprim, aminoglycosides, chloramphenicol or tetracyclines. Class 1 and/or class 2 integrons have been reported in clinical isolates of the *Enterobacteriacea* family (Leverstein van Hall *et al.*, 2001), in bacteria from food (Sunde, 2005) and also in aquatic environments (Roe *et al.*, 2003).

As a result of the direct selection pressure that antibiotics exert on organisms carrying antibiotic resistance genes (ARGs), the transport pathways of antibiotic-resistant microorganisms and the ARGs that they carry are expected to be similar to the pathways of antibiotics used as pharmaceuticals. In fact, it is likely that ARGs persist further in the pathway, considering that in many cases they are maintained in the microbial populations even after the antibiotic selection pressure has been removed (Bjorkman *et al.*, 2000 and Manson *et al.*, 2004).

Also, horizontal gene transfer (HGT) is a major mechanism for sharing ARGs between microbes and has been documented to occur between non pathogens, pathogens, and even distantly related organisms, such as Gram-positive and Gram-negative bacteria (Courvalin, 1994 and Kruse and Sorum, 1994). In many cases, ARGs have been discovered to occur as part of multiple antibiotic resistant (MAR) super integrons, which may contain over 100 ARG cassettes (Mazel, 2004). These MAR super integrons cause multiple-drug resistance in organisms, meaning that even when very different antibiotics are used, one antibiotics may co-select for resistance to other antibiotics (Beekmann *et al.*, 2005). MAR gene cassettes and ARGs are notorious for being associated with plasmids and/or transposons that facilitate horizontal gene transfer. However, even if cells carrying ARGs have been killed, the DNA released by this organism to the environment has been observed to persist, to be protected from DNAse, especially by certain soil/clay compositions, and to be eventually transformed into other cells (Hill and Top, 1998 and

Crecchio *et al.*, 2005). For all of these reasons, ARGs in and of themselves can be considered to be emerging "contaminants" for which mitigation strategies are needed to prevent their widespread dissemination. Moreover, many studies have addressed the presence of antibiotic resistant bacteria from water, food and clinical samples and have also observed the presence of antibiotics residue in animal and food samples from Nigeria. But there is paucity of report on the mechanism of antibiotic resistance among bacteria from Nigeria and in particular southwestern part. Many authors focused on phenotypic expression and not on the molecular basis of antibiotic resistance in these bacteria.

1.1. Objectives of the study

This study focused on:

i. Determination of the physico-chemical properties of water samples.
ii. Isolation and molecular characterisation of bacteria from selected dams from southwestern Nigeria using 16S rDNA.
iii. Determination of the antibiotic resistance profiles of the bacteria and selection of multi-drug resistance (MDR) bacteria.
iv. Determination of the presence of plasmid among the MDR bacteria.
v. Molecular characterisation of integron and gene cassettes and other antibiotic resistance genes using PCR and Microarray genotyping.

CHAPTER TWO

Literature Review

2.1 . Dams construction and their uses

Dams are barriers constructed across a stream or river to harness water for one or more of the following uses: concentrate the natural fall of a river at a given site to generate electricity; direct water from rivers into water supply systems; direct water into canals for irrigation systems; increase depths for navigational purposes; control flow during time of flood and droughts and; create artificial lakes for fisheries and recreational use. Many dams are multipurpose and fulfill several of these functions (Uyigue, 2005).

In Nigeria, there has been an upsurge in dam construction in the past three decades. A total of 323 dams were identified in literature out of which 246 (76.2%) were constructed between 1970 and 1995 (Ofoezie, 2002). The effect of the sahelian drought of 1972-1975 aggravated the already stressed food security situation in the country prompting the various levels of government to embark on a rigorous policy to increase food production. To achieve this, impoundment of river basins was seen as inevitable to provide sufficient water for year-round irrigation 'which led to the construction of over 246 dams (Imevbore *et al.*, 1986). Many benefits have been derived from the services of dams, however, their construction and operation have also led to many significant negative social, health and human impacts (World commission on Dams (WCD), 2000).

2.2. Water borne diseases

Water borne diseases remain a major cause of death and illness in developing countries. The global spatial distribution shows that Africa and Asia account for a large percentage of these diseases, which include cholera, typhoid fever, paratyphoid, bacillary dysentery, amoebic dysentery, gastro-enteritis, and infective hepatitis (Lucas and Gilles, 1973). Children less than five years are particularly affected adversely since they can experience as many as 10 episodes of diarrhoea in a year. Among this age group, 15-18% of mortality is attributed to diarrhoea. Nevertheless, adults are not spared the scourge of the same diseases (United State Agency for

International Development (USAID), 2005). Generally, water-borne diseases are transmitted through the ingestion of water and food that are contaminated by faecal materials that carry infective dose of various pathogens. Some of these diseases that are acquired through the gastro-intestinal tract occur in epidemic form. This is because they tend to affect people who share a common source of water supply over a wide area (Oguntoke *et al.,* 2009).

The prevalence of these diseases are particularly linked to the dearth of potable water in most parts of the developing countries. According to USAID (2005), diarrhoeal diseases are largely caused by unsafe water, inadequate sanitation and poor hygiene among human population.

Worried by the increasing rate of water-borne diseases in this part of the world, researchers have investigated the quality of water samples drawn from private wells and springs in Oregun, Lagos in Nigeria (Oguntoke *et al.,* 2009). *Escherichia coli*, *Citrobacter freundii* and *Klebsilla pneumoniae* were found as the most frequently isolated coliform from water samples (Lamka *et al*., 1980). *Staphylococcus aureus* and *Aeromonas hydrophila* were also isolated from the water samples. Similarly, in a different study *E. coli*, *Klebsilla aerogenes, K. edwardsii, K. rhhonsleromatic, K.stlantae, Entrobacter* spp and *Citrobacter* spp. were identified in water samples collected from wells in Ago-Iwoye town, Nigeria (Fagade and Osho, 1996).

2.3. Environment and antibiotics

2.3.1. Antibiotics in the environment

Although antibiotics have been applied in large quantities for some decades, until recently the existence of these substances in the environment was accorded very little attention. Studies conducted in various countries have detected a number of antibiotics in the low microgram per litre to nanogram per litre range in different environmental compartments, e.g. hospital wastewater, municipal waste water, effluent from sewage treatment plants, surface water and in some cases ground water (Golet *et al.,* 2001; Kolpin *et al.,* 2002; Kummerer, 2003 and Kummerer, 2004a). The compounds detected are from different important antibiotic classes such as macrolides, tetracyclines, sulphonamides, quinolones and others as far as analytical methods are available. Beta-Lactams have not been detected yet despite the fact that b-lactams are used in the highest amounts (Molstad *et al.,* 2002). Obviously, most of the antibiotics are not fully eliminated during the sewage purification process. The results of investigations using test systems indicate that a number of antibiotics and disinfectants are not biodegradable in the

aquatic environment (Al-Ahmad *et al.*, 1999 and Kummerer *et al.*, 2000). In soil, tetracycline concentrations in the range of several hundred micrograms per kilogram have been detected some months after manure application (Hamscher *et al.*, 2002 and De Liguroro *et al.*, 2003). The main processes of substance elimination in the environment, especially in waste water, sediments, and soil are through biodegradation. The concentration of antibiotics may be higher if the active compounds are persistent and accumulate. It is not known how strongly the antibiotics are sorbed and under what circumstances they are still available and active after sorption (Kummerer, 2004a).

2.3.2. Bacteria resistance in the environment

The occurrence and spread of antibiotic resistant bacteria is a pressing public health problem worldwide and aquatic ecosystems are a recognized reservoir for antibiotic-resistant bacteria (ARB) (Kummerer, 2004b; Baquero *et al.*, 2008; Martinez, 2008 and Zhang *et al.*, 2009). Naturally occurring ARB and antibiotic-resistant genes (ARGs) in the aquatic environment are selected for and enriched by antibiotics found in sewage and agricultural runoff, which result from the widespread and increased use of antibiotics (Kummerer, 2004b; Baquero *et al.*, 2008; Martinez, 2008 and Zhang *et al.*, 2009). A large number of these bacteria host antibiotics resistance genes, which ultimately find their way to mobile genetic elements and disseminate among water and soil bacterial populations (Alonso *et al.*, 2001). These antibiotic resistant bacteria are serious contaminants of water environments (Huber, 1971). Resistance genes as well as resistant bacteria in the environment are increasingly seen as an ecological public health threat. The most prominent medical examples are vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* (MRSA), and multi-resistant pseudomonads. With respect to the causes of resistance, the focus had been on the use of antimicrobials in hospitals, by medical practitioners and in animal husbandry. They play a role in the stimulation of resistance and its transfer by genetic material in bacteria (Kummerer, 2004b).

According to Murray (1997) the transfer as well as the emergence of new combinations of resistance genes will happen most frequently in compartments with high bacterial density, e.g. biofilms. Bacterial density is very high both in aerobic and anaerobic septic tanks of sewage treatment works, and in biofilms, e.g. in drinking water pipes, sediments, and soils. Bacteria are either resistant by nature or they may have become resistant by the use of antibiotics as well as in the environment by uptake of genetic material encoding resistance (e.g. in hospital waster or

manure) before they reach a sewage treatment plant or the soil. Research is still on-going on whether they may also become resistant in the sewage treatment plant or soil itself. The transfer of resistant bacteria to humans may occur via water or food if plants are watered with surface water or sewage sludge, if manure is used as a fertilizer or if resistant bacteria are present in meat (Salyers, 2002).

2.3.3. Sources of antibiotic resistance in the environment

2.3.3.1. Hospital wastewater

Antibiotics used in medicine for the treatment of infections are mainly released non-metabolized into the aquatic environment via wastewater (Kummerer, 2004a). This can only have an effect if an active compound is present. Unused therapeutic drugs are sometimes disposed of down the drains. Amongst other active compounds used, antibiotics and disinfectants are present in the wastewater of hospitals (Kummerer, 2001). Ciprofloxacin, for example, was found in concentrations of between 0.7 and 124.5mg/L in hospital wastewater and was assumed to be the main source of genotoxic effects measured with the umuC test in hospital wastewater (Hartmann, 1998). Ampicillin was found in concentrations of between 20 and 80 mg/L in the effluent of a large German hospital (Kummerer and Henninger, 2004). Gentamicin resistance genes were found in *Acinetobacter*, *Pseudomonas* and Enterobacteriaceae in hospital sewage (Schwartz *et al.*, 2003). Resistant and multi-resistant pathogenic bacteria such as *Acinetobacter sp* (Heuer *et al.*, 2002 and Feuerpfeil *et al.*, 1999) have been detected in waste water and sewage treatment plants (STPs) while horizontal transfer of these resistant has also been observed. (Marcinek *et al.*, 1998; Puhler, 1999 and Heuer *et al.*, 2002). Exchange of genes encoding for resistance between *Pseudomonas* and *E. coli* in sewage sludge (Schwartz *et al.*, 2003) has also been reported.

2.3.3.2. Surface water

Bacteria resistant to antibiotics are present in surface water (Schwartz *et al.*, 2003). Goni-Urrizza *et al.* (2000) observed a correlation between resistant bacteria in rivers and urban water input. Schwartz *et al.* (2003) were able to amplify *AmpC* beta-lactamase gene sequences by PCR in surface water. Genetic transformations have been reported for *E. coli* (Baur *et al.*, 1996).

Antimicrobial resistance was also found in marine bacteria and bacteria living in estuaries (Barkay *et al.,* 1995). Gentamicin resistance genes were found in *Acinetobacter, Pseudomonas,* Enterobacteriaceae, and in phylogenetically distant bacteria such as members of alpha and beta proteobacteria in coastal water polluted with sewage water (Kummerer and Henninger, 2004).

2.3.3.3. Ground water

Antibiotics are rarely found in ground water and if they do occur, they are far below the microgram per litre range. Leaching from fields fertilized with animal slurry or passing through sediments into the ground water might be a source of antibiotics in ground water. However, the volume load of antibacterial agents in ground water in rural areas with high concentrations of livestock has proved to be small (Hirsch *et al.,* 1999). Antibiotic-resistant *E. coli* have been found with a surprisingly high incidence in rural ground water (McKeon *et al.,* 1995). Manure runoff from farms or leakages from septic tanks are clear possibilities for the input of resistant bacteria into this ground water.

2.3.3.4. Drinking water

Antibiotic-resistant bacteria were detected in drinking water as early as 1980s and later in the 1990s (Armstrong *et al.,* 1981 and Kolwzan *et al.,* 1991). These authors discovered that resistant bacteria identified using classical microbiological methods, i.e. standard plate count, occurred within the distribution network of drinking water supply systems. They concluded that the treatment of raw water with chlorine and its subsequent distribution could selects for antibiotic-resistant bacteria. In agreement with these, increased phenotypic resistance rates were also detected at the drinking water sampling points in the study by Schwartz *et al.* (2003). These authors also found *vanA* and *ampC* genes in heterotrophic bacteria in drinking water biofilms. Enterococci were not detected. The authors concluded that this is an indication of the possible resistance transfer to autochthonous bacteria.

2.3.3.5. Sediments

Resistant bacteria may be present because of the application of antibiotics in aquaculture or because of selective pressure exert on the bacteria by the antibiotics present in sediments. High antibiotic load in sediments in concentrations sufficiently potent to inhibit the growth of bacteria were reported for aquaculture (Hektoen *et al.,* 1995; Jacobsen and Berglind, 1998). . The fact

that the exposure is highly locally concentrated has to be considered critical. The substances used in fish farming can enter sediments directly from the water without undergoing any kind of purification process. Some investigations have demonstrated the presence and persistence of antibiotics applied extensively in fish farming in sediments beneath fish farms (Hektoen *et al.,* 1995; Jacobsen and Berglind, 1998). Quinolones, sulphonamides and tetracyclines are sorbed by organic matter and can therefore accumulate in the sediment with organic matter. It is not yet known to what degree and under what circumstances the compounds are effective after sorption or whether they are released and may contribute to resistance. Antibiotics may have qualitative and quantitative effects upon the resident microbial community of the sediments (Samuelson *et al.,* 1992). Bacteria resistant against these compounds have been detected in sediments (Samuelson *et al.,* 1992). Kummerer. (2004b) posit that an increased antibacterial resistance in sediment bacteria is often the most sensitive environmental indicator of past antibacterial use.

2.4. Mechanisms of antibiotic resistance

At least 17 different classes of antibiotics have been produced to date. Unfortunately for each one of these classes, at least one mechanism of resistance (and many times more than one) has developed over the years. In fact, in some cases these bacteria have been able to develop simultaneous resistance to two or more antibiotic classes, making the treatment of infections caused by these microorganisms extremely difficult, very costly and in many instances associated with high morbidity and mortality (Sefton, 2002; Levy and Marshal, 2004).

Thus it seems that the dream that some clinicians had and the predictions that many others made in the middle of the 20th century about the future eradication of most common infectious diseases from humanity may not be achievable. Recent experience with the emergence of totally new infectious diseases (AIDS, SARS, etc.), and the epidemiological trends of antibiotic resistance observed thus far, tend to indicate that we will continue to move in the opposite direction, towards an environment with an ever-growing number of new infectious diseases. Common bacteria will also develop antibiotic resistance and more bacteria becoming resistant to several antibiotics at the same time, and some of these bacteria will continue to transfer their resistance genes from the hospital setting to the community through mobile genetic elements like plasmid, transposon and integrons. The net result could be even higher morbidity, higher mortality, higher costs, and the potential for the rapid spread of these bacteria and overall a

decreasing number of useful antimicrobial agents to combat the infections they cause (Alfonso, 2005).

Gaining a good understanding of the molecular basis for the development of resistance is important because it allows us to develop new approaches to manage the infections caused by these bacteria and to create new strategies for the development of new treatments against these bacteria. In general, it can be said that bacterial resistance has its foundation at the genetic level. This means that in most cases of bacterial resistance, changes in the genetic makeup of the previously susceptible bacteria take place, either via a mutation or by the introduction of new genetic information. The expression of these genetic changes in the cell result in changes in one or more biological mechanisms of the affected bacteria and ultimately determine the specific type of resistance that the bacteria develops, resulting in a myriad of possible biological forms of resistance (Levy and Marshal, 2004; Sefton, 2002).

2.4.1. Genetic mechanisms of transmission

The development of antibiotic resistance tends to be related to the degree of simplicity of the DNA present in the microorganism becoming resistant and to the ease with which it can acquire DNA from other microorganisms. For antibiotic resistance to develop, it is necessary that two key elements combine: the presence of an antibiotic capable of inhibiting the majority of bacteria present in a colony and a heterogeneous colony of bacteria where at least one of this bacterium carries the genetic determinant capable of expressing resistance to the antibiotic (Levy and Marshall, 2004).

Once this happens, susceptible bacteria in the colony will die whereas the resistant strains will survive. These surviving bacteria possess the genetic determinants that codify the type and intensity of resistance to be expressed by the bacterial cell. Selection of these bacteria results in the selection of these genes that can now spread and propagate to other bacteria (Levy and Marshall, 2004). Resistance to antibiotics can be natural (intrinsic) or acquired and can be transmitted horizontally or vertically. Whereas the natural form of antibiotic resistance is caused by a spontaneous gene mutation and is far much less common than the acquired one, it can also play a role in the development of resistance. For the most part, however, the micro-ecological pressure exerted by the presence of an antibiotic is a potent stimulus to elicit a bacterial

adaptation response and is the most common cause of bacterial resistance to antibiotics (Sefton, 2002). Susceptible bacteria can acquire resistance to antimicrobial agents by either genetic mutation or by accepting antimicrobial resistance genes from other bacteria.

2.4.1.1. Conjugation

Conjugation is the most important and the most common mechanism of transmission of resistance in bacteria. This mechanism is normally mediated by plasmids (circular fragments of DNA) that are simpler than chromosomal DNA and can replicate independently of the chromosome. The mechanism of transmission of plasmids among bacteria is via the formation of a ‘‘pilus’’ (a hollow tubular structure) that forms between bacteria when they are next to each other, thus connecting them temporarily and allowing the passage of these DNA fragments (Alfonso, 2005). To facilitate conjugation steps, cell-to-cell contact is made between donor and recipient cell, followed by the transfer of DNA from the donor to the recipient (Murray and Hodel-Christian, 1991). Conjugation can also mobilize the resident chromosome and nonconjugative plasmids (Berger-Bachi and McCallum, 2006). Both Gram-positive and Gram-negative bacteria have been reported to contain conjugative plasmids and transposons which carry one or more antibiotic resistance genes (Catry *et al.*, 2003 and Grohmann *et al.*, 2003).

2.4.1.2. Transformation

Transformation is another form of transmission of bacterial resistance genes and takes place when there is direct passage of free DNA (also known as ‘‘naked DNA’’) from one cell to another. The ‘‘naked DNA’’ usually originates from other bacteria that have died and broken apart close to the receiving bacteria. The receiving bacteria then simply introduce the free DNA into their cytoplasm and incorporate it into their own DNA (Alfonso, 2005). Transformation is considered to play a minor role under *in vivo* conditions as free DNA originating from lysed bacteria is usually degraded rapidly under most environmental conditions (Schwarz *et al.*, 2001). Few bacteria exhibit a natural ability to take up DNA from their environment such as *Streptococcus pneumoniae*, *Bacillus* sp., *Pneumococci*, *Haemophilus,* and *Neisseria* (Berger-Bachi and McCallum, 2006).

2.4.1.3. Transduction

Transduction is a third mechanism of genetic transfer and occurs via the use of a ‘‘vector’’, most often viruses capable of infecting bacteria also known as ‘‘bacteriophages’’ (or simply

‘‘phages’’). The virus containing the bacterial gene that codifies antibiotic resistance (the ‘‘resistant DNA’’) infects the new bacterial cell and introduces this genetic material into the receiving bacteria. Most times, the infecting bacteriophage also introduces to the receiving bacteria its own viral DNA, which then takes over the bacterial replication system forcing the cell to produce more copies of the infecting virus until the bacterial cell dies and liberates these new bacteriophages, which then go on to infect other cells (Alfonso, 2005). Although it is thought that the importance in spread of resistance genes via the transduction mechanism is underestimated (Brabban *et al.*, 2005), both chromosomal or plasmid DNA of donors can be transduced (Schwarz and Chaslus-Dancla, 2001). In addition, transducing phages have been detected in a wide variety of bacteria (Rabsch *et al.*, 2002; Brabban *et al.*, 2005). A study by Schmieger and Schicklmaier (1999) demonstrates that antibiotic resistance genes of *Salmonella enterica* serovar Typhimurium definitive phage type 104 (DT104) strain can be efficiently transduced by P22-like phage ES18 and by phage PDT17, which are usually released by DT104 strains. The transfer of antibiotic resistance determinants by transduction has been demonstrated *in vitro* and therefore has the potential to occur *in vivo* (Schmieger and Schicklmaier, 1999). However, the limitation of transduction is that bacteriophages are highly specific to their bacterial hosts (Brabban *et al.*, 2005; Berger-Bachi and McCallum, 2006). Transduction is considered to play a major role in resistance transfer of *Staphylococci* clinical strains which most of them harbor several lysogenic phages and constantly produce phage particles (Berger-Bachi and McCallum, 2006).

2.4.2.0 Elements involved in horizontal transfer of resistance genes

Mobile elements, including plasmids, transposons and integrons are known to be involved in spreading of resistance genes (Sefton, 2002). They all consist of double-stranded DNA, but differ distinctly in their sizes, structures, biological properties and mechanisms of spread (Schwarz and Chaslus-Dancla, 2001).

2.4.2.1. Plasmid

Plasmid are extra chromosomal genetic element that have replication systems and are capable of autonomous replication. Large plasmids can carry the transfer gene (*tra* gene) which enables them to move from one host cell to another by themselves, termed conjugative plasmids (Schwarz and Chaslus-Dancla, 2001). The conjugative plasmids can mobilise others smaller, non-conjugative plasmids (Carattoli, 2003). Plasmid conjugation can occur at high frequency, capability of co-transfer of several resistance genes and can occur between bacterial species and

between different species. Therefore the transfer of conjugative plasmids is considered to be the most common mechanism for genetic exchange (Carattoli, 2003; Rice *et al.*, 2003). In addition, plasmids may intergrate themselves into the chromosome of recipient strains, thus resistance determinants can be stably maintained in their hosts even in the absence of selective pressure (Waters, 1999; Rice *et al.*, 2003). Plasmid-mediated mechanisms confer resistance to a variety of classes of clinically important antimicrobials, such as β-lactams, aminoglycosides, macrolides, tetracyclines, amphenicols, sulphonamides and trimethoprim (Yan *et al.*, 2004; Li, 2005). Recently, there has been the emerge of plasmid-mediated resistance to expanded-spectrum cephalosporins encoded by the *CMY-2 AmpC* beta-lactamase in human and animal strains of *E. coli* and *Salmonella* spp. (Yan *et al.*, 2004; Batchelor *et al.*, 2005; Donaldson *et al.*, 2006; Hopkins *et al.*, 2006). The worldwide spread of plasmid-mediated quinolone resistance in *Enterobacteriaceae* (Rodriguez-Martinez *et al.*, 2006; Castanheira *et al.*, 2007) also highlights the significance of emerging plasmid-mediated mechanisms.

2.4.2.2. Transposon

In contrast to plasmids, transposons do not possess replication systems. They must insert themselves into replication-proficient vector molecules such as chromosomal DNA or plasmids in the cell for their stable maintenance (Schwarz and Chaslus-Dancla, 2001). Transposons can encode genes for resistance to a variety of antibiotics and may have self transfer and mobilising capabilities, thus playing a role in disseminating antibiotic resistance among bacteria (Waters, 1999; Prescott, 2000).

Among the more interesting and important classes of transposons are the conjugative transposons. As their name implies, conjugative transposons are mobile elements that possess the genetic machinery to facilitate their own transfer between bacterial cells. Several prototypes of conjugative transposons have been described. Conjugative transposons encoding the *tet Q* tetracycline resistance determinants are widespread in clinical *Bacteroides* isolates and have been the subject of several reviews (Salyers and Shoemaker, 1996). The two most extensively studied conjugative transposons are Tn*916* from *Enterococcus faecalis* (Franke and Clewell, 1981) and Tn*1545* from *S. pneumoniae* (Courvalin and Carlier, 1987). These two transposons differ in size (18 versus 25.2 kb, respectively) and in the antimicrobial resistance determinants that they encode {tetracycline-minocycline [*tet*(M)] by Tn*916* and erythromycin [*ermAM*], kanamycin [*aphA-3*], and tetracycline-minocycline [*tet*(M)] by Tn*1545*}. Despite these differences, the two transposons are similar and are even identical in many respects. Their

termini are identical for at least 250 bp on each end (Caillaud and Courvalin, 1987 and Clewell *et al.*, 1988). Moreover, their integrase and excisase genes that encode transposition functions differ by only one nucleotide over ca. 2,000 bp (Flannagan *et al.*, 1994 and Poyart-Salmeron *et al.*, 1990). The *tetM* genes from the two transposons exhibit roughly 94.5% nucleotide identity over 2 kb (Oggioni *et al.*, 1996), a difference that probably reflects divergent evolution of the *tetM* determinant in disparate genera.

Conjugative transposons are now widely prevalent in gram positive bacteria and have made substantial inroads into certain species of gram-negative bacteria as well. Their role in the spread of resistance to tetracycline and minocycline and, to a lesser extent, erythromycin and kanamycin is well established and indisputable (Rice, 1998a). Their ability to increase the level of expression of tetracycline resistance and their own transferability in response to environmental exposure to tetracycline has positioned them to thrive in human and animal gastrointestinal tracts, where significant quantities of tetracycline and its analogs may frequently be found (Rice, 1998a). Moreover, the ability of these novel elements to establish genetic connections between widely disparate species makes them likely to be principal players in the ongoing dissemination of a wide variety of antimicrobial resistance determinants (Rice, 1998a).

2.4.2.3. Integrons and gene cassettes

Integrons are capable of mobilizing or integrating gene cassettes encoding antibiotic resistance determinants such as resistance to trimethoprim, aminoglycosides, chloramphenicol or tetracyclines (Leverstein-van Hall *et al.*, 2001). Among the three classes of integrons that have been identified, class 1 integrons are prevalent among clinical isolates. Class 1 integrons were originally defined as being composed of two conserved segments, the 5' conserved segment (5'-CS) and the 3' conserved segment (3'-CS), and an internal variable region which contains gene cassettes encoding antibiotic resistance determinants (Hall and Collis, 1995 and Strokes and Hall, 1989). The 5'-CS contains the *intI1* gene, which encodes the type 1 integrase. This integrase is responsible for site-specific insertion and excision of gene cassettes (Collis *et al.*, 1993). Also, the 5'-CS contains the *attI1* site, which is responsible for recombination. The 3'-CS contains the *qacEΔ1* and *sul1* genes, which encode resistance to quaternary ammonium compounds and to sulfonamides, respectively. However, class 1 integrons do not always contain the entire 3'-CS (Recchia and Hall, 1995 and Sundstrom, 1998). Class 1 and/or class 2 integrons have been reported in clinical isolates of the *Enterobacteriacea* family (Leverstein-van Hall *et*

al., 2001), in bacteria from food (Sunde, 2005). Integrons have been identified in both gram-negative and gram-positive bacteria, and they seem to confer high-level multi-drug resistance to the bacteria that carry and express them (Levy and Marshall, 2004).

In a review by Fluit and Schmitz. (2004) they reported that integrons can now be divided into two major groups: the resistance integrons (RI) and the super integrons (SI). RI carries mostly gene cassettes that encode resistance against antibiotics and disinfectants, and can be located either on the chromosome or on plasmids. The large chromosomally-located integrons, which contain gene cassettes with a variety of functions, belong to the SI group.

Three classes of RI are known (Radstrom *et al.*, 1994; Hall and Collis, 1995 and Senda *et al.*, 1996). SI is not given a specific name. The integron originally designated as class 4 is now named *Vibrio cholera* SI. Most RI belong to class 1, and class 1 integrons have been reported in many Gram negative genera including *Acinetobacter* (Oh *et al.*, 2002), *Aeromonas* (Severino and Magalhaes, 2002), *Alcaligenes* (Nordmann and Poirel, 2002), *Burkholderia* (Crowley *et al.*, 2001), *Campylobacter* (Gibreel and Skold, 2000), *Citrobacter* (Norskov-Lauritsen *et al.*, 2001), *Enterobacter* (Kartali *et al.*, 2002), *Escherichia* (Sanchez *et al.*, 2002), *Klebsiella* (Girlich *et al.*, 2000), *Pseudomonas* (Pallecchi *et al.*, 2001 and Lee *et al.*, 2002), *Salmonella* (Gebreyes and Altier, 2002), *Serratia* (Centron and Roy, 2002), *Shigella* (Sundin, 2002) and *Vibrio* (Dalsgaard *et al.*, 2000). They have also been found in other bacteria such as *Corynebacterium glutamicum* (Nesvesra *et al.*, 1998) and *Mycobacterium fortuitum* (Martins *et al.*, 1990), and a gene cassette has been discovered in *Enterococcus faecalis* (Clark *et al.*, 1999).

Class 2 integrons are embedded in the Tn7 family of transposons and consist of an integrase gene followed by gene cassettes (Radstrom *et al.*, 1994). Class 2 integrons have been found in *Acinetobacter* (Mclver *et al.*, 2002), *Shigella* (Gonzalez *et al.*, 1998) and *Salmonella* (Orman *et al.*, 2002). Class 3 integrons have been described in *Pseudomonas aeruginosa, Serratia marcescens, Alcaligenes xylosoxidans*, *Pseudomonas putida* and *Klebsiella pneumoniae* isolates from Japan. Class 3 integrons is comparable to that of class 2 integrons (Arakawa *et al.*, 1995 and Senda *et al.*, 1996). SI have been described in *Geobacter sulfurreducens* (Nield *et al.*, 2001), *Listonella pelagia* (Rowe-Magnus *et al.*, 2001), *Nitrosomonas europaea* (Rowe-Magnus *et al.*, 2001), *Pseudomonas alcaligenes* (Vaisvila *et al.*, 2001), *Vibrio anguillarum* (Clark *et al.*, 2000), *Xanthomonas campestris* (Rowe-Magnus *et al.*, 2001) and several others. Several studies support the hypothesis that integrons play a major role in the spread of multi-drug resistance in Gram-

negative bacteria due to their ability to capture gene cassettes from the environment and incorporate them by site-specific recombination (Ploy *et al.*, 2000 and White and Mciver, 2001). Thus, Henriques *et al.* (2006) attests that detection and characterisation of integrons containing antibiotic resistance genes are key steps in evaluating the potential of a certain environment to represent a reservoir of antibiotic resistance.

Gene cassettes consist of a coding sequence, which usually lacks a promoter, followed by a repeat sequence. In RI the repeat sequence is called a 59-base element (59-be) or attC. The repeat sequences of SI are named after the species in which the SI resides, e.g., the repeat of a *V. cholerae* gene cassette is called VCR. The repeats of SI are generally species-specific, but some direct or indirect exchange of repeats between some species has occurred (Clark *et al.*, 2000 and Rowe-Magnus *et al.*, 2001). Several new antibiotic resistance cassettes in RI have been described in recent years. Remarkably, until 1999, nearly all the known gene cassettes encoded resistance to the oldest groups of antibiotics, but an increasing number of new gene cassettes defining resistance against newer groups of antibiotics have now appeared. RI, like In53, may contain as many as eight gene cassettes. One gene cassette in *In 53* was composed of a fusion between two previously known gene cassettes (*oxa-10* and *aadA1*) encoding a beta-lactamase and an adenylyltransferase, respectively. In addition, two other new cassettes (*cmlA5* and *qacI*) were found. All three newly discovered cassettes had their own promoter sequences, a feature in contrast to most other known cassettes (Naas *et al.*, 2001). It should also be noted that a large proportion of the RI contain gene cassettes encoding resistance against streptomycin and spectinomycin, despite the fact that use of these antibiotics (at least in a clinical setting) has long ago been discontinued. In fact, new gene cassettes encoding resistance to aminoglycosides have also been discovered (Peters *et al.*, 2001 and Partridge *et al.*, 2002). In the case of *aadA8*, the gene cassettes may have arisen following recombination between the *aadA2* and *aadA3* cassettes. Although new gene cassettes seem at first sight to be rather distantly related, this relationship may be much closer upon better scrutiny. Sometimes minor sequence differences between gene cassettes have been found, which may result in larger differences at the protein level. For example, the *ereA2* and *ereA1* genes are nearly identical, but a one-nucleotide deletion in the *ereA1* sequence results in a predicted protein that is 62 amino-acids shorter than its *ereA2* counterpart. Whether these differences are real or reflect sequence errors is a matter of debate (Peters *et al.*, 2001). However, larger deletions may sometimes occur and new fusion cassettes arise, which may yield a functional fusion protein. An example is the product of the fused

aac(3)-Ib and *aac(6')-Ib* genes. Not only is the fusion protein functional, but also are the separately cloned products of the genes, despite the lack of the last four aminoacids in the *AAC(3)-I* enzyme. This enzyme confers resistance to gentamicin and fortimicin.

2.4.3. Biochemistry of antibiotic resistance

Understanding the mechanisms of resistance has become a significant biochemical issue over the past several years and nowadays there is a large pool of information about how bacteria can develop drug resistance (Mobashery and Azucena, 1999). Although the manner of acquisition of resistance may vary among bacterial species, resistance is created by only a few mechanisms which include (i) Antibiotic inactivation i.e. direct inactivation of the active antibiotic molecule (Wright, 2005); (ii) Target modification i.e. alteration of the sensitivity to the antibiotic by modification of the target (Lambert, 2005); (iii) Efflux pumps and outer membrane (OM) permeability changes i.e. reduction of the concentration of drug without modification of the compound itself (Kumar and Schweizer, 2003); or (iv) Target bypass i.e. some bacteria become refractory to specific antibiotics by bypassing the inactivation of a given enzyme. This mode of resistance is observed in many trimethoprim-and sulfonamide-resistant bacteria. The example is in bypassing inhibition of dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) enzymes (involved in tetrahydrofolate biosynthesis). They are inhibited by trimethoprim and sulfonamides, respectively. In several trimethoprim- and sulfonamide-resistant strains, a second enzyme that has low affinity for the inhibitors is produced (Mobashery and Azucena, 1999; Happi *et al.,* 2005). There is an amazing diversity of antibiotic resistance mechanisms within each of these four categories and a single bacterial strain may possess several types of resistance mechanisms. The mechanisms that will be used by the bacteria depends on the nature of the antibiotics, its target site, the bacterial species and whether it is mediated by a resistance plasmid or by a chromosomal mutation (Dzidic *et al.,* 2008).

2.4.3.1. Antibiotics inactivation

The defense mechanisms within the category of antibiotic inactivation include the production of enzymes that degrade or modify the drug itself. Biochemical strategies are hydrolysis, group transfer, and redox mechanisms (Dzidic *et al.,* 2008). This occurs through the following processes:

2.4.3.1.1. Antibiotic inactivation by hydrolysis

Many antibiotics have chemical bonds that are susceptible to inactivation by hydrolysis (*e.g.* esters and amides). Several enzymes are known to destroy antibiotic activity by targeting and cleaving these bonds. These enzymes can often be excreted by the bacteria, inactivating antibiotics before they reach their target within the bacteria. The classical hydrolytic amidases are the beta-lactamases that cleave the beta-lactam ring of the penicillin and cephalosporin antibiotics. Many Gram-negative and Gram-positive bacteria produce such enzymes, and more than 200 different beta-lactamases have been identified. beta-lactamases are classified into four groups on the basis of functional characteristics, including preferred antibiotic substrate. Clinical isolates often produce beta-lactamases belonging to different functional groups (Bonnet, 2004 and Poole, 2004). Extended-spectrum beta-lactamases (ESBLs) mediate resistance to all penicillins, third generation cephalosporins (*e.g.* ceftazidime, cefotaxime, ceftriaxone) and aztreonam, but not cephamycins (cefoxitin and cefotetan) and carbapenems. ESBLs are very diverse: more than 180 different ESBLs have been identified. They are most commonly detected in *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis*, but have also been found in other *Enterobacteriaceae* (Bradford, 2001; Shah *et al.*, 2004). Other hydrolytic enzyme examples include esterases that have been linked to macrolide antibiotic resistance and ring-opening epoxidases causing resistance to fosfomycin (Kim *et al.*, 2002; Nakamura *et al.*, 2000a; Fillgrove *et al.*, 2003).

2.4.3.1.2. Antibiotics inactivation by group transfer

The most diverse family of resistant enzymes is the group of transferases. These enzymes inactivate antibiotics (aminoglycosides, chloramphenicol, streptogramin, macrolides or rifampicin) by chemical substitution (adenylyl, phosphoryl or acetyl groups are added to the periphery of the antibiotic molecule). The modified antibiotics are affected in their binding to a target. Chemical strategies include *O*-acetylation and *N*-acetylation (Vetting *et al.*, 2004 and Schwarz *et al.*, 2004), *O*-phosphorylation (Nakamura *et al.*, 2000b and Matsuoka and Sasaki, 2004), *O*-nucleotidylation (Pedersen *et al.*, 1995), *O*-ribosylation (Houang *et al.*, 2003), *O*-glycosylation, and thiol transfer. These covalent modification strategies all require a co-substrate for their activity (ATP, acetyl-CoA, NAD+, UDP-glucose, or glutathione) and consequently these processes are restricted to the cytoplasm (Dzidic *et al.*, 2008).

2.4.3.2. Target modification

The second major resistance mechanism is the modification of the antibiotic target site so that the antibiotic is unable to bind properly. However, it is possible for mutational changes to occur in the target that reduce susceptibility to inhibition whilst retaining cellular function (Spratt, 1994). In some cases, the modification in target structure needed to produce resistance requires other changes in the cell to compensate for the altered characteristics of the target. This is the case in the acquisition of the penicillin-binding protein 2a (PBP2a) transpeptidase in *Staphylococcus aureus* that results in resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) and to most other beta-lactam antibiotics. To save the efficiency of peptidoglycan biosynthesis, PBP2a needs alterations in the composition and structure of peptidoglycan, which involves functioning of a number of additional genes (Enright, 2003; Happi *et al.*, 2005 and Leski and Tomasz, 2005).

2.4.3.2.1. Peptidoglycan structure alteration

The peptidoglycan component of the bacterial cell wall provides an excellent selective target for antibiotics. It is essential for the growth and survival of most bacteria. Consequently, enzymes involved in synthesis and assembly of the peptidoglycan component of the bacterial cell wall provide excellent targets for selective inhibition. The presence of mutations in the penicillin-binding domain of penicillin-binding proteins (PBPs) results in decreased affinity to beta-lactam antibiotics. Alterations among PBPs result in ampicillin resistance among *Enterococcus faecium*, and penicillin resistance among *Streptococcus pneumoniae* (Dowson *et al.*, 1994; Nagai *et al.*, 2002 and Kasowska *et al.*, 2004). Resistance to methicillin and oxacillin in *S. aureus* is associated with acquisition of a mobile genetic element called SCC*mec*, which contains the *mecA* resistance gene. The *mecA* determinant encodes PBP2a, a new penicillin-binding protein distinct from the PBPs normally found in *S. aureus.* PBP2a is highly resistant to inhibition by all clinically used beta-lactams and remains active to maintain cell wall synthesis at normally lethal beta-lactam concentrations (Tenover, 2006). Glycopeptides such as vancomycin inhibit cell wall synthesis of Gram-positive bacteria by binding C-terminal acyl-D-alanyl-D-alanine (acyl-D-Ala-D-Ala)-containing residues in peptidoglycan precursors. Resistance is achieved by altering the target site by changing the D-Ala-D-Ala to D-alanyl-D-lactate (D-Ala-D-Lac) or D-alanyl-D-serine (D-Ala-D-Ser) at the C-terminus, which inhibits the binding of vancomycin (Cooper *et al.*, 2000 and Hiramatsu, 2001). As a consequence, the affinity of vancomycin for the new terminus is 1000 times lower than for the native peptidoglycan precursor in the case of D-Ala-D-Lac. Dissemination of glycopeptide resistance in Gram-positive cocci can occur at the level of the

bacteria (clonal spread), replicons (plasmid epidemics) or of the genes (transposons). Glycopeptide (vancomycin) resistance can be intrinsic (VanC-type resistance) or acquired, present only in certain isolates belonging to the same species (*VanA, B, D, C, E* and *G* types of vancomycin resistance) (Courvalin, 2005).

2.4.3.2.2. Protein synthesis interference

A wide range of antibiotics interfere with protein synthesis on different levels of protein metabolism. The resistance to antibiotics that interfere with protein synthesis (aminoglycosides, tetracyclines, macrolides, chloramphenicol, fusidic acid, mupirocin, streptogramins, oxazolidinones) or transcription *via* RNA polymerase (the rifamycins) is achieved by modification of the specific target (Happi *et al.*, 2005). The macrolide, lincosamide and streptogramin B group of antibiotics block protein synthesis in bacteria by binding to the 50S ribosomal subunit (Weisblum, 1998; Spigaglia and Mastrantonio, 2002 and Ackermann *et al.*, 2003). Resistance to these antibiotics is referred to as MLS (B) type resistance and occurs in a wide range of Gram-positive bacteria. It results from a post-transcriptional modification of the 23S rRNA component of the 50S ribosomal subunit (Weisblum, 1995). Mutations in 23S rRNA close to the sites of methylation have also been associated with resistance to the macrolide group of antibiotics in a range of organisms. In addition to multiple mutations in the 23S rRNA, alterations in the L4 and L22 proteins of the 50S subunit have been reported in macrolide-resistant *S. pneumoniae* (Canu *et al.*, 2002). The mechanism of action of oxazolidinones (for example, inezolid) involves multiple stages in the protein synthesis (Bozdogan and Appelbaum, 2004). Although they bind to the 50S subunit, the effects include inhibition of formation of the initiation complex and interference with translocation of peptidyl- tRNA from the A site to the P site. Resistance has been reported in a number of organisms including enterococci and is linked to mutations in the 23S rRNA resulting in decreased affinity for binding (Wang and Taylor, 1998). Mutations in the 16S rRNA gene confer resistance to the aminoglycosides (Suzuki *et al.*, 1998). Chromosomally acquired streptomycin resistance in *M. tuberculosis* is frequently due to mutations in the *rpsL* gene encoding the ribosomal protein S12. Microorganisms that produce resistance to aminoglycosides have developed mechanism of high level antibiotic resistance by posttranscriptional methylation of 16S rRNA in the aminoglycoside binding site. This mechanism of resistance has recently been reported in human pathogens from nosocomial infections and animal isolates (Vlahovicek *et al.*, 2008).

2.4.3.2.3. DNA synthesis interference

Fluoroquinolones interact with the DNA gyrase and topoisomerase IV enzymes and prevent DNA replication and transcription. Resistance is conferred by mutations in specific regions of the structural genes that sufficiently alter these enzymes preventing the binding of antibiotics (Khodursky *et al.,* 1995 and Ince *et al.,* 2002). The most common mutations in this region cause resistance through decreased drug affinity for the altered gyrase–DNA complex (Willmott and Maxwell, 1993).

2.4.3.3. Efflux pumps and outer membrane (OM) permeability

The efflux pumps are the membrane proteins that export the antibiotics out of the cell and keep its intracellular concentrations at low levels. Reduced outer membrane (OM) permeability results in reduced antibiotic uptake. The reduced uptake and active efflux induce low level resistance in many clinically important bacteria (Nikaido, 1994).

2.4.3.3.1. Efflux pumps

Efflux pumps affect all classes of antibiotics, especially the macrolides, tetracyclines, and fluoroquinolones because these antibiotics inhibit different aspects of protein and DNA biosynthesis and therefore must be intracellular to exert their effect. Efflux pumps vary in both their specificity and mechanism (Nikaido and Zgurskaya, 1999). Although some are drug-specific, many efflux systems are multi-drug transporters that are capable of expelling a wide spectrum of structurally unrelated drugs, thus contributing significantly to bacterial multi-drug resistance (MDR) (Van Veen and Konings 1997). Inducible multi-drug efflux pumps are responsible for the intrinsic antibiotic resistance of many organisms, and mutation of the regulatory elements that control the production of efflux pumps can lead to an increase in antibiotic resistance. For example, the MexAB-OprM efflux pump in *Pseudomonas aeruginosa* is normally positively regulated by the presence of drugs, but mutations in its regulator (*mexR*) lead to the over expression of MexAB-OprM, which confers increased resistance to antibiotics such as beta-lactams (Poole, 2001). Both Gram-positive and Gram-negative bacteria can possess single-drug and/or multi-drug efflux pumps (Langton *et al.,* 2005). Bacterial drug efflux transporters are currently classified into five families (Pao *et al.,* 1998 and Van Veen and Konings, 1998). The major facilitator superfamily (MFS) and the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily are very large and the other three are smaller families: the small multi-drug resistance (SMR) family, the resistance-nodulation-cell division (RND)

superfamily and the multi-drug and toxic compound extrusion (MATE) family. Efflux transporters can be further classified into single or multicomponent pumps (Ma and Chang, 2004). Single component pumps transport their substrates across the cytoplasmic membrane. Multicomponent pumps, found in Gram-negative organisms, function in association with a periplasmic membrane fusion protein (MFP) component and an outer membrane protein (OMP) component, and efflux substrates across the entire cell envelope. Furthermore, the regulators of efflux systems may be attractive drug targets themselves. The regulators involved in efflux gene expression are either local or global regulators. Many pump component-encoding operons contain a physically linked regulatory gene. Some efflux pumps are known to be regulated by two-component systems. These systems mediate the adaptive responses of bacterial cells to their environment. Expression of various efflux pumps is also controlled by different global regulators. So far, several global transcriptional activators, including MarA, SoxS and Rob, have been shown to be involved in the regulation of expression of this system (Ma and Chang, 2004).

2.4.3.3.2. Outer membrane (OM) permeability changes

Gram-negative bacteria possess an outer membrane consisting of an inner layer containing phospholipids and an outer layer containing the lipid A moiety of lipopolysaccharides (LPS). This composition of the outer membrane (OM) slows down drug penetration, and transport across the OM is achieved by porin proteins that form water-filled channels. Drug molecules can penetrate the OM employing one of the following modes: by diffusion through porins, by diffusion through the bilayer or by self-promoted uptake. The mode of entry employed by a drug molecule largely depends on its chemical composition. For example, hydrophilic compounds either enter the periplasm through porins (*e.g.* beta-lactams) or self-promoted uptake (aminoglycosides). Antibiotics such as beta-lactams, chloramphenicol and fluoroquinolones enter the Gram-negative outer membrane *via* porins. As such, changes in porin copy number, size or selectivity will alter the rate of diffusion of these antibiotics (Denyer and Maillard, 2002 and Nikaido, 2003). The role of LPS as a barrier to antibiotics is well documented. Mutations in LPS that result in antibiotic hypersusceptibility have been reported. Strains of *E. coli* and *S. enterica* defective in LPS have been found to be at least 4-fold more susceptible to erythromycin, roxithromycin, clarithromycin and azithromycin than the wild-type strains (Vaara, 1993).

2.5. Modes of antibiotics action

Three conditions must be met for an antibiotic to be effective against bacteria: (i) a susceptible antibiotic target must exist in the cell; (ii) the antibiotic must reach the target in sufficient quantity; and (iii) the antibiotic must not be inactivated or modified (Sutcliffe *et al.,* 1999). Understanding antibiotic resistance mechanisms requires an understanding of where antibiotics exert their effect. There are five major modes of antibiotic mechanisms of action and here are some examples (Dzidic *et al.,* 2008).

2.5.1. Interference with cell wall synthesis

Beta-lactam antibiotics such as penicillins and cephalosporins interfere with enzymes required for the synthesis of the peptidoglycan layer. Glycopeptides (vancomycin, teicoplanin, oritavancin) target the bacterial cell wall by binding to the D-alanyl-D-alanine termini of the peptidoglycan chain, thereby preventing the cross-linking steps. Telavancin, a novel rapidly bactericidal lipoglycopeptide, inhibits peptidoglycan biosynthesis through preferential targeting of transglycosylation (Strohl, 1997 and Benton *et al.,* 2007).

2.5.2. Inhibition of protein synthesis

Macrolides bind to the 50S ribosomal subunit and interfere with the elongation of nascent polypeptide chains. Aminoglycosides inhibit initiation of protein synthesis and bind to the 30S ribosomal subunit. Chloramphenicol binds to the 50S ribosomal subunit blocking peptidyltransferase reaction. Tetracyclines inhibit protein synthesis by binding to 30S subunit of ribosome, thereby weakening the ribosome-tRNA interaction. The semisynthetic tetracycline derivatives, colloquially termed the glycylglycines, act at the bacterial ribosome to arrest translation. The glycylglycines bind the ribosome more tightly than previous tetracyclines, so that the *TetM* resistance factor is unable to displace them from this site. Hence *Tet M* is unable to protect the ribosomes from the action of these new drugs. The *Tet A*-mediated efflux system is ineffective against the glycylglycines, as they are not substrates for the transporter. The oxazolidinones, one of the newest classes of antibiotics, interact with the A site of the bacterial ribosome where they should interfere with the placement of the aminoacyl-tRNA (Strohl, 1997 and Leach *et al.,* 2007).

2.5.3. Interference with nucleic acid synthesis

Rifampicin interferes with a DNA-directed RNA polymerase. Quinolones disrupt DNA synthesis by interference with type II topoisomerases DNA gyrase and topoisomerase IV during replication and by causing double strand breaks (Strohl, 1997).

2.5.4. Inhibition of metabolic pathway

The sulfonamides (*e.g.* sulfamethoxazole) and trimethoprim each block the key steps in folate synthesis, which is a cofactor in the biosynthesis of nucleotides, the building blocks of DNA and RNA (Strohl, 1997).

2.5.5. Disorganizing of the cell membrane

The primary site of action is the cytoplasmic membrane of Gram-positive bacteria, or the inner membrane of Gram-negative bacteria. It is postulated that polymyxins exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial content. The cyclic lipopeptide daptomycin displays rapid bactericidal activity by binding to the cytoplasmic membrane in a calcium-dependent manner and oligomerizing in the membrane, leading to an efflux of potassium from the bacterial cell and cell death (Tenover, 2006 and Straus and Hancock, 2006).

2.6. Selected Molecular techniques for characterisation of antibiotic resistance genes

Detection of genetic determinants using modern molecular techniques confirm antimicrobial resistance based on the organism's genotype, rather than relying on the variability of phenotypic expression of the resistance. Moreover, these tests can be done within hours, providing clinically relevant information days before conventional susceptibility test results become available. Molecular assays to detect antimicrobial resistance directly from clinical and environmental samples have been developed (Fluit and Schmitz, 2001).

2.6.1. PCR method

PCR is well known and the first description of PCR was in 1987 by Mullis and Faloona. While in Saiki *et al.*, 1988 published the first diagonsitic application of the technique. The technique became broadly used after the introduction of a thermostable DNA polymerase from *Thermus aquaticus Taq* DNA polymerase (Saiki *et al.* 1988) and the development of automated oligonucleotide synthesis and thermocyclers. PCR involves cycles of heating the sample for denaturing, annealing of the primers, and elongation of the primers by a thermostable DNA

polymerase. In theory, each round of amplification gives a doubling of the number of DNA target molecules, but the process is seldom 100% efficient because of the presence of inhibitors, and in later rounds of amplification DNA polymerase may become limited (Fluit *et al.*, 2001).

However, during the last few years, new developments in labeling technology have expanded the applicability of PCR. One of such development was the use of 5'-fluorescence-labeled oligonucleotides that were blocked at their 3' ends, thereby preventing elongation by DNA polymerase. Besides this special oligonucleotide, PCR has two traditional oligonucleotides which function as primers and are chosen in regions flanking the special oligonucleotide. The special oligonucleotide hybridizes with the target and is removed by the 5' $\rightarrow$3' exonuclease activity of *Taq* DNA polymerase during primer extension, resulting in enhanced fluorescence that can easily be detected (Holland *et al.*, 1991).

Molecular beacons are advanced development which include hairpin-shaped oligonucleotide probes with a fluorophore attached and a molecule that quenches this fluorescence when it is next to the fluorophore. On hybridization with the target, the fluorophore and quenching molecule are spatially separated and fluorescence is possible (Tyagi *et al.*, 1998 and Tyagi and Kramer, 1996). In principle, the use of fluorophores with different emission spectra makes it possible to discriminate multiple targets. The addition of molecular beacons to PCR amplifications makes possible real-time monitoring of amplification. Furthermore, it allows a relatively easy quantitative PCR (Tyagi and Kramer, 1996 and Tyagi *et al.*, 1998). This technology has now been commercially realized with the TaqMan (ABI/Perkin-Elmer Corp., Foster City, Calif.) and Lightcycler (Roche Molecular Biochemicals, Mannheim, Germany) systems. A variation on this theme is the Scorpions primer (Oswell Research Products, Southampton, United Kingdom) (Whitcombe *et al.*, 1999). In this PCR-based method, the primer, probe, and fluorescent label are integrated into one molecule and form part of a homogeneous (closed-tube) assay (Fluit *et al.*, 2001).

2.6.2. DNA microarray technology

DNA microarrays offer the latest technological advancement for multi-gene detection and diagnostics. They were conceived originally to examine gene expression for large numbers of genes (DeRisi *et al.*, 1996; Lockhart *et al.*, 1996 and Schena *et al.*, 1996), but have also been applied to DNA sequence analysis (Pease *et al.*, 1994), immunology (Heller *et al.*, 1997), genotyping and diagnostics (Yershov *et al.*, 1996; Drmanac *et al.*, 1998; Call, 2001). In the latter context, they can be used to distinguish between DNA sequences that differ by as little as a

single nucleotide (Wang *et al.*, 1998). In addition, their flexibility and high throughput capabilities hold tremendous potential for pathogen detection, identification, and genotyping in molecular diagnostic laboratories. DNA microarrays are typically composed of DNA "probes" that are bound to a solid substrate such as glass. Each spot (50 to 150 μm) in the array lattice is composed of many identical probes that are complementary to the gene of interest. During hybridization, DNA "targets" diffuse passively across the glass surface, while sequences complementary to a probe will anneal and form a DNA duplex. Hybridised targets can then be detected using one of many reporter molecule systems. In essence, a microarray is a reverse dotblot that employs the same principles of hybridisation and detection used for many years with membrane-bound nucleic acids (e.g. Southern and Northern blots) (Call, 2001).

Several studies have demonstrated that DNA microarrays can be used to detect resistance genes as effectively as standard techniques such as polymerase chain reaction, sequencing, conjugation, and southern hybridization (Batchelor *et al.*, 2008; and Zou *et al.*, 2009). Most of these microarrays as reported in the previous paragraph rely on short to medium-length (20mer–80mer) oligonucleotides as detection probes because they are easily synthesized without the requirement of template DNA and can be cheaply made and arrayed onto a variety of substrates such as glass slides (Nickelsen and Sparling, 1981 and Frye *et al.*, 2006). Modified microscope glass slides have become the most widespread format for custom microarrays, with most Universities and Research Institutes having access to printing robots and scanners designed to manufacture and analyze these arrays. With the advent of this technology, it should be theoretically possible to design microarrays for the detection of all known sequenced antibiotic resistance genes available in the public domain and cheaply construct them in many research facilities world wide (Frye *et al.*, 2006).

It is important to note that this microarray is not intended to replace phenotypic testing in diagnostic and clinical settings, although there has been considerable progress in the development of diagnostic microarrays (Holland and Kiechle, 2005 and Monecke *et al.*, 2008). Microarray data are difficult to interpret in a clinical setting because the detection of a gene is a potential unclear result. Indeed, genes detected may not be functional or expressed, and negative hybridization results are even more difficult to interpret, as previously uncharacterized resistance mechanisms could lead to failure of a selected treatment. In the clinical setting, the goal is to select a successful treatment regimen, thus making phenotypic testing more informative than

gene detection. However, when the goal is to study the molecular epidemiology of AR, DNA microarrays are an exceptional tool for detecting multiple AR genes in a single assay (Frye *et al.*, 2010).

The major advantage of DNA microarray technology over convectional PCR technique is that it allows for the simultaneous analysis of a large amount of genetic information in a single assay (Ramsay, 1998), and has been developed to analyze specific bacterial species or test for a few microbial antibiotic resistance determinants (Call *et al.*, 2003 and Volokhov *et al.*, 2003; Giammarinaro *et al.*, 2005). For example Zhu *et al.* (2007) have developed a new multiplex PCR-based DNA microarray for detection of six antibiotic resistance genes in staphylococci, including *mecA*, *blaZ*, *aac*(6)-Ie-*aph*(2), *ermA*, *ermC*, and *msrA*, using one sequence-specific probe for each gene. The microarray also had probes specific to a variable region of 16S rRNA gene, simultaneously differentiating between *S. aureus* and other coagulase-negative staphylococci (CoNS) isolates. Validation of the microarray with 415 nonduplicate staphylococci isolates has demonstrated that this platform will be a suitable complement for phenotypic susceptibility testing and will provide a rapid guide for appropriate antimicrobial therapy as well as infection control.

CHAPTER THREE

Materials and Methods

3.1. Site description

3.1.1. Osun State, Nigeria

3.1.1.1. Obafemi Awolowo University Dam: This dam is located in the University Campus of Obafemi Awolowo University which is situated in Ile-Ife, Osun State. The dam was constructed in 1979 by damming Opa River for potable water supply and freshwater fisheries research in the University. It has an embankment length of 233.3m, a crest width of 6.7m. It is equipped with mechanical and auxiliary spillways.

3.1.1.2. Ede Water Works: This dam is located in Ede town in Osun state. It is also known as Ede Erinle dam. It is an extension of old Ede dam from Erinle River in the state. It lies between Lat $7^{o}44'$ 30.44'' and $7^{o}57'$ 00.79''N and Long $4^{0}26'21.71''$ and $4^{o}41'23.48''$ East of the Greenwich Meridian (Adediji and Ajibade, 2008). The dam was constructed to improve existing water supply system of cities such as Osogbo, Ede, Ife, Gbongan, Erin-Osun, Ilobu and Ifon as well as other town and rural communities in Osun central, Osun west and Ife areas of Osun State. In fact, the dam was designed by the old Oyo State Water Corporation to improve and expand existing Ede headwater work. The reservoirs created behind the dam extend some 12 km northward along the Erinle River. The reservoir covers about 14.0 km^2 at the normal water level, and about 15 km^2 at maximum water level. The gross capacity and safe annual yield of the impounding reservoir on Erinle River was estimated at 92.5 and 94 Million Cubic Metre (MCM).

3.1.2. Oyo State, Nigeria

3.1.2.1. Eleyele Water Works: Eleyele dam was constructed in 1942 in a quest to create a modern water supply system to meet the challenge of water supply and solve the problem of water scarcity for the emerging Ibadan metropolis. It was constructed on the main River Ona with a reservoir storage capacity of 29.5 million litres. The dam is located along Eleyele wetland in the northeastern part of Ibadan, southwestern Nigeria within longitude $N07^{o}25'00$" and $N07^{0}27'00$" as well as latitude $E03^{0}50'00$" and $E03^{o}53'00$". The wetland and the associated

Eleyele dam receive water from River Alapata and the head stream of River Ona. The catchment area of Eleyele wetland is relatively well drained with a network of River Ona and its tributaries (such as Ogbere, Alapata and Ogunpa). River Ona flows roughly in a north south direction (Tijani *et al.*, 2011)

3.1.2.2. Asejire Water Works: This is a reservoir that is located in Asejire via the Osun River, about 30 kilometers east of Ibadan. The reservoir was built in the late 1960s. Farming is totally banned in the catchment area, and trees have been planted on the banks so as to prevent erosion and silting. With abundant water supply, the reservoir remains full throughout the year (African Development Bank, 2010). The reservoir provides water to the Asejire and Osegere water treatment plants in Ibadan. (NLEVD, 2009). The water supply project was completed in 1972, and has a capacity of about 80 million liters per day, of which 80% is used for domestic purposes (Central Bank of Nigeria, 1999).

3.1.3. Ondo State, Nigeria

3.1.3.1. Owena-Idanre: This dam was constructed and commissioned by the Ondo State government in 1965 with an installed capacity of 19600 m^3/day to supply water to a population of 787867 people.

3.1.3.2. Owena-Ondo: This dam was constructed and commissioned by the Ondo State government in 1971. It was designed for an estimated populace of 192340 with an installed capacity of 5450 m^3/day. The dam gets its water supply from the Owena River and it covers an approximate surface area of 7.8km^2.

3.2. Sample collection

Water samples for microbiological and chemical analysis were obtained four times between December, 2010 to January, 2011 as well as between June, 2011 to July, 2011 from six selected dams in southwestern Nigeria. Samples were obtained from the untreated water (raw water), treated water and from municipal taps (distribution points) for microbial analysis while samples were obtained only from the raw water and treated water of each of the dams for chemical analysis. Points where the municipal tap samplings were carried out for each of the dams are indicated on Table 3.1. Sampling of the water was carried out as described by American Public health Association (APHA), 1998. Sterile sample bottles (50ml) were used for the sampling. Samples of the treated water (dams final output and municipal taps) was collected by opening of

the taps and allowing the water to run for 3 to 4minutes before collection. Collected samples were afterwards kept in the box packed with ice and transported to the laboratory for analysis within six hours. Samples from the raw water were taken from the dam output before getting to the treatment plants. Samples for chemical analysis were taken with 500ml sample bottles from the raw water and final water of the dams and then transported into the laboratory for chemical analysis.

Table 3.1: Locations of Municipal Tap Samplings of Selected Dams in southwestern, Nigeria.

Dams	Municipal Tap Code	Tap Location
Dam 1(Ife, Osun State), Municipal Tap 1 (M1)	IFM1	Awo Hall, OAU, Ife.
Dam 1(Ife, Osun State), Municipal Tap 2 (M2)	IFM2	Fajuyi Hall, OAU, Ife.
Dam 2 (Ede, Osun State), Municipal Tap 1 (M1)	EDM1	Muslim Grammar School, Ede.
Dam 2 (Ede, Osun State), Municipal Tap 2 (M2)	EDM2	Obada, Ede.
Dam 3 (Asejire, Oyo State), Municipal Tap 1 (M1)	AM1	Asejire
Dam 3 (Asejire, Oyo State), Municipal Tap 2 (M2)	AM2	Agodi Gate Booster Station
Dam 4 (Eleyele, Oyo State), Municipal tap 1 (M1)	EM1	Eleyele Water Tap
Dam 4 (Eleyele, Oyo State), Municipal tap 2 (M1)	EM2	Eleyele Water Tap
Dam 5 (Owena-Ondo, Ondo State), Municipal Tap 1 (M1)	OWODM1	NEPA, Akure
Dam 5 (Owena-Ondo, Ondo State), Municipal Tap 2 (M2)	OWODM2	Arakale, Akure

Dam 6 (Owena-Idanre, Ondo State), Municipal Tap 1 (M1)	OWIM1	Owena-Igbara Oke
Dam 6 (Owena-Idanre, Ondo State), Municipal Tap 1 (M2)	OWIM2	Owena-Ijesha

3.3. Determination of the physico-chemical properties of water samples.

Water samples were transported to the analytical laboratory of the Department of Chemistry; University of Ibadan, Oyo State, Nigeria. The following procedures were carried out.

3.3.1. Determination of pH

pH of the water samples was determined by the use of digital pH meter (Mettler Toledo FE20 Desktop)

3.3.2. Determination of Dissolved Oxygen (DO)

Two hundred mililitre of water sample was used to determine the DO. The DO meter (YS pro 20) was switched on and the probe was inserted into the water sample to read the value of the DO after 2 minutes.

3.3.3. Determination of Biochemical Oxygen Demand (BOD)

Samples were pipetted into BOD bottles containing aerated diluted water. The DO content was determined with DO probe and bottles were incubated in the dark for five days at 20^{o}C. At the end of five days, the final DO content was determined and the difference between the final DO reading and the initial DO reading was calculated. The difference in DO represented the BOD of the samples.

3.3.4. Determination of Chemical Oxygen Demand (COD)

In order to determine the COD of water sample 2.5 ml of water samples was taken with a pipette into a tube. Into another tube, 2.5 ml of distilled water was also pipetted which acted as blank. Then 1.5 ml of Potassium chromate was added into each tube. Into this same tube 3.5 ml of sulphuric acid reagent was added and covered with a screw cap. The tube was then placed in a COD digester at 150^{o}C for 2 hours. Afterwards tube was removed and cooled to room temperature before transferring it into a conical flask. Later a burette ~~burette~~ was filled with freshly prepared Ferrous Ammonium Sulphate (FAS). Two drops of Ferroin indicator were added to the samples in the conical flask and then titrated against ammonium sulphate until the colour changed to reddish brown. The COD was calculated as follows:

SN	Samples	Volume of Samples	Burette Reading (mL)		Volume of 0.1N FAS (mL)
			Initial	Final	
1	Blank	2.5			
2	Sample 1	2.5			

Specimen calculation:

$$COD = \frac{(A-B*N*8*1000)}{\text{Volume of sample taken}}$$

Where A= Volume of FAS for blank

B= Volume of FAS for Sample 1

N= Normality of FAS

V=Volume of sample

3.3.5. Determination of Total Organic Carbon (TOC)

The different water samples were acidified and the inorganic carbon (IC) was removed prior to analysis for organic carbon (OC) content using a TOC instrument system. The organic carbon in the water sample was oxidized to produce carbon dioxide (CO_2) by combustion which is then measured by a detection system. The carbon dioxide formed by oxidation is determined directly. A calibration curve was established by analyzing potassium hydrogen phthalate standard solutions of adequate concentration. Each sample solution and the blank solution were analyzed. A calibration curve was plotted using the mass concentrations of TOC in milligrams per litre of carbon against the instrument-specific response units. The reciprocal value of the slope of the resulting calibration line was the calibration faction (F) in milligram per litre of carbon.

3.3.6. Determination of Total Solid (TS)

A clear dry crucible of 150ml capacity was kept at 103^{o}C in an oven for 1 hour. A capacity and appropriate identification mark was placed on it. After 100ml of the thoroughly mixed sample was added into the beaker using measuring cylinder. The beaker was placed in an oven maintained at 103^{o}C for 24 hours. Afterwards, the beaker was cooled and weighed. The weight of the solid in the beaker was determined by subtracting the weight of the clean beaker from the weight determined after addition and drying of the sample in the beaker. Total solid (TS) was then determined as follows:

$$\text{Total solid, TS (mg/l)} = \frac{\text{mg of Solids in the beaker x 1000}}{\text{Volume of sample}}$$

3.3.7. Determination of Total Dissolved Solid (TDS)

A clear dry glass beaker of 150ml capacity was kept at 103^{o}C in an oven for 1hour. A capacity and appropriate identification mark was placed on it. With a measuring cylinder 100ml of the sample was filtered through a double layered filter paper (whatman no 2) and the filtrate was collected in the beaker. The beaker was placed in an oven maintained at 103^{o}C for 24hours. Soon after, it was cooled and weighed after 24 hours. The weight of the solids in the beaker was then determined by subtracting the weight of the clean beaker from the weight after drying with the sample. The total dissolved solid (TDS) was determined as follows:

Total dissolved solids, TDS (mg/l) = mg of solid in the beaker x 1000

Volume of sample

3.3.8. Determination of Total Suspended Solid (TSS)

This was calculated as follows: Total suspended solid, TSS (mg/l) = TS (mg/l) – TDS (mg/l)

3.3.9. Determination of conductivity

The conductivity of the water samples was determined with the aid of a conductivity probe and meter (YSI Pro 30). This was achieved by calibrating the meter with 0.1N Potassium chloride solution in a beaker. Afterwards, the electrode which has been switch on 30 minute before the experiment in order to stabilize the instrument is thoroughly rinsed with deionized water and carefully wiped with a tissue paper. Then it was dipped into 200ml of water sample in a beaker and steady reading was recorded.

3.3.10. Determination of Residual chlorine

The residual chlorine of the treated water samples were determined with the use of Diethyl Paraphenylene Diamine (DPD) indicator test, using a comparator. A tablet of the DPD is added to a sample of water, colouring it red. The strength of colour was measured against standard colours on a chart to determine the chlorine concentration. The stronger the colour the higher the concentration of chlorine in the water.

3.4 Determination of total plate count, total enterobacteriaceae plate count and isolation of bacteria

A total of 96 water samples were purposively collected aseptically in sterile screw cap bottles four times between December 2010 and July 2011 and then transported to the laboratory of the Environmental and Biotechnology Unit, Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria. Serial dilutions of the water samples were carried out aseptically up to 10^{-4} dilution in order to obtain countable bacteria colonies on the agar plate. Dilution 10^{-2} and 10^{-4} were plated out for enumeration and isolation of bacteria on different agar media. Nutrient agar was used for Total bacteria count while Eosin methylene blue (EMB) agar and deoxycholate agar were used as selective agar for isolation of *E. coli* and other species. For all treated water samples (treated dam water samples and the municipal samples) undiluted samples of the water and samples diluted to 10^{-1} were plated out. Colonies with different morphologies were observed

on the plates and streaked out on Nutrient Agar plate for purification. Colonies were later stored at 4^{o}C on Nutrient Agar (NA) slant.

3.5. Catalase test

This test was carried out by smearing overnight culture on a microscope slide. Afterward, a drop of 3% hydrogen peroxide was added to the smear. If copious bubbles were observed, the microbe is positive for catalase.

3.6. Gram Stain reaction

This test was carried out in order to separate the bacteria isolates into gram positive and gram negative before storing in freezing medium. This was achieved by transferring a drop of overnight culture from agar plate to a loopful of sterile distilled water on glass slide. The culture was then air dried and heat fixed over a gentle flame while moving the slide in a circular fashion to avoid overheating. Thereafter, crystal violet was added to the fixed culture and it was allowed to stand for 60 seconds. The excess stain was then rinsed with a stream of water from a plastic water bottle. Afterwards, iodine solution was added to the smear as a mordant while excess was also rinsed off with a running water. A few drop of decolorizer (95% ethanol) was then added and rinsed off after 5 seconds. It was then counterstained with safranin solution for 60 seconds and washed off with water. Excess water was removed by blotting with bibulous paper. The slide was then observed under oil immersion microscope. Gram positive bacteria retain the blue colour of the crystal violet while gram negative bacteria retain the red colour of the basic safranin.

3.7 Storage of bacteria isolates in phosphate buffer glycerol (freezing medium)

A total number of 296 bacteria isolates were obtained from all the samples points and were transferred into cariole bottles and shipped to the laboratory of Dr. Call Douglas at Paul G Allen School for Global Animal Health, Washington State University, Pullman, United States of America for analysis. Over there, the bacteria were stored in freezing medium at -80^{o}C. The freezing medium was prepared as follows: The chemical used were Na_2HP0_4, NaH_2P0_4 and glycerol. Phosphate buffer was prepared by combining 11.14ml solution of 0.2M Na_2HP0_4 with 8.46ml of 0.2M NaH_2PO_4 in 100ml glass bottle. Glycerol (29.4ml) was added into the phosphate buffer (final proportion of glycerol to buffer was 60:40) which was then autoclaved at 121^{0}C for 15minutes to make phosphate buffered glycerol. In order to make freezing medium 1:3 of the phosphate buffered glycerol to the bacteria solution in Luria Bertani (LB) broth were combined together in 96 agar well plate and grown overnight. One well was left un-inoculated in order to

serve as control. The well was afterwards sealed with cello tape properly, covered with the lid, wrapped with foil paper, labeled and stored at -80^{o}C.

3.8 Molecular characterisation of bacteria using 16S rDNA sequencing

The molecular characterization of all the bacteria isolates was carried out as described below.

3.8.1. Extraction of Total genomic DNA from bacteria using chelex 100 extraction method

Total genomic DNA was extracted from a single colony of 292 bacteria isolates using plastic sterile loop to pick each of the bacteria grown on Luria Bertani (LB) agar at 37^{o}C overnight into 200µl of 5% Chelex 100 (0.5g chelex 100 into 10ml pcr water) in a sterile boil proof microfuge tube. The mixture was then vortex very well. The suspension was boiled at 100^{o}C for 10minute and then centrifuged at 14000 rpm for 1minute at 4^{o}C to obtain the supernatant. The supernatant was used as template for PCR reactions (Suenaga and Nakamura, 2005)

3.8.2. Amplification of 16S rDNA of bacteria isolates

In order to identify all the bacteria the 16S rDNA of the bacteria was amplified using 16s-8F (AGAGTTTGATCMTGGCTCAG) as forward primer and 16s- 517R (ATTACCGCGGCTGCTGG) as backward primer which are designed to target the conserved regions of the 16S rDNA. Then 5µl of the chelex extracted DNA was used as template for the PCR reaction with 1x PCR buffer, 2mM $MgCl_2$, 0.8mM dNTPs and 0.1µM each of the forward and reverse primers. The amplification condition for the 16S rDNA were, 1minute of denaturation at 95^{o}C followed by 30 cycles of 96^{o}C for 30seconds, 60^{o}C for 30seconds and 72^{o}C for 30seconds and a final extension of 72^{o}C for 10minutes in a thermal cycler (Bio-Rad Laboratories, Richmond, CA, USA). The PCR products were analyzed on 1% agarose gel electrophoresis (Basica Le Agarose, Prona and 0.5X TBE prepared from 50X TBE, FERMENTAS) and run on gel electrophoresis system (Gel XL Ultra V-2, Colepalmer, Labnet International, Inc.). Amplified DNA was run on 1% agarose gel and the protocols were as follows: 1% agarose was prepared using 1% agarose (Invitrogen) in Tris-Borate (TBE) buffer solution (pH 8.5, 5X See Appendix 6) in a 500ml Erlenmeyer flask and shake carefully. The solution was heated in a microwave and allowed to boil until there was no particle in the solution. Afterwards 0.01% of ethidium bromide was added into the solution and allowed to cool. The solution was poured into a casting tray whose end had been sealed with a paper tape. Gel comb with 50 combs was placed on the solution in the casting tray and then allowed to solidify. The comb was removed from the solidified gel, it was placed in an electrophoretic tank into which TBE buffer had been poured. A five microlitre of the PCR product was mixed with

1µL of 6X loading dye (0.25% w/v bromophenol blue, 0.25% w/v xylene cyanol FF, 40% w/v sucrose) and then loaded into the well that had been created by the comb on the gel. Thereafter, 5µL DNA ruler ready to use 1kb plus DNA ladder was loaded into the first and last well. A voltage of between 90V and 100V was applied for 30 to 45minutes with an alternate current. The gel was taken out and a photograph was taken with a camera attached to an ultra-violet ray cupboard.

Running the PCR product on the electrophoresis gel was to confirm the gene amplification. The amplified products were sequenced (Eurofins MWG, USA). However, prior to sequencing the amplified products were purified by adding 1µl of shrimp alkaline phosphate and 0.5µl of exonuclease I to 5 µL of the amplified product and incubated at 37^{o}C for 15 minutes, and 80^{o}C for 15 minutes in order to terminate the reaction. In order to sequence the purified product, the forward primer was divided into four batches and each batch was labeled with different fluorescent dyes. Each dye-labeled primer was used in a sequencing reaction with one of dideoxynucleotides which include dideoxyadenisine, dideoxycytidine, dideoxyguanosine and dideoxythymidine. The reaction products were then pooled and analyzed using capillary electrophoresis for nucleotide sequence determination (Applied Biosystems (ABI) prism 310 Genetic Analyzer). The sequence obtained was then trimmed using gene sequential software and were compared with online genomic database using BLAST programme (http: www.ncbi.nlm.nih.gov/BLAST/blast) by submitting the sequence obtained to Gen Bank of National Centre for Biotechnology Information (NCBI) website.

3.9. Antimicrobial drugs susceptibility of bacteria isolates

The susceptibility of all the 292 bacteria isolates was determined. Specific concentration according to CLSI standard breakpoint of the antibiotics whose profile was to be determined was added into the LB agar after it had been cooled to 55^{o}C in a water bath. The agar was poured into petri dishes (150mm x 15mm) and then allowed to solidify. Bacteria culture from the selected dams already stored in 96 well plate in phosphate buffer glycerol at -80^{o}C was brought out from the freezer, subcultured in sterile LB broth in another 96 well plate at 25^{0}C overnight. The subcultured bacteria were inoculated on the surface of the solidified agar by point inoculation using a sterile replicator. The bacteria was allowed to grow overnight at 37^{o}C in an incubator. Bacteria growth at each of the points of inoculation was termed resistant while those termed as sensitive will not grow at the point of inoculation on the agar. The antibiotics used and their

various concentrations according to CLSI standard for both gram positives and gram negative organisms are shown in Table 2.1.

Table 3.2: Antibiotic concentrations tested against gram positive and gram negative bacteria

Antibiotics for gram negative with concentration (µg/ml)			**Antibiotics for gram positive with concentration (µg/ml)**		
Code	**Name**	**Concentration**	**Code**	**Name**	**Concentration**
FF	Florfenicol	16	SU	Sulfamethoxazole	512
T	Tetracycline	16	AM	Ampicillin	0.5
S	Streptomycin	16	T	Tetracycline	16
G	Gentamycin	16	SXT	Sulfamethoxazole/Trimethoprim	76/4
K	Kanamycin	64	G	Gentamycin	16
C	Chloramphenicol	32	E	Erythromycin	8
N	Nalidixic Acid	30	RIF	Rifampin	4
AMC	Amoxillin/Clavulanic Acid	32/16	LIN	Lincomycin	4
CEF	Ceftiofur	12	CIP	Ciprofloxacin	4
SU	Sulfamethoxazole	512			
SXT	Sulfamethoxazole/Trimethoprim	76/4			

3.10. Molecular characterisation of antibiotic resistance genes using microarray analysis

Microarray analysis was carried out on 19 multi-drug resistance bacteria selected randomly from all the locations. The following steps were taken for the analysis.

3.10.1. Microarray construction

PCR products containing complementary DNA of interest (gene probes) were diluted to 75 ng/µl in print buffer (100 mM Na_2HPO_4, 200 mM NaCl, 0.01% sodium dodecyl sulfate [SDS]; pH - 11); denatured (95°C, 5 minutes) with a thermal cycler, and allowed to cool to room temperature. Each probe was then deposited at a fixed location within each masked well by a robotic spotter (BioRobotics Microgrid II; Woburn, Mass). Each probe was printed as four replicate spots within each array, and every array included an arbitrary oligonucleotide probe (25-mer) conjugated with biotin. These biotin pseudoprobes served as positive controls for the detection chemistry and served as orientation points for image processing. Thereafter, slides were baked for 1hour at 130°C under vacuum (22 Hg) and stored at room temperature in a dark cupboard.

3.10.2. Genomic DNA extraction for microarray hybridization

The genomic DNA of the bacteria was extracted using Qiagen DNeasy genomic extraction kit (Qiagen, Valencia, CA; Cat No 6950). A single colony of the bacteria that has been grown and incubated at 37^{o}C overnight on Luria Bertani (LB) agar was picked with plastic inoculation loop

and inoculated into LB broth and grown overnight in an incubator at 37^{o}C. Water bath and heating block were set at 55^{o}C and 70^{o}C respectively before commencement of the process. In a microcentrifuge, 3.0ml of the bacteria culture grown in LB broth was transferred and spinned for 10 minutes at 5000 x g to pellet the cells. Supernatant was removed by decanting and pellet blotted in order to remove the last bit. After the pellet was completely re-suspended in 180µl of Buffer ATL (Tissue lysis buffer). Afterwards, 20µl proteinase K was added, mixed by vortexing and incubated at 55^{o}C for 1 hours until the tissue was completely lysed. Thereafter, 20µl RNase A (10mg/ml) was added and mixed by vortexing, and allowed to cool for 2 minutes at room temperature. The mixture was vortexed again for 15 seconds, and 20µl Buffer AL (lysis buffer) was added into the sample mixture, thoroughly vortexed and incubated at 70^{o}C for 10 minutes. After incubation, 200µl of 100% ethanol was added to the mixture, and thoroughly mixed by vortexing. All the white precipitate formed in the mixture were pipetted out into Dneasy mini Column sitting in a 2ml collection tube. This was thereafter centrifuged for 1 minutes at 10000 x g. The flow through in the collection tube was then discarded. The Dneasy column was placed in a new 2ml collection tube and 500µl Buffer AW1 (Wash buffer 1) was added and centrifuged for 1 minutes at 10000 x g. The flow through in the collection tube was again discarded. Dneasy column was placed in another 2ml collection tube and 500µl buffer AW2 (Wash buffer 2) was added and centrifuged for 3 minutes at full speed to dry the Dneasy membrane. The flow through in the collection tube was discarded again. The Dneasy column was afterwards placed in a clean 1.5ml tube and 200µl buffer AE (Elution buffer) was pipetted into the membrane. It was incubated at room temperature for 1 minutes, and centrifuge for 1min at 10000 X g for elution of the DNA from the membrane.

DNA extraction was confirmed by measuring its concentration with a spectrophotometer. After measuring the DNA, the concentration observed was less than 25ng/ul. The DNA was then precipitated using Ethanol precipitation into 1.5 ml micro centrifuge tube containing the DNA to be concentrated 1/10 volume of 3M sodium acetate (20µl) solution was added along with 2 volumes of 100% ethanol (440µl). The mixture was mixed by inversion which was followed by incubation at -80^{o}C for 30 minutes. The mixture was centrifuged at a maximum speed of 30 minutes at 4^{o}C. The supernatant was then carefully decanted off and blotted with paper towels. Ethanol (100%) of volume 400µl was then added. The solution was centrifuged again at a maximum speed of 30 minutes at 4^{o}C. The supernatant was carefully decanted and blotted with

paper towel. The DNA was then re-suspended in 50µl of PCR water. The DNA was afterwards quantitated using a spectrophotometer to measure the concentration.

3.10.3. Nick translation: biotinylation and fragmentation of DNA

This was achieved by combining the following in 0.2ml PCR tubes on ice:

i. 1µl (up to 40µl) DNA
ii. 5µl 10X dNTP mix (from Nick Translation Kit)
iii. 5µl 10X enzyme mix (from Nick translation Kit)

It should be noted that a total of 1µg of DNA was used in a total volume of 40µl. The mixture was incubated at 16^{o}C in a thermal cycler (Bio-Rad Laboratories, Richmond, CA, USA) for 2 hour and held at 4^{o}C. The samples were then transferred into 1.5ml microcentrifuge tubes.

3.10.4. Slide pre-hybridization preparation

The microarray slides were prepared by immersing in 50ml 1% Bovine Serum Albumin (BSA) blocking solution (See Appendix 2.2) in a coplin staining jar. The slides were incubated for 10 minutes at room temperature by shaking at 80rpm to eliminate bubbles on the slide surface. The slides were removed and dipped to rinse 20 times in double de-ionised or double distilled water. The water was discarded, and the slide was washed 5 times with new double distilled water.The back and edges of the slide were wiped with a kimwipe and spun to dry for 15 seconds using a slide centrifuge. It should be noted that slides should be handled using forceps on the frosted end. The slides can then be stored for up to 1hour before adding the samples.

3.10.5. Sample application/hybridization

The nick translated DNA, i.e. DNA that has been labeled with biotin from session, 3.7.3 was boiled for 3 minutes and left to cool on ice. The fairly cold DNA was then vortexed and centrifuged slightly before adding the DNA samples on the microarray slides. The microarray slides were placed on the humidified chamber. On each of the microarray well containing the gene probe, 45µl of each sample was added (Note: 2 well per nick translated sample). When adding the samples, care was taken to spread the droplets fully on the well in order for the samples to fully cover the probes on the well. The slides were carefully sealed (face-up and frosted end toward the cap) in a hybridization chamber. Caution was taken to avoid touching the well with a damp filter paper in the humidity chamber. The lid of the chamber was properly tightened. Afterwards, the humidity chamber was plated in the rack with lead weight plated on

the rack before the rack was submerged in a water bath at 55^{o}C. The slides were then hybridized overnight for about 12 to 16 hours.

3.10.6. Post hybridization stringency washes

Low stringency wash of the slides was carried out by pre-warming 1X solution containing Saline Sodium citrate (SSC), 0.2% Sodium dodecyl sulfate (SDS) in a coplin jar to 55^{o}C (See Appendix 2.3c). The slides were removed from the hybridization chamber and completely immersed in the pre-warmed solution with the frosted end up in the coplin jar for 4 minutes (plate 3.1). The slides were then transferred to 0.1X SSC, 0.2% SDS (See Appendix 2.3b) in a coplin jar and rotated on an orbital shaker at 80 rpm for 4 minutes at room temperature. The slides were transferred to 0.1X SSC in a coplin jar for high stringency wash by shaking at 80rpm for 4min at room temperature. Low and high stringent wash was followed by Tris-NaCl- Tween (TNT) Buffer wash where the slides were transferred to horizontal staining jar (Plate 3.2) that contained TNT buffer (See Appendix 2.4) to cover the slides. This was followed by shaking the slides on an orbital shaker for 1 minutes at 80rpm room temperature. The slides were washed two more times in TNT solution. Care was taken not to allow the slides get dry. The slides were then incubated with 45µl of 1:100 streptavidin-horseradish peroxidase (SA-HRP) in TNB (See Appendix 2.5 (i)) for 30 minutes and washed three times in TNT solution in horizontal staining jar by shaking it with an orbital shaker at 80rpm for 1 minute. Slides were incubated with 45µl of 10% FES, 2X SSC (See Appendix 2. 5(ii)) for 30 minutes and washed again three times with TNT in horizontal staining jar at 80rpm for 1 minute.

The slides were removed from the TNT and incubated with 1:50 biotinyl tyramide (BioT), 1x A mplification Diluent (Amp Dil) (See Appendix 2.5(iii)) for 10 minutes and washed three times in horizontal staining jar with TNT solution. The light in the room was dimmed before inoculating the slide with 45µl of 1:500 SA Alexa 555 (Molecular Pro bes, Eugene, Oregon), 1XSSC, 5X Denhardt's solution (See Appendix 2.5(iv)) for 1hour in the dark. The slides were afterwards washed again three times with TNT solution in a horizontal staining jar by shaking it at 80 rpm for 1 minute. The slides were spinned dry in a slide centrifuge for 15 minutes.

After hybridization, the slides were scanned with an arrayWoRx^{e} scanner (Applied Precision, Issaquah, Washington). Alexa555 used as the fluorescence dye, hads an optimal excitation wavelength of 555nm and an emission wave length of 565nm. The scanner used in this study had a white light source and an emission filter of cy3 that functioned well with Alexa555. Hence,

excitation wavelength of 540nm (25 nm bandwidth) and an emission wavelength of 595nm (50nm bandwidth) were used. Image quantification was accomplished with softWoRx software (Applied Precision). Median pixel values were reported as signal intensity (averaged for replicate probes).

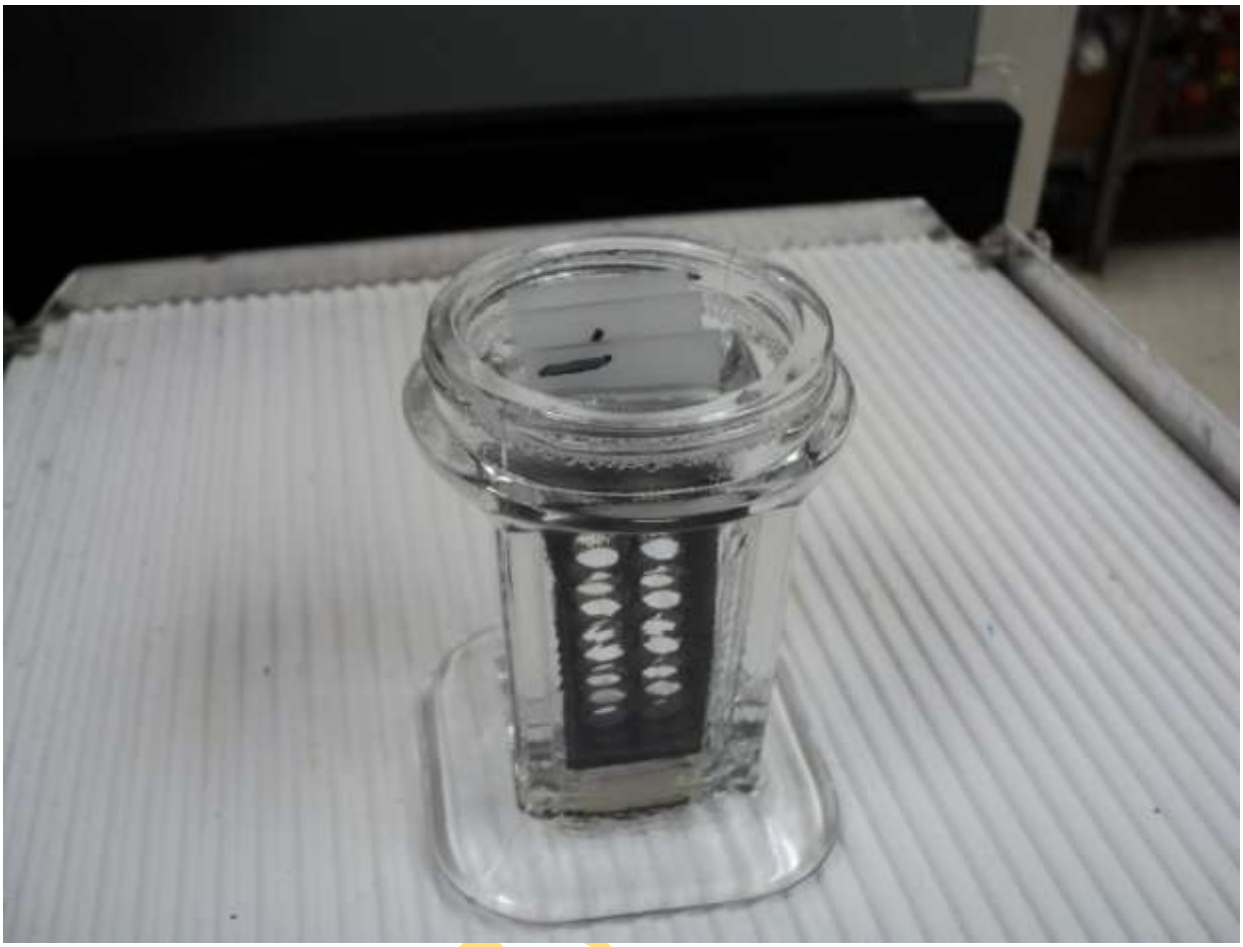


Plate 3.1: Microarray slide in pre-warmed solution of 1X SSC, 0.2% SDS at 55^{o}C

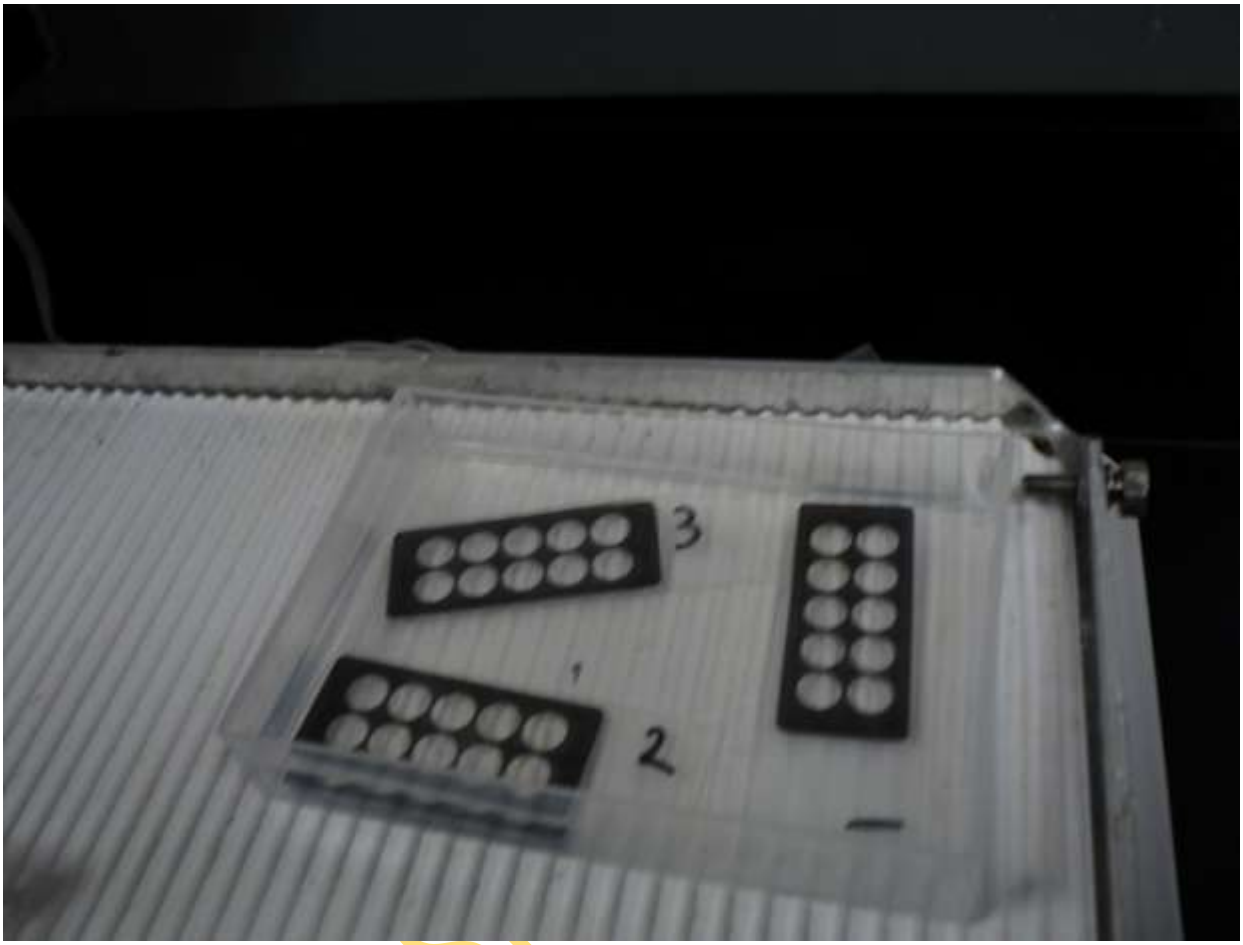


Plate 3.2: Microarray slides in horizontal staining jar

3.11. Molecular characterisation of antibiotics resistance genes

3.11.1. Characterisation of class 1 and class 2 integron and sequencing of gene cassettes

A total of 191 bacteria isolates that were multi-drug resistant (i.e. resistant to at least 3 classes of antibiotics) were selected from the bacteria isolates derived from all the selected dams and were tested for the presence of class 1 and class 2 integrons. Total genomic DNA obtained from Session 3.5.1 by chelex 100 extraction methods was used as template for PCR amplification of class 1 and class 2 integrons. Integrase genes were detected using primer sets intI1_F/intI1_R and intI2_F/intI2_R, for class 1 and class 2 integrons respectively (Table 3.3). Isolates that gave a positive result using integrase-specific primers were later tested for the presence of inserted gene cassettes. Previously described primer sets 5´-CS/3´-CS and hep_F/hep_R (Table 3.3), with homology to the 5´ and 3´ conserved regions (CS), was applied to detect and determine the size of the gene cassettes (for class 1 and class 2 integrons respectively). The primer set, *qacEΔ1* and *Sul 1* as shown on Table 3.3 directed at the 3'-CS of class 1 integrons was used to detect the presence of these genes which encodes for resistance to disinfectants i.e. quaternary ammonium compounds and sulfonamides respectively for class 1 integron.

The amplification of all the genes was carried out using 5µl of the chelex extracted DNA as temp late for the PCR reaction mixture with 5µl of PCR buffer (1x), 2µl of $MgCl_2$ (2mM), 1µl of dNT Ps 0.8mM and 1ul (0.2 µl) each of the forward and reverse primers in a thermal cycler (Model: Bio Rad Laboratories, Richmond, CA, USA). The condition for the amplification of the *int1* gene of class 1 integron was as follow: 1 minute of Denaturation at 95^{o}C followed by 30 cycles of 96^{o}C for 30 seconds, 60^{o}C for 30 seconds and 72^{o}C for 30 seconds and a final extension of 72^{o}C for 10min. The same condition was also used for the *intI* 2 integron of class 2 integron, 5´-CS/3´-CS of class 1 variable region of gene cassette and hep_F/hep_R variable region of class 2 gene cassette except that the annealing temperature of the PCR machine was changed to 50^{o}C, 58.5^{o}C and 60^{o}C respectively as shown in Table 3.3. PCR products (5 µl) were analyzed by electrophoresis on a 1% agarose gel and stained with ethidium bromide as described in session 3.5.2. Primers previously used for amplification of each DNA fragment of the amplified variable regions of the gene cassettes were used in the sequencing reactions they were sent (Eurofins MWG, USA). Protocol used for the sequencing is reported in the 16S rDNA amplification in ses sion 3.5.2. Online similarity searches were performed with the BLAST software in the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/).

Table 3.3: Primers used in this study for amplification of class 1 and class 2 integrons and other antibiotic resistant genes

Primer pair	Target	Sequence (5´-3´)	Annealing temperature (◦C)	Amplicon size (bp)	Reference
IntI1_F	Class 1 integrase gene	CCTCCCGCACGATGATC	55	270	Houang *et al.*, 2003
IntI1_R		TCCACGCATCGTCAGGC			
IntI2_F	Class 2 integrase gene	TTATTGCTGGGATTAGGC	50	233	Roe *et al.*, 2003
IntI2_R		ACGGCTACCCTCTGTTATC			
5´_CS	Class 1 integron variable region	GGCATCCAAGCAGCAAG	58.5	variable	Hall and Collis, 1995
3´_CS		AAGCAGACTTGACCTGA			
Hep_F	Class 2 integron variable region	CGGGATCCCGGACGGCATGCACGATTTGTA	60	variable	White and Mciver, 2001
Hep_R		GATGCCATCGCAAGTACGAG			
Sul1-F	*sul*1	CGGCGTGGGCTACCTGAACG	60	433	Vinue *et al.*, 2010
Sul1-R		GCCGATCGCGTGAAGTTCCG			
Sul2-F	*sul*2	GCGCTCAAGGCAGATGGCATT	60	293	,,
Sul2-R		GCGTTTGATACCGGCACCCGT			

Table 3.3 (Cont'd): Primers used in this study for amplification of class 1 and class 2 integrons and other antibiotic resistant genes

Primer pair	Target	Sequence (5´-3´)	Annealing temperature (◦C)	Amplicon size (bp)	Reference
pVP440sul3F	*sul*3	TCAAAGCAAAATGATATGAGC	55	787	Vinue *et al.*, 2010
pVP440sul3 R		TTTCAAGGCATCTGATAAAGAC			
qacEΔ1F	*qacE*Δ1F	ATC GCA ATA GTT GGC GAA GT	58	800	Stokes and Hall, 1989
sul1-B	*sul*1-B	GCA AGG CGG AAA CCC GCG CC	58		Sundstrom, 1998
aph (3´´)[c] -F	*aph* (3´´)[c]	GCTCAAAGGTCGAGGTGTGG	55	515	Van Overbeek *et al.*, 2002
aph (3´´)[c] -R		CCAGTTCTCTTCGGCGTTAG	55	515	,,
ant (3´´)[b] -F	*ant* (3´´)[b]	CAGCGCAATGACATTCTTGC	55	295	,,
ant (3´´)[b] -F		GTCGGCAGCGACA(C/T)CCTTCG	55	295	,,
aph(6)-1d[d] –F	*aph*(6)-1d[d]	GACTCCTGCAATCGTCAAGG	55	560	,,
aph(6)-1d[d] -R		GCAATGCGTCTAGGATCGAG	55	560	,,
tet(A)- F	*tet*(A)	TTGGCATTCTGCATTCACTC	60	494	,,
tet(A)- R		GTATAGCTTGCCGGAAGTCG	60	494	,,

Table 3.3 (Cont'd): Primers used in this study for amplification of class 1 and class 2 integrons and other antibiotic resistant genes

Primer pair	Target	Sequence (5´-3´)	Annealing temperature (◦C)	Amplicon size (bp)	Reference
tet(B)- F	*tet*(B)	CAGTGCTGTTGTTGTCATTAA	60	571	Call *et al.*, 2003
tet(B)- R		GCTTGGAATACTGAGTGTAA	60	571	,,
tet(E)- F	*tet*(E)	TATTAACGGGCTGGCATTTC	55	544	,,
tet(E)- R		AGCTGTCAGGTGGGTCAAAC	55	544	,,
tet(M)- F	*tet*(M)	ACACGCCAGGACATATGGAT	55	536	,,
tet(M)- R		ATTTCCGCAAAGTTCAGACG	55	536	,,
tet(30)- F	*tet*(30)	CCGTCATGCAATTTGTGTTC	55	550	,,
tet(30)- R		TAGAGCACCCAGATCGTTCC	55	550	,,
SHV_F	bla_{SHV}	GCGAAAGCCAGCTGTCGGGC	62	538	Henriques *et al., 2006*
SHV_R		GATTGGCGGCGCTGTTATCGC	62	538	,,
CTX_F	bla_{CTX-M}	GTGCAGTACCAGTAAAGTTATGG	55	538	,,
CTX_R		CGCAATATCATTGGTGGTGCC		538	,,
TEM_F	bla_{TEM}	AAAGATGCTGAAGATCA	44	425	,,
TEM_R		TTTGGTATGGCTTCATTC		425	,,

3.11.2. Molecular characterisation of tetracycline resistance genes (*tet A, tet B, tet E, tet M, tet O* and *tet 30*)

The diversity of tetracycline resistant genes encoded in the genome of 120 tetracycline and multi antibiotic resistant bacteria isolates selected among isolates from all the sample areas were tested for the presence of specific tetracycline resistant genes *tet* A, *tet* B, *tet* E, *tet* M, *tet* O and *tet* 30. Primers specific for these tetracycline resistant genes were used for PCR amplification (Table 3.3). The amplification for all the genes were carried out using 5µl of the chelex extracted DNA of tetracycline and multi antibiotic resistant isolates or bacteria as template for the PCR reaction, using PCR machine (Bio-Rad Laboratories, Richmond, CA, USA). The PCR reaction mixture is described in the amplification of class 1 and class 2 integron in session 3.8.1 above. The annealing conditions for each of the primers were 60^0C for *tet A* and *tet B* and 55^0C for *tet E*, *tet M* and *tet 30* as shown in Table 3.3.

3.11.3. Molecular characterisation of sulfonamide resistance genes (*sul* 1, *sul* 2, *sul* 3)

A total of 179 bacteria that were sulfamethoxazole and multi antibiotic resistant were characterized for *sul 1, sul 2* and *sul 3* for this test. Primers specific for sulfonamide resistant genes *sul* 1, *sul* 2 and *sul* 3 were used in the amplification of these genes. The chelex extracted bacteria DNA (5µl) were used as a template in the PCR reaction. The reaction mixture and PCR condition were as described in session 3.8.1. While annealing temperature of each of the primer was as shown in Table 3.3 that is 60^oC for *sul 1, sul 2* and 55^oC for *sul 3.*

3.11.4. Molecular characterisation of extended β- lactamase resistance genes (bla_{SHV}, bla_{CTX} and bla_{TEM})

A total of 172 bacteria isolates that were multi antibiotic and ampicillin resistant were selected for this amplification reaction. Primers specific for these resistant genes were used for this amplification. The chelex extracted Genomic DNA (5µl) were used for this amplification. The PCR reaction mixture is also described in session 3.8.1. The amplification condition is also as described in the session while annealing temperature is shown on Table 3.3

3.11.5 Molecular characterisation of streptomycin/spectinomycin resistance genes (*aph* $(3'')^c$, *ant* $(3'')^b$, *aph* (6)-$1d^d$)

Three genes conferring resistance on aminoglycoside streptomycin, aminoglycoside phosphotransferases *aph (3´´), aph(6)-1d*^{d} and adenylases *ant* $(3'')^b$ were amplified in 101 Gram negative bacteria isolates that were resistant to streptomycin. Chelex extracted DNA (5µl) was used for the amplification as described in session 3.5.1 while reaction mixture and PCR reaction condition were as described in session 3.8.1.

3.12. Determination of presence of plasmid on bacteria isolates

Bacteria were grown on Luria Bertani (LB) agar overnight and a single colony was picked and inoculated overnight as well in LB Broth. A micropipette 150µl of the culture was later pelleted by centrifugation for 10 minutes at 1000xg. The supernatant was removed and re-suspended in 100µl of lysis buffer (3% SDS, 50mM Tris pH 12.6 with 50mM Tris adjusted by 1.6ml 2N NaOH up to 100ml final volume). The mixture was incubated at 55^{o}C for 1 hour. The resulting plasmid was extracted with 150µl of Phenol: chloroform (1:1, v/v, pH 7.9) and mixed by inversion several times before spinning at highest speed for 10 minutes. A micropipette 50µl of the supernatant was transferred into a new tube and mixed with 10µl of a loading dye. The mixture containing the plasmid was run on 1% agarose gel electrophoresis in 1X TAE (See Appendix 7b) buffer for more than 3 hours at a voltage of 8v/cm.

3.12. Conjugation studies on bacteria isolates

Recipient bacteria *E. coli* strain DH5α that is sensitive to all antibiotics except nalidixic acid and 19 donor bacteria that are resistant to tetracycline and carrying various tetracycline resistant genes (*tet A, tet B* and *tet 30*) but sensitive to nalidixic acid were inoculated separately in Luria Bertani (LB) broth overnight. Nitrocellulose membrane papers were then cut into 2 x 1 cm and sterilized by dry cycle autoclave at 121^{o}C for 30 minutes. Afterwards, the sterile papers were placed aseptically using sterile forceps on the surface of solidified sterile Luria Bertani agar. Ten microliter of the overnight grown broth culture of both the donor and recipient bacteria were inoculated and incubated together on the same spot on the nitrocellulose paper at 37^{o}C overnight. Thereafter, 500µl of sterile phosphate buffer saline was used to wash the bacteria from the nitrocellulose paper into sterile petri dish. Afterwards, micropipette was used to transfer the culture on to the surface of LB agar containing 20µl/ml of nalidixic acid and 16µl/ml of tetracycline. It was then incubated overnight at 37^{o}C. Bacteria that grow on the Nalidixic and tetracycline agar were the recipient bacteria (i.e transconjugants) that have successfully received the tetracycline resistant genes from the donor. The transconjugants DNA were extracted by chelex extraction method as described in session 3.5.1 and used as DNA template in the amplification of the transferred genes by PCR so as to confirm the transfer of the tetracycline resistance genes.

CHAPTER FOUR

Results

4.1. Physico-chemical properties of selected water samples

4.1.1 Physico-chemical properties of raw water from selected Dam water samples in southwestern Nigeria.

The results of physico-chemical properties of raw water are shown in Table 4.1. Some of the parameters tested were compared with WHO limits and it was discovered that Eleyele water had BOD of 12.30mg/l during the December/January sampling, which was above the WHO limit (6 to 9ml/l). It was also observed that the TSS of the water sampled from Ede raw water was 769mg/l during June/July sampling, a value higher then WHO limit (500mg/l). Other parameters were within the recommended WHO limits. The lowest COD was 11.00mg/l which was obtained from December/January sample from Ede Dam while the highest value was 88.6mg/l, obtained from December/January water sample from Eleyele Dam. The TSS of the water samples during June/July sampling ranged from the lowest of 47.0mg/l at Asejire to the highest of 769.0mg/l in Ede.

4.1.2. Physico-chemical properties of treated water from selected water samples in southwestern Nigeria.

The results of the physico-chemical properties of treated water are shown in Table 4.2. The pH of 5.7, 5.4 and 5.2 were obtained in December/January sample from Asejire, June/July sample of Owena-Ondo and December/January sample from Owena-Idanre respectively. These values are all below the WHO limits signifying that these water samples are more acidic than the recommended range. All other parameters were observed to be within the recommended WHO limits. The BOD of all samples ranged from the lowest of 0.43mg/l observed in December/January for Ede water sample to the highest of 3.99mg/l in June/July Asejire water sample. The DO of all the sample points was in the range of 3.11mg/l to 6.82mg/l. The lowest TOC value of 0.49mg/l was obtained in December/January sample of Eleyele water while 2.76mg/l was observed as the highest value during December/January samples from Ife Dam. The highest level of 1.71mg/l residual chlorine was obtained in December/January sample from Ife compared to 0mg/l value observed in June/July samples. No residual chlorine was also observed in Ede water sample during in June/July.

Table 4.1: Physico-chemical properties of raw water samples of selected dams in southwestern Nigeria

Parameters	Dam 1 (IFRW)		Dam 2 (EDRW)		Dam 3 (ARW)		Dam 4 (ERW)		Dam 5 (OWODRW)		Dam 6 (OWIRW)		WHO Limit
	June/July	Dec/Jan	June/July	Dec/Jan	June/July	Dec/Jan	June/July	Dec/Jan	June/July	Dec/Jan	June/July	Dec/Jan	
pH	6.90	7.40	7.10	7.40	7.10	8.20	6.70	6.90	6.50	7.40	7.10	7.30	6.5-8.5
BOD (mg/l)	5.38	4.36	3.89	1.21	2.38	4.82	7.32	12.30	4.83	6.83	6.62	5.72	6-9
COD (mg/l)	64.70	28.20	37.60	11.00	13.60	28.30	65.20	88.60	53.60	49.80	64.60	56.10	-
DO (mg/l)	3.40	5.61	4.21	5.39	4.82	3.84	2.66	3.28	3.11	1.45	3.21	2.34	-
TOC (mg/l)	2.65	2.17	5.21	2.08	3.03	4.31	2.65	5.32	6.22	1.34	6.43	1.43	-
TDS (mg/l)	100.00	97.00	60.00	42.00	80.00	64.00	120.00	76.00	66.00	62.00	50.00	58.00	500
TS (mg/l)	260.00	124.00	840.00	81.70	130.00	120.00	362.00	280.00	420.00	190.00	430.00	360.00	-
TSS (mg/l)	158.00	23.40	769.00	38.40	47.00	50.00	240.00	200.00	350.00	120.00	370.00	287.00	500
Conductivity (µs/cm)	65.00	55.30	48.00	24.80	41.60	125.00	67.20	138.00	42.00	34.00	32.50	34.80	500

(CODE: BOD= Biochemical Oxygen Demand, COD= Chemical Oxygen Demand, DO= Dissolved Oxygen, TOC= Total Oxygen Carbon, TDS= Total Dissolved Solid, TS= Total Solid, TSS= Total suspended Solid, IFRW= Ife Raw Water, EDRW= Ede Raw Water, ARW= Asejire Raw Water, ERW= Eleyele Raw Water, OWODRW= Owena-Ondo Raw Water, OWIRW= Owena-Idanre Raw Water)

Table 4.2: Physico-chemical properties of treated water of selected dams in southwestern Nigeria

Parameters	Dam 1 (IFFW)		Dam 2 (EDFW)		Dam 3 (AFW)		Dam 4 (EFW)		Dam 5 (OWODFW)		Dam 6 (OWIFW)		WHO Limit
	June/July	Dec/Jan	June/July	Dec/Jan	June/July	Dec/Jan	June/July	Dec/Jan	June/July	Dec/Jan	June/July	Dec/Jan	
pH	6.90	7.60	7.30	7.90	7.20	5.70	6.10	7.10	5.40	7.70	7.40	5.20	6.5-8.5
BOD (mg/l)	1.28	1.33	1.68	0.43	3.99	2.10	2.66	1.12	1.32	2.11	3.11	1.87	6-9
COD (mg/l)	43.10	34.10	41.30	21.70	28.70	23.00	74.10	19.80	47.40	81.30	34.80	78.00	-
DO (mg/l)	5.32	4.20	5.81	5.66	3.11	5.18	5.34	5.72	5.21	4.83	4.33	6.82	-
TOC (mg/l)	1.32	2.76	1.22	1.54	2.11	0.83	1.22	0.49	1.65	1.90	2.45	1.75	-
TDS (mg/l)	120.00	129.00	70.00	61.00	110.00	82.00	160.00	103.0	80.00	96.00	100.00	83.00	500
TS (mg/l)	182.00	138.00	110.00	83.00	150.00	110.00	384.00	140.0	220.00	130.00	320.00	93.00	-
TSS (mg/l)	61.00	6.29	39.00	19.20	37.00	27.00	220.00	30.00	130.00	32.80	210.00	9.20	500
Conductivity (µs/cm)	67.00	71.00	37.10	34.20	78.10	148.00	80.00	194.0	40.00	59.50	52.00	5.60	500
Residual Chlorine (mg/l)	0.00	1.71	0.00	1.00	0.00	0.61	0.05	2.32	0.09	0.03	0.04	0.05	0.5

(CODE: BOD= Biochemical Oxygen Demand, COD= Chemical Oxygen Demand, DO= Dissolved Oxygen, TOC= Total Oxygen Carbon, TDS= Total Dissolved Solid, TS= Total Solid, TSS= Total suspended Solid, IFFW= Ife Treated Water, EDFW= Ede Treated Water, AFW= Asejire Treated Water, EFW= Eleyele Treated Water, OWODFW= Owena-Ondo Treated Water, OWIFW= Owena-Idanre Treated water)

4.2. Coliform and Total bacteria count from water samples

4.2.1 Coliform plate count from selected water samples in southwestern Nigeria

In the December/January sampling, as shown in Figure 4.1 there was no coliform in the treated water and municipal water samples from Dam 3 (Asejire) and Dam 4 (Eleyele) while coliform count of 5.49 log cfu/ml and 2.81 log cfu/ml were found in the raw water of these dams respectively. Coliform count of above 2.0 log cfu/ml was obtained from the treated water of Dam 1 (Ife) and its municipal tap 1 which was lower than the 3.79 log cfu/ml that, was obtained in its raw water sample. Coliform count of 3.00 log cfu/ml was obtained in the treated water of Dam 2 (Ede) and its first municipal distribution tap. There was no coliform on the municipal taps 2 of Dam 1 (Ife) and Dam 2 (Ede). No coliform was also found from the municipal distribution point tap 1 of Dam 6 (Owena-Ijesha) while a count of 2.6 log cfu/ml was observed from the municipal tap 2 of the same dam.

In June/July samples, it was only in Dam 4 (Eleyele) that coliform was not observed from the treated water sampled. Other five dams showed the presence of coliform in all their treated water samples. Coliform counts of water samples from Dam 3 (Asejire) were the lowest (2.2 log cfu/ml) in the first municipal sample and highest (3.56 log cfu/ml) in the second municipal sample. At Dam 1 (Ife) the lowest count (2.2 log cfu/ml) was observed from the treated water while the highest count (3.53 log cfu/ml) was observed from the second municipal sample. No coliform was observed from the two municipal taps of Dam 5 (Owena-Ondo) as shown in Figure 4.3.

4.2.2. Total heterotrophic bacteria count of selected water samples in southwestern Nigeria

No bacteria was obtained from Dam 4 (Eleyele) treated water while bacterial count of 0.6 log cfu/ml was found in the two municipal taps samples of this dam respectively in the December/January samples. Higher bacteria counts were observed in all the raw water samples compared to the treated water samples. Bacterial count of almost 6 log cfu/ml was observed in the raw water sample of Dam 3 (Asejire) compared to a lower count of 1.17 log cfu/ml, 1.9 log cfu/ml and 0.48 log cfu/ml observed from its treated water and its municipal 1 and 2 respectively, as shown in Figure 4.2. Bacteria were not observed from the two municipal samples during the June/July sampling at Dam 4 (Eleyele) and Dam 5 (Owena-Ondo). The lowest bacteria count of 2.51 log cfu/ml in the raw water and highest of 4.43 log cfu/ml in the treated water was obtained from the water distribution shystem of Dam 3 (Asejire). Bacteria count above 1 cfu/ml was found at the treated water of Dam 6 (Owenna Ijesha) and it two municipal water sampling. The heterotrophic bacterial count obtained at the municipal and treated water

samples of dam 3 (Asejire) were above 3.0 log cfu/ml which was higher than 2.51 log cfu/ml observed at it raw water sample as shown on Figure 4.4.

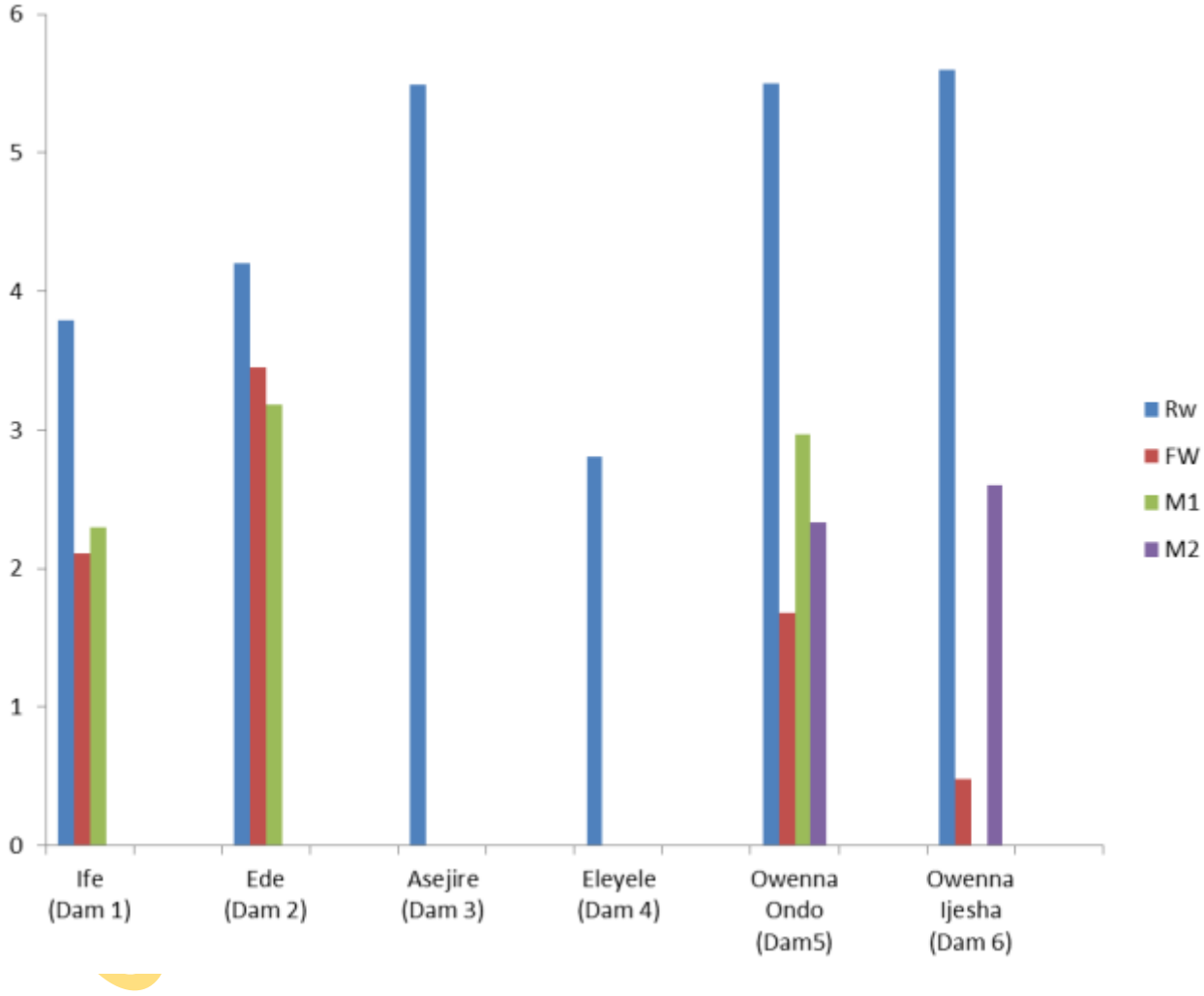


Fig 4.1: Coliform count (in Log cfu/ml) of selected water samples in southwestern Nigeria Rw= Raw Water, Fw= Treated Water, M1= Municipal 1, M2= Municipal 2

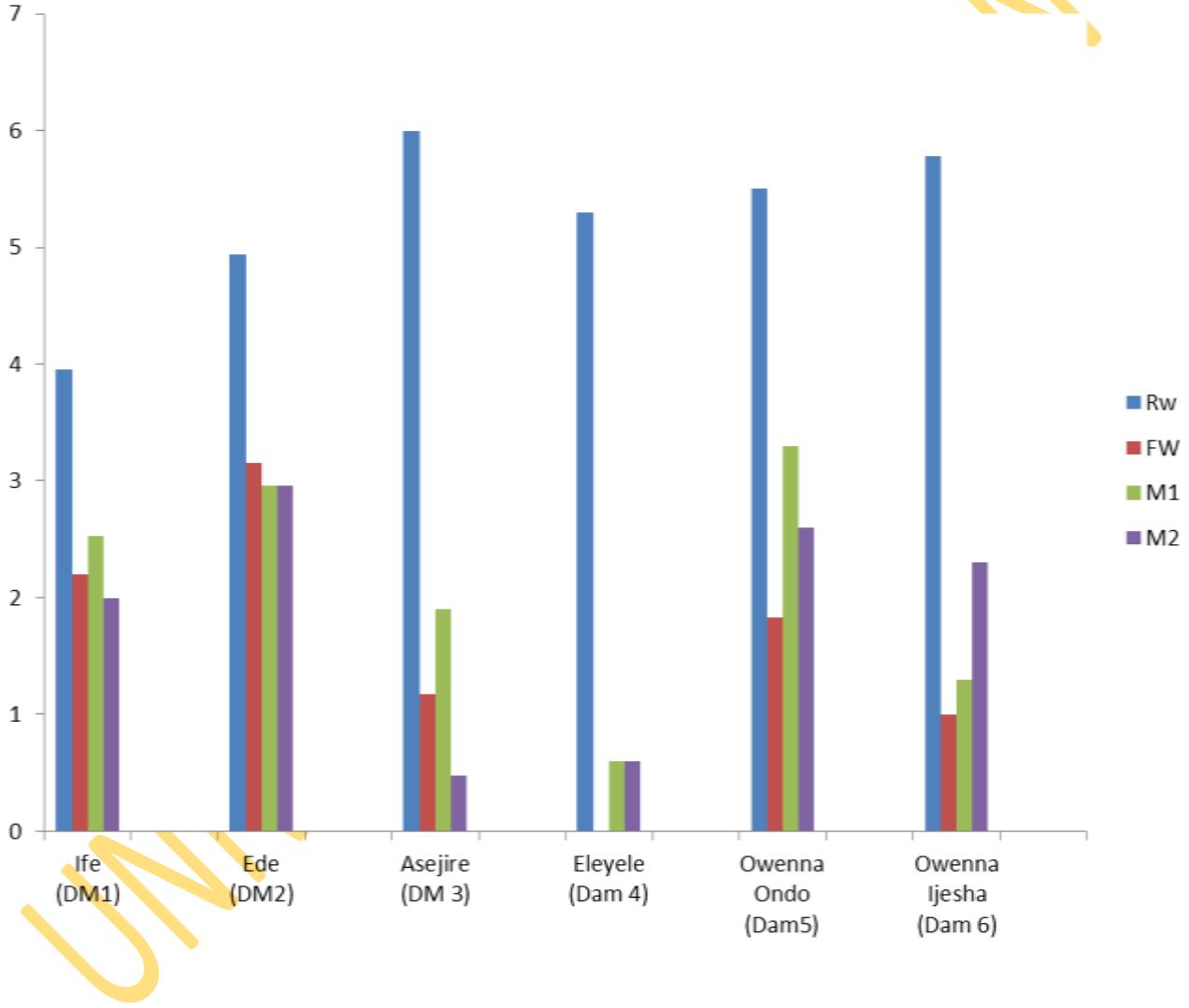


Fig 4.2: Total heterotrophic bacteria count (in Log cfu/ml) of selected water samples in southwestern Nigeria
Rw= Raw Water, Fw= Treated Water, M1= Municipal 1, M2= Municipal 2

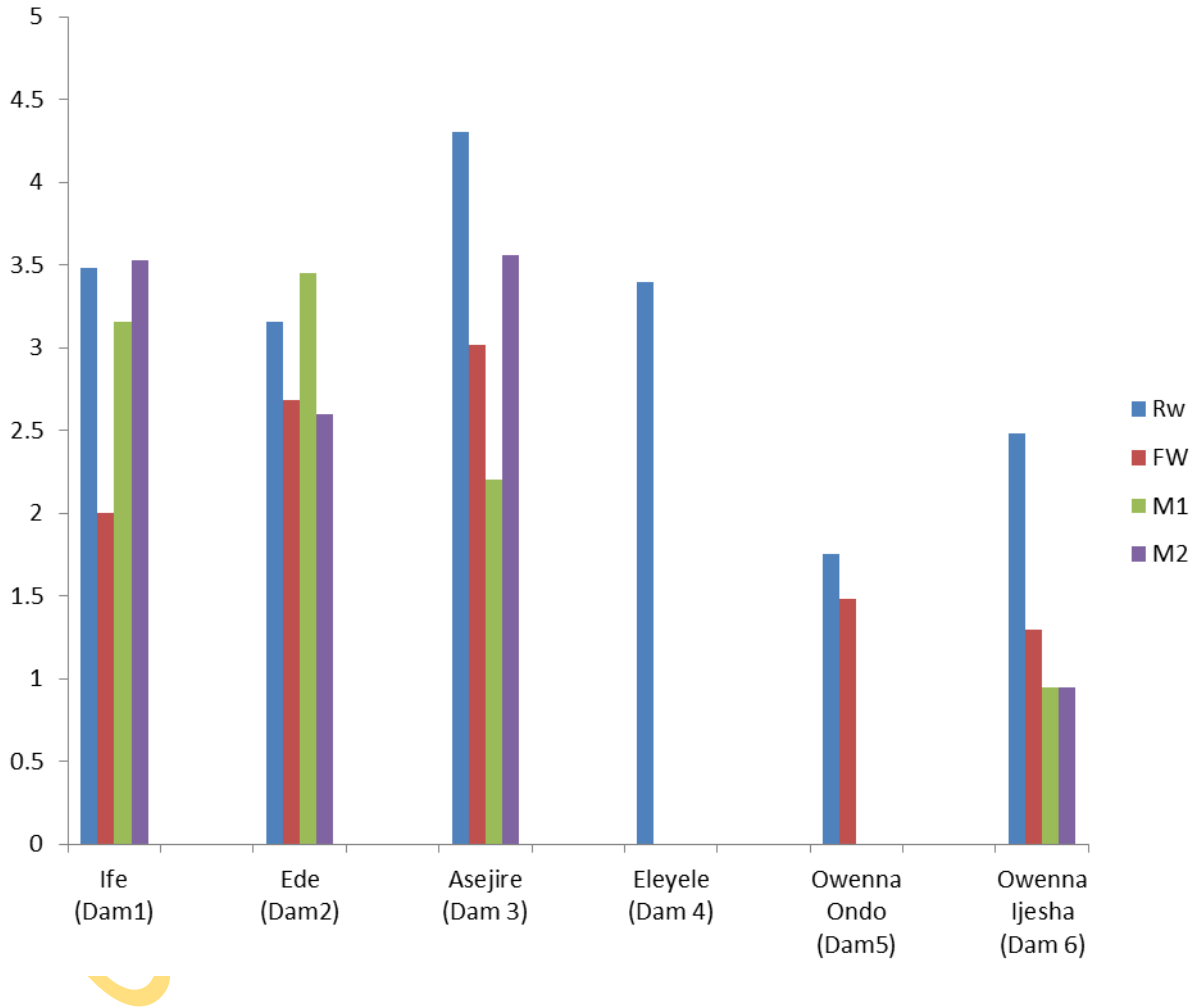


Fig 4.3: Coliform count (in Log cfu/ml) of selected water samples from southwestern Nigeria

Rw= Raw Water, Fw= Treated Water, M1= Municipal 1, M2= Municipal 2

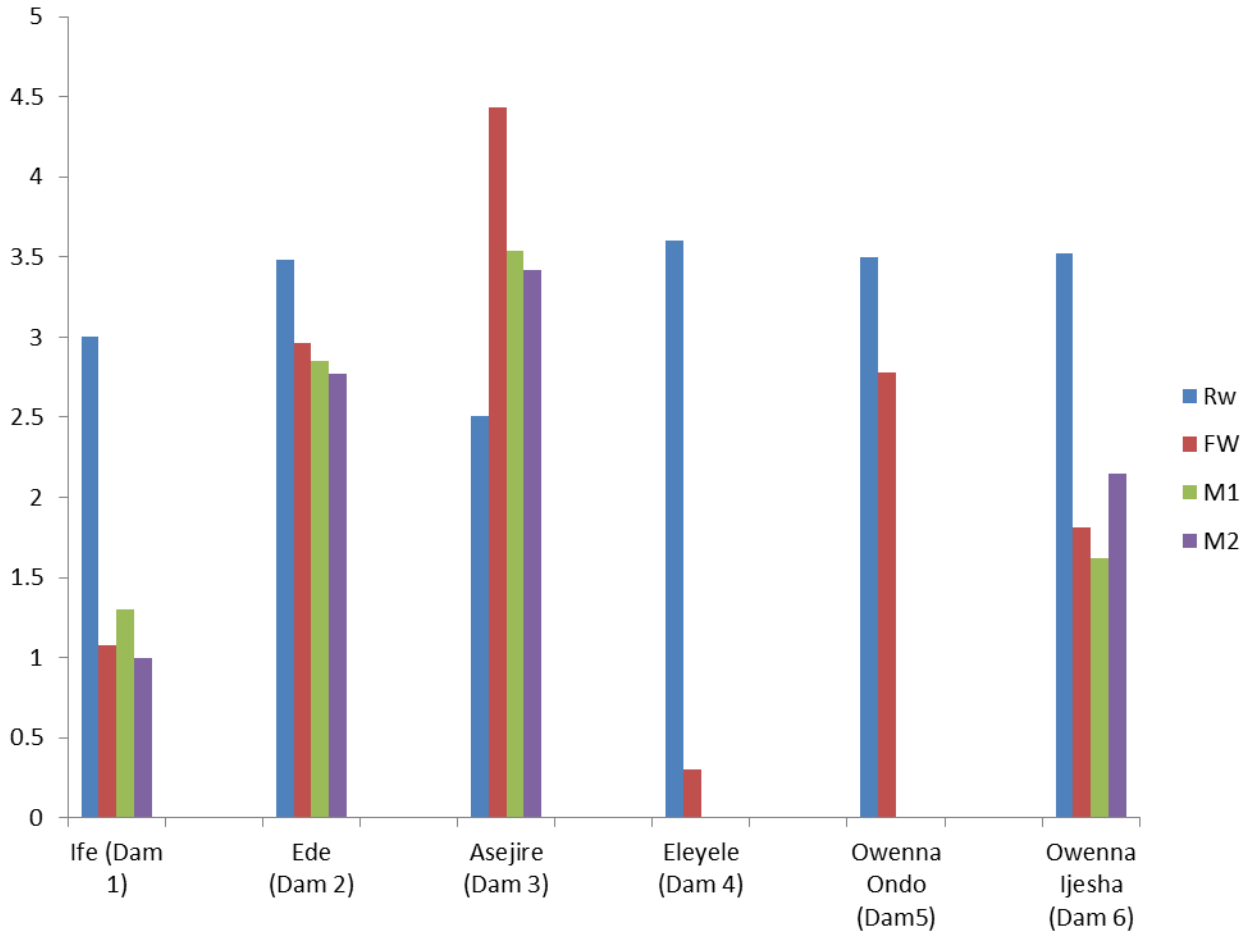


Fig 4.4: Total heterotrophic bacteria count (in Log cfu/ml) of selected water samples in southwestern Nigeria
Rw= Raw Water, Fw= Treated Water, M1= Municipal 1, M2= Municipal 2

4.3. Bacteria isolated from selected dams and their water distribution taps in southwestern Nigeria

The bacteria obtained from Ife (Dam 1) and Ede (Dam 2), raw and treated as well as each of their municipal water distribution points, are shown on Table 4.3. With respect to the total bacteria in each of the sample location, *Bacillus sp* had the highest frequencies in Dam 1 (Ife) raw water (31.25%), treated water (31.25%), municipal tap (50.00%), and Dam 2 (Ede) raw water (33.33%) but the treated water and municipal 1 tap of Ede (Dam 2), *Alcaligenes sp* (50%) and *Pseudomonas sp* (42.86%) had the highest frequencies. Bacteria observed in the raw water of Dam 1 (Ife) include *Aeromonas sp, Acinetobacter sp, Camamonas sp, Escherichia sp, Klebsiella sp, Providencia sp, Proteus sp, Pseudomonas sp* and *Stenotrophomonas sp* while bacteria observed from the treated water include *Alcaligenes sp, Brevundimonas sp, Chromobacterium sp, Pseudomonas sp, Proteus sp, Lysinibacillus sp* and Uncultured bacterium clone. *Alcaligenes sp, Bacillus sp* and *Morganella sp* were the only bacteria genera isolated from the treated water of Dam 2 (Ede).

Bacteria isolated from all sample points of Dam 3 (Asejire) and Dam 4 (Eleyele) are shown in Table 4.4. It was observed that in the raw water from Dam 3, *Alcaligenes sp* had the highest occurrence (26.67%) while *Pseudochrobactrum sp, Klebsiella sp, Leucobacter sp, Lysinibaccillus sp* and Uncultured bacterium strain each had the least occurrence with 6.67%. From the treated water of Dam 3 *Bacillus sp* had the highest frequency of occurrence (27.27%) out of 11 bacteria isolated from this point followed by *Proteus sp* and *Pseudomonas sp* having 18.18% each. From the first municipal tap, out of 11 bacteria isolated from this point 45.45% was *Bacillus sp* while 18.18% was *Chromobacterium sp.* From second water distribution tap, out of 7 bacteria isolates the highest was *Acinetobacter sp* (6.82%) while 2.27% each were *Klebsiella sp, Proteus sp, Pseudomonas sp* and *Staphylococcus sp*. From Eleyele (Dam 4), out of a total of 19 bacteria from the raw water, the highest occurrence was *Klebsiella sp* (26.32%) followed by *Alcaligenes sp* (21.05%). Two bacteria were isolated from the treated water from Dam 4 which included *Klebsiella sp* and *Proteus sp*. Two bacteria were obtained from the first

municipal water distribution tap of Eleyele dan and were identified as *Alcaligenes sp* and *Staphylococcus sp*.

Bacteria isolated from Owena-Ondo (Dam 5) and Owena-Idanre (Dam 6) are shown in Table 4.5. A total of 13 bacteria were obtained in the raw water from Owena-Ondo and these included, *Escherichia sp, Klebsiella sp, Leucobacter sp, Bacillus sp* and Uncultured bacteria strain while 11 bacteria were isolated from the treated water which were identified as *Alcaligenes sp, Aquitalea sp, Klebsiella sp, Pseudomonas sp, Proteus sp, Morganella sp, Bacillus sp* and *Staphylococcus sp*. From Owena-Idanre (Dam 6), 32 bacteria were got out of which *Proteus sp, Pseudomonas sp* and *klebsiella sp* had the highest occurrence with each having 15.63% occurrence followed by *Alcaligenes sp* with 12.50% occurrence. From the treated water 5 bacteria were isolated which included *Ralstonia sp* (1), *Proteus sp* (1) and *Bacillus sp* (3). Twelve bacteria were isolated from each of the two municipal water distribution channels. Bacteria observed from the first municipal tap were *Alcaligenes sp, Acinetobacteria sp, Citrobacter sp, Enterobacter sp, Klebsiella sp, Pseudomonas sp, Bacillus sp* and *Enterococcus sp*.

4.4. Classification of total bacteria from all water sampled points to various groups

In this study, a total of 292 bacteria were isolated from all sampled locations (Table 4.6). They were classified into uncultured bacteria and 6 bacteria groups made up of alpha proteobacteria (4), beta proteobacteria (39), gamma proteobacteria (131), bacteroidetes (4), Actinobacteria (2), firmicutes (105) and uncultured bacteria (7). Bacteria genera belonging to alpha proteobacteria included *Brevundimonas* and *Pseudochrobactrum*; beta proteobacteria were *Alcaligenes, Chromobacterium, Bordetella, Camamonas, Aquitalea* and *Rolstonia*; gamma proteobacteria were made up of *Aeromonas, Acinetobacter, Escherichia, Klebsiella, Pseudomonas, Providencia, Proteus, Stenotrophomonas, Morganella, Psychrobacter, Trabulsiella, Pantoea* etc. Two bacteria genera belonging to bacteroidetes were *Myroides* and *Sphingobacterium* and one belonged to Actinobacteria which was *Leucobacter* while those belonging to Firmicutes group were *Bacillus, Lysinibacillus, Staphylococcus* and *Enterococcus*.

Table 4.3: Bacteria isolated from Ife (Dam 1) and Ede (Dam 2) water samples identified through 16S rDNA sequencing

Sampled Dam	Location	Bacteria	No of Isolates	Percentage of bacteria isolated from location (%)
Ife (Dam 1)	**Raw water**	*Aeromonas sp*	2	12.5
		Acinetobacter sp	1	6.25
		Bacillus sp	5	31.25
		Camamonas sp	1	6.25
		Escherichia sp	2	12.5
		Klebsiella sp	1	6.25
		Providencia sp	1	6.25
		Proteus sp	1	6.25
		Pseudomonas sp	1	6.25
		Stenotrophomonas sp	1	6.25
		Total bacteria (Raw water)	**16**	
	Treated water	*Alcaligenes sp*	2	11.76
		Bacillus sp	5	31.25
		Brevundimonas sp	1	5.88
		Chromobacterium sp	1	5.88
		Pseudomonas sp	2	11.76
		Proteus sp	4	23.53
		Uncultured bacteria clone	1	5.88
		Lysinibacillus sp	1	5.88
		Total (Treated water)	**17**	
	Municipal Tap 1	*Klebsiella sp*	1	16.67
		Bacillus sp	3	50.00
		Chromobacterium sp	1	16.67
		Uncultured bacterium clone	1	16.67
		Total (Municipal Tap 1)	**6**	
Total bacteria from Ife (39)				
Ede	**Raw water**	*Acinetobacter sp*	2	9.52
		Aeromonas sp	1	4.76
		Bordetella sp	1	4.76
		Bacillus sp	7	33.33
		Camamonas sp	1	4.76
		Chromobacterium sp	1	4.76
		Proteus sp	2	9.52
		Pseudomonas sp	3	14.29
		Stenotrophomonas sp	1	4.76
		Sphingobacterium sp	1	4.76
		Staphylococcus sp	1	4.76
		Total (Raw water)	**21**	
	Treated water	*Alcaligenes sp*	2	50.00
		Bacillus sp	1	25.00
		Morganella sp	1	25.00
		Total (Treated water)	**4**	

Table 4.3 (cont'd): Bacteria isolated from Ife (Dam 1) and Ede (Dam 2) water samples identified through 16S rDNA sequencing

Sampled Dam	Location	Bacteria	No of Isolates	Percentage of bacteria isolated from location (%)
Ede (Cont)	**Municipal Tap 1**	*Acinetobacter sp*	3	21.43
		Bacillus sp	5	35.71
		Pseudomonas sp	6	42.86
		Total (Municipal Tap 1)	**14**	
Ede (Cont'd)	**Municipal Tap 2**	*Alcaligenes sp*	2	14.29
		Bacillus sp	5	35.71
		Chromobacterium sp	2	14.29
		Escherichia sp	2	14.29
		Lysinibacillus sp	1	7.14
		Psychrobacter sp	1	7.14
		Providencia sp	1	7.14
		Total (Municipal Tap 2)	**14**	
Total bacteria from Ede (53)				

Table 4.4: Bacteria isolated from Asejire (Dam 3) and Eleyele (Dam 4) water samples identified through 16S rDNA sequencing

Sampling Dam	Location	Bacteria	No of Isolates	Percentage of bacteria isolated from location (%)
Asejire	**Raw water**	*Alcaligenes sp*	4	26.67
(Dam 3)		*Bacillus sp.*	3	20.00
		Klebsiella sp	1	6.67
		Leucobacter sp	1	6.67
		Lysinibacillus sp	1	6.67
		Proteus spp	3	20.00
		Pseudochrobactrum sp	1	6.67
		Uncultured bacterium clone	1	6.67
		Total (Raw water)	**15**	
	Treated water	*Alcaligenes sp*	1	9.09
		Bacillus sp	3	27.27
		Klebsiella sp	1	9.09
		Myroides sp	1	9.09
		Proteus spp	2	18.18
		Pseudomonas sp	2	18.18
		Uncultured bacterium clone	1	9.09
		Total (Treated water)	**11**	
	Municipal Tap 1	*Bacillus sp*	5	45.45
		Chromobacterium sp	2	18.18
		Pseudochrobactrum sp	1	9.09
		Pseudomonas sp	1	9.09
		Staphylococcus sp	1	9.09
		Uncultured bacterium clone	1	9.09
		Total (Municipal Tap 1)	**11**	
	Municipal Tap 2	*Acinetobacter sp*	3	6.82
		Klebsiella sp	1	2.27
		Proteus sp	1	2.27
		Pseudomonas sp	1	2.27
		Staphylococcus sp	1	2.27
		Total (Municipal Tap 2)	**7**	
Total bacteria from Asejire 44				

Table 4.4 (Cont'd): Bacteria isolated from Asejire (Dam 3) and Eleyele (Dam 4) water samples identified through 16S rDNA sequencing

Sampling Dam	Location	Bacteria	No of Isolates	Percentage of bacteria isolated from location (%)
Eleyele	**Raw water**	*Alcaligenes sp*	4	21.05
		Aeromonas sp	3	15.79
		Bacillus sp	1	5.26
		Klebsiella sp	5	26.32
		Morganella sp	1	5.26
		Proteus sp	2	10.53
		Providencia sp	1	5.26
		Trabulsiella sp	1	5.26
		Staphylococcus sp	1	5.26
		Total (Raw water)	**19**	
	Treated water	*Klebsiella sp*	1	50.00
		Proteus sp	1	50.00
		Total (Treated water)	**2**	
	Municipal Tap 1	*Alcaligenes sp*	1	50.00
		Staphylococcus sp	1	50.00
		Total (Municipal Tap 1)	**2**	
Total bacteria from Eleyele 23				

Table 4.5: Bacteria isolated from Owena-Ondo (Dam 5) and Owena-Idanre (Dam 6) water samples identified through 16S rDNA sequencing

Sampled Dam	Location	Bacteria	No of Isolates	Percentage bacteria isolated from location (%)
		Bacillus sp	7	53.85
Owena-Ondo	**Raw water**	*Escherichia sp*	1	7.69
		Klebsiella sp	3	23.08
		Leucobacter sp	1	7.69
		Uncultured bacteria clone	1	7.69
		Total bacteria (Raw water)	**13**	
	Treated water	*Alcaligenes sp*	2	18.18
		Aquitalea sp	1	9.09
		Bacillus sp	3	27.27
		Klebsiella sp	1	9.09
		Morganella sp	1	9.09
		Pseudomonas sp	1	9.09
		Proteus sp	1	9.09
		Staphylococcus sp	1	9.09
		Total (Treated water)	**11**	
	Municipal Tap 1	*Alcaligenes sp*	2	9.09
		Bacillus sp	10	45.45
		Escherichia sp	2	9.09
		Lysinibacillus sp	2	9.09
		Proteus sp	2	9.09
		Pseudomonas sp	2	9.09
		Providencia sp	2	9.09
		Total (Municipal Tap 2)	**22**	
	Municipal Tap 2	*Acinetobacter sp*	1	3.85
		Bacillus sp	13	50
		Lysinibacillus sp	1	3.85
		Morganella sp	4	15.38
		Myroides sp	1	3.85
		Proteus sp	5	19.23
		Serratia sp	1	3.85
		Total (Municipal Tap 3)	**26**	
Total bacteria from Owena-Ondo (72)				

Table 4.5 (Cont'd): Bacteria isolated from Owena-Ondo (Dam 5) and Owena-Idanre (Dam 6) water samples identified through 16S rDNA sequencing

Sampling Dam	Location	Bacteria	No of Isolates	Percentage of bacteria isolated from location (%)
Owena-Idanre	**Raw water**	*Alcaligenes sp*	4	12.50
		Acinetobacter sp	2	6.25
		Aeromonas sp	1	3.12
		Morganella sp	1	3.12
		Proteus sp	5	15.63
		Providencia sp	2	6.25
		Pseudomonas sp	5	15.63
		Klebsiella sp	5	15.63
		Serratia sp	1	3.12
		Myroides sp	1	3.12
		Bacillus sp	3	9.38
		Lysinibacillus sp	1	3.12
		Uncultured bacterium clone	1	3.12
		Total (Raw water)	**32**	
	Treated water	*Ralstonia sp*	1	20.00
		Proteus sp	1	20.00
		Bacillus sp	3	60.00
		Total (Treated water)	**5**	
	Municipal Tap 1	*Alcaligenes sp*	2	16.67
		Acinetobacter sp	1	8.33
		Bacillus sp	2	16.67
		Citrobacter sp	2	16.67
		Enterobacter sp	1	8.33
		Klebsiella sp	1	8.33
		Pseudomonas sp	1	8.33
		Enterococcus sp	2	16.67
		Total	**12**	
	Municipal Tap 2	*Alcaligenes sp*	2	16.67
		Bacillus sp	5	41.67
		Brevundimonas sp	1	8.33
		Chromobacterium sp	1	8.33
		Proteus sp	1	8.33
		Pseudomonas sp	1	8.33
		Lysinibacillus sp	1	8.33
		Total	**12**	

Total bacteria from Owena-Idanre (61)

Table 4.6: Classification of total bacteria from all sample points` to various groups

Bacteria Group	Bacteria genera	Occurrence	Percentage of Total bacteria
Alpha Proteobacteria	*Brevundimonas*	2	0.68
	Pseudochrobactrum	2	0.68
	Total	**4**	1.37
Beta Proteobacteria	*Alcaligenes*	26	8.90
	Chromobacterium	8	2.74
	Bordetella	1	0.34
	Camamonas	2	0.68
	Aquitalea	1	0.34
	Rolstonia	1	0.34
	Total	**39**	13.36
Gamma Proteobacteria	*Aeromonas*	7	2.40
	Acinetobacter	15	5.14
	Escherichia	7	2.40
	Klebsiella	21	7.19
	Pseudomonas	26	8.90
	Providencia	7	2.40
	Proteus	29	9.93
	Stenotrophomonas	2	0.68
	Morganella	8	2.74
	Psychrobacter	2	0.68
	Trabulsiella	1	0.34
	Pantoea	1	0.34

Table 4.6 (Cont'd): Classification of total bacteria from all sample point to various groups

Bacteria Group	Bacteria genera	Occurrence	Percentage of Total bacteria
Gamma Proteobacteria (Cont'd)	*Serratia*	2	0.68
	Citrobacter	2	0.68
	Enterobacter	1	0.34
	Total	**131**	44.86
Bacteroidetes	*Myroides*	3	1.03
	Sphingobacterium	1	0.34
	Total	**4**	1.37
Actinobacteria	*Leucobacter*	2	0.68
	Total	**2**	0.68
Firmicutes	*Bacillus*	90	30.82
	Lysinibacillus	7	2.40
	Staphylococcus	6	2.05
	Enterococcus	2	0.68
	Total	**105**	35.96
	Uncultured bacteria clone	**7**	2.40
	Grand Total bacteria	**292**	

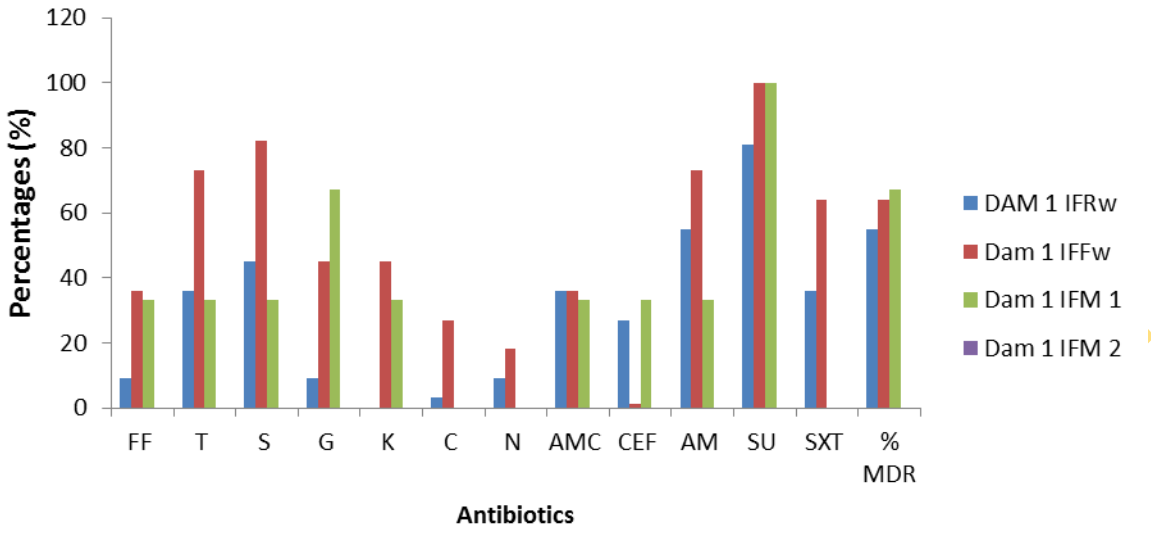
4.5. Drug resistant pattern of bacteria isolated from dam water samples and their distribution systems

There were high levels of resistance in the bacteria isolated from the selected water samples from Ife (Dam 1). Figure 4.5a shows the percentage of Gram-negative bacteria from water distribution systems of Ife (Dam 1) that were resistant to antibiotics. There were between 0-18% resistances to nalidixic acid among bacteria isolated from this location. It was observed that between 81-100% of bacteria isolated from this location were resistant to sulfamethoxazole as shown in Fig 4.5a. It was also observed that 73% of the total bacteria from treated water were resistant to ampicillin and tetracycline while 82% were resistant to streptomycin. The range of multi-drug resistant bacteria (MDR) at all the sampling points within this location was 55% from the raw water, to 67% at the first water distribution tap. Fig. 4.5b shows the percentage resistance of the Gram positive bacteria from Ife Dam 1 water distribution system to various antibiotics. The results showed that there were no bacteria resistant to ciprofloxacin while 100% resistance to ampicillin was found among bacteria isolates from the second water distribution tap system samples. Among the bacteria isolated from the raw water and first distribution tap 100% resistance to lincomycin was observed. In addition, 20% of the bacteria from the raw water were resistant to erythromycin, the combination of sulfamethoxazole and trimethoprim as well as riframprim. The range of MDR bacteria was from 20% from the raw water to100% of bacteria isolated from the second water distribution tap.

Results of antibiotic-resistant profiles of gram negative bacteria from Ede dam and its water distribution taps are shown in Fig 4.6a. It was observed that bacteria from treated water showed 100% resistance to tetracycline, streptomycin and sulfamethoxazole. On the contrary, the bacteria from this same sampling point showed no resistance to florfenicol, chloramphenicol or the combination of amoxicillin and clavulanic acid. While resistance to nalidixic acid ranged from 8% of bacteria from the raw water sampling to 33% of bacteria from the treated water. The percentages of antibiotic-resistant gram positive bacteria isolates from this dam and its water distribution systems are shown in Fig. 4.6b. None of the total bacteria from all the sample location showed resistance to ciprofloxacin. Similar results were also observed in the resistance of the bacteria from treated water and the two municipal taps to gentamicin. MDR bacteria were found to be between 20% from Municipal 2 to 44% in raw water sample

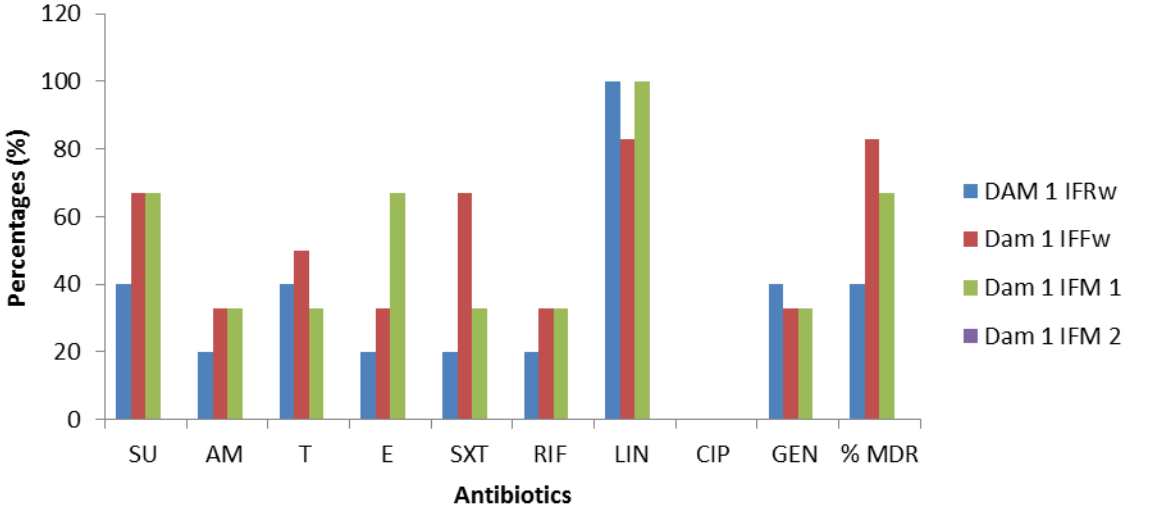
The results of percentage resistance to various antibiotics by gram negative bacteria from Asejire (Dam 3) is shown in Fig 4.7a. It was observed that 100% of the bacteria isolated from the treated water were resistant to streptomycin and ampicillin. No bacteria from the first distribution tap

A



(IFM1= Awo Hall, IFM2= Fajuyi Hall)
(Rw= 11, Fw= 11, M1= 3, M2= 0, Total no of Bacteria= 25)

B

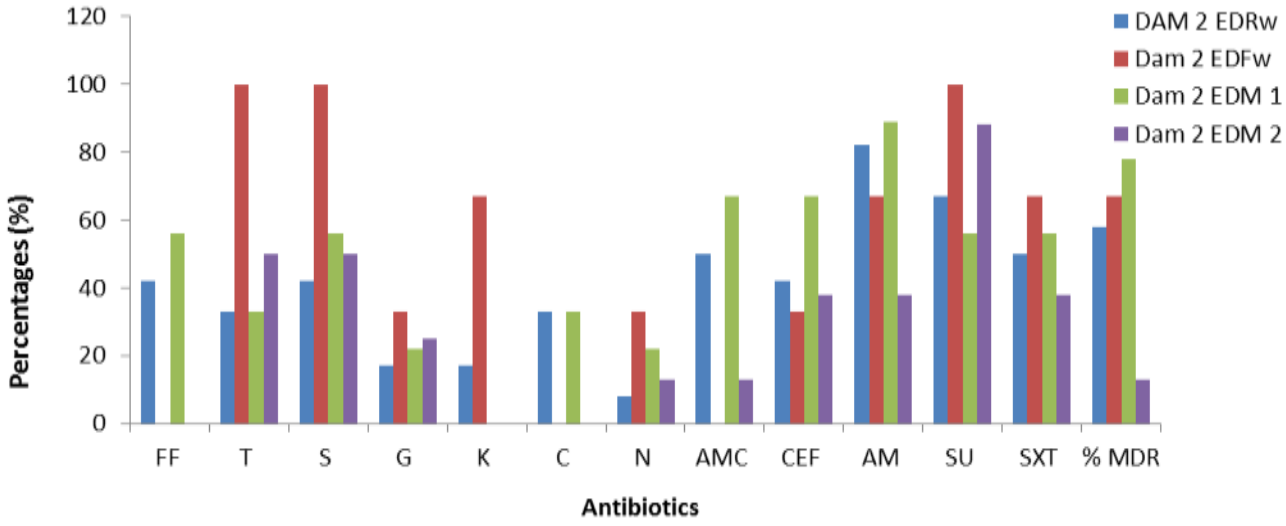


(IFM1= Awo Hall, IFM2= Fajuyi Hall)
Rw= 5, Fw= 6, M1= 3, M2= 0, Total no of Bacteria= 14)

Fig 4.5: Bacteria that were resistant to antibiotics in Ife water samples A= Gram negative and B= Gram positive

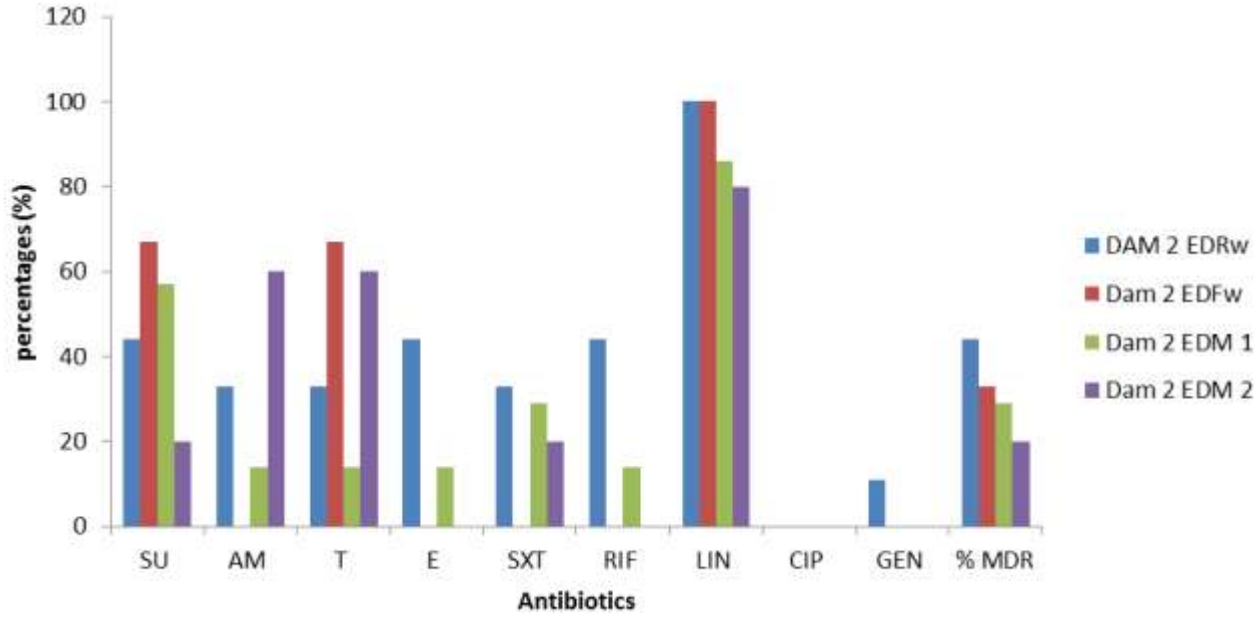
Class and codes of Antibiotics: Beta-lactam, Ampicillin (AM); **Cephalosporin**, Ceftiofur (CEF); **Phenicol**, Chloramphenicol (C) and Florfenicol (FF); **Aminoglycocide;** Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); **Tetracycline,** Tetracycline (T); **Quinolone,** Nalidixic Acid (N); **Sulpha Drug,** Sulfamethoxazole (SU); **Sulpha drug Combo,** Sulfamethoxazole/ Trimethoprim (SXT); **Beta lactam + Inhibitor Combo,** Amoxicillin/Clavulanic Acid (AMC); **Macrolide,** Erythromycin (E); **Rifamycin,** Riframprim (RIF); **Lincosamides,** Lincomycin (LIN); **Fluroquinolone,** Ciprofloxacin (CIP)

A



EDM1= Muslim Grammar School, EDM2= Obada)
(Rw= 13, Fw= 3, M1= 9, M2= 8, Total no of Bacteria= 33)

B



(EDM1= Muslim grammar School, EDM2= Obada)
Rw= 8, Fw= 1, M1= 5, M2= 6, Total no of Bacteria= 20)

Fig 4.6: Bacteria that were resistant to antibiotics in Ede water samples A= Gram negative and B= Gram positive

Class and codes of Antibiotics: Beta-lactam, Ampicillin (AM); **Cephalosporin**, Ceftiofur (CEF); **Phenicol**, Chloramphenicol (C) and Florfenicol (FF); **Aminoglycocide;** Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); **Tetracycline,** Tetracycline (T); **Quinolone,** Nalidixic Acid (N); **Sulpha Drug,** Sulfamethoxazole (SU); **Sulpha drug Combo,**

Sulfamethoxazole/ Trimethoprim (SXT); **Beta lactam + Inhibitor Combo,** Amoxicillin/Clavulanic Acid (AMC); **Macrolide,** Erythromycin (E); **Rifamycin,** Riframprim (RIF); **Lincosamides,** Lincomycin (LIN); **Fluroquinolone,** Ciprofloxacin (CIP)

sampled were resistant to florfenicol, tetracycline and chloramphenicol. It was also observed that 50% of the bacteria from each of the two municipal distribution taps were MDR while 100% of bacteria isolated from the treated water were MDR. Percentage resistances to antibiotics among the gram positive bacteria from Asejire (Dam 3) is shown in Fig. 4.7b. No bacteria from the two water distribution taps sampled showed resistance to erythromycin while at all sampled points, no bacteria was observed to be resistant to ciprofloxacin. However, 100% of bacteria from the treated water were resistant to sulfamethoxazole and lincomycin. At the second municipal distribution tap, it was also observed that all bacteria isolates were also resistant to ampicillin and lincomycin. MDR bacteria from these sites ranged 25% in the raw water to 67% in the treated water.

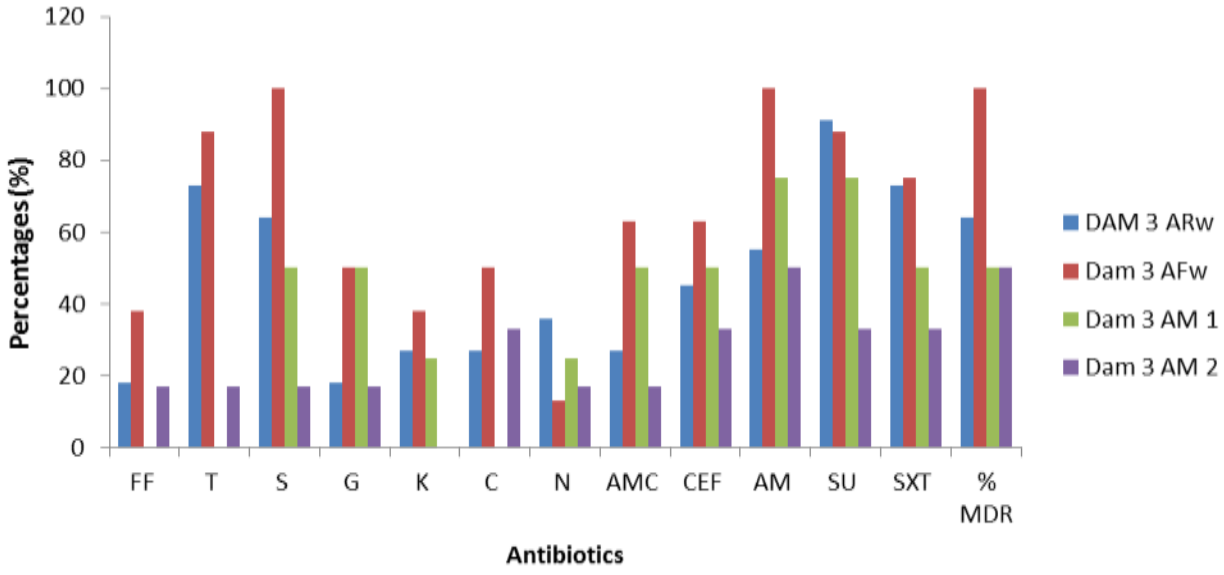
Results of the percentages of antibiotic-resistance among gram negative bacteria from each of the sample points of Eleyeye water (Dam 4) is shown in Fig. 4.8a. All isolated gram negative bacteria (100%) in the first water distribution tap were resistance to tetracycline, streptomycin, kanamycin, combination of amoxicillin and clavulanic acid, ampicillin, sulfamethoxazole and combination of sulfamethoxazole and trimethoprim. Bacteria from the treated water also showed 100% resistance to ampicillin and sulfamethoxazole. No bacteria from the two municipal distribution taps showed resistance to nalidixic acid. It was observed that 50% of the total bacteria from the treated water were MDR while all bacteria from the first municipal distribution tap were MDR. The results of the percentage resistant to antibiotics among gram positive bacteria from Eleyele (Dam 4) as shown in Figure 4.8b revealed that all bacteria from the raw water showed resistance to lincomycin while 50% of the bacteria from the raw water showed resistance to sulfamethoxazole, ampicillin and erythromycin. The result showed that 50% bacteria from the raw water were MDR bacteria.

The results of the susceptibility tests of gram negative bacteria from Owena-Ondo water samples shown in Figure 4.9a revealed that there were high antibiotic-resistant bacteria from each sampling point. All the bacteria in the raw and treated water were susceptible to nalidixic acid and gentamicin while 100% of the bacteria from Municipal tap 1 were resistant to sulfamethoxazole. From first Municipal distribution tap, it was observed that 86% of bacteria were resistant to tetracycline. However, 20% of the bacteria from the raw water were observed to be MDR. Figure 4.9b shows the percentage resistances of gram positive bacteria. It was observed

that 100% of the bacteria from each of all the municipal sampling points were resistant to lincomycin. No bacteria from all the sampling points were resistant to ciprofloxacin.

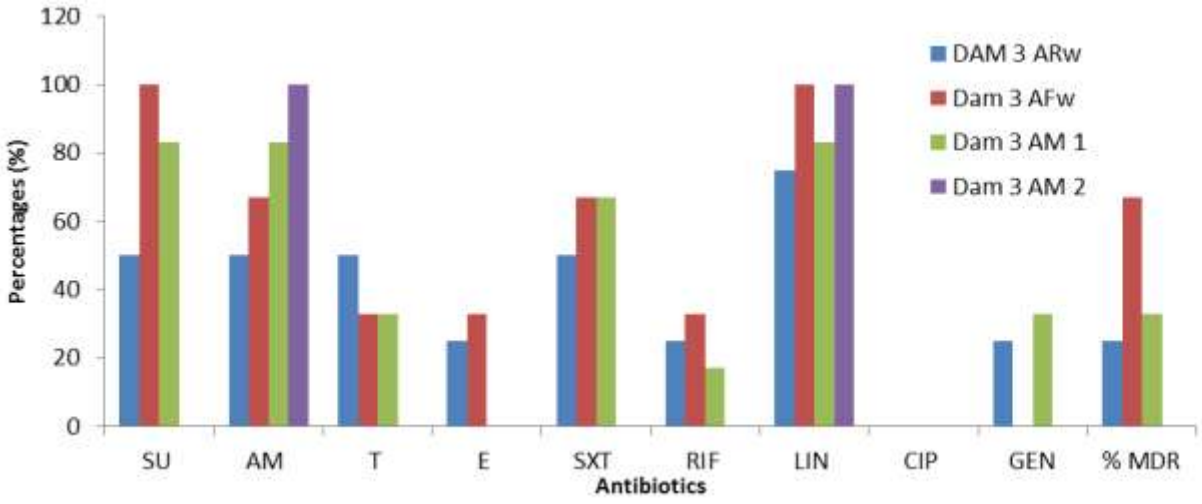
High resistance to antibiotics among the gram negative bacteria isolates from each of the selected sampling points of Owena-Idanre were observed and shown in Fig. 4.10a. All the bacteria were (100%) resistant to sulfamethoxazole. Similar results were also observed among the bacteria from the second Municipal tap in resistance to streptomycin. All bacteria from the treated water were susceptible to tetracyclin, gentamicin, kanamycin, nalidixic acid and combination of amoxicillin and clavulanic acid. The results of susceptibility of gram positive bacteria from Owena-Idanre (Dam 6) are shown in Fig 4.10b. None of the bacteria isolates from all the sampling locations showed resistance to ciprofloxacin. Similar results were also observed in the bacteria from each of the treated samples and the two municipal water samplings in their resistance to gentamycin. It was also observed that all bacteria from all the sampling points were resistant to lincomycin. In addition, 83% of the total bacteria from the second distribution tap were resistant to sulfamethoxazole, ampicillin and the combination of sulfamethoxazole and trimethoprim.

A



(AM1= Asejire, AM2= Agodi gate boaster station)
(Rw= 11, Fw= 8, M1= 5, M2= 6, Total no of Bacteria= 30)

B



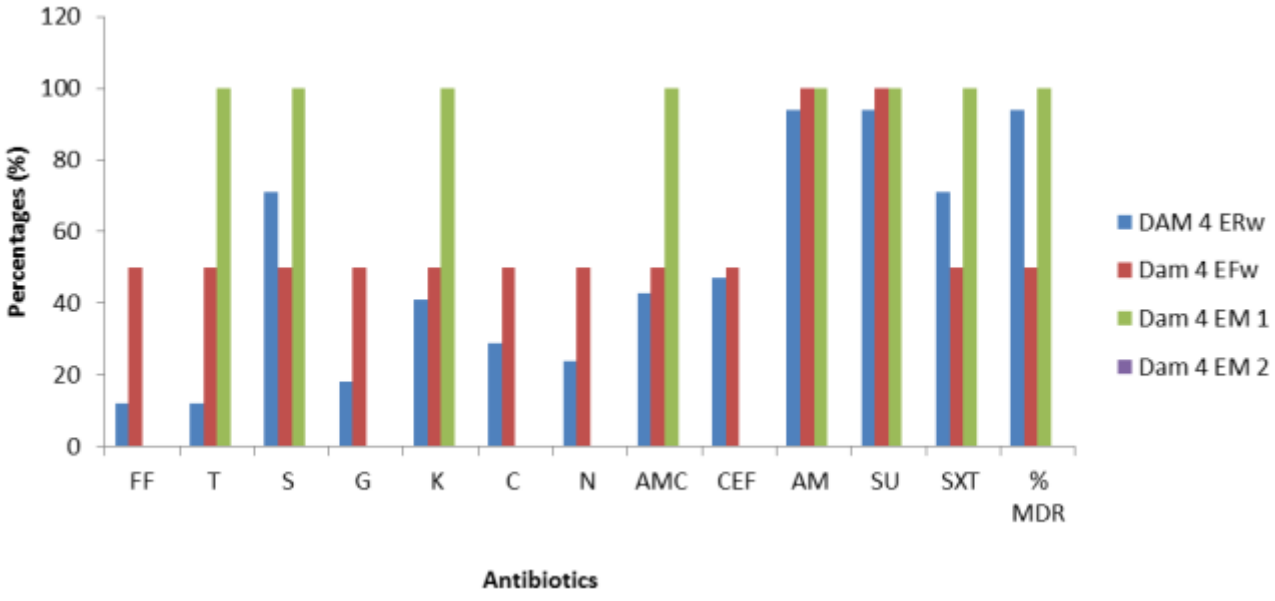
(AM1= Asejire, AM2= Agodi boaster station)
(Rw= 4, Fw= 3, M1= 6, M2= 1, Total no of Bacteria= 14)

Fig 4.7: Bacteria that were resistant to antibiotics in Asejire water samples A= Gram negative and B= Gram positive

Class and codes of Antibiotics: Beta-lactam, Ampicillin (AM); **Cephalosporin**, Ceftiofur (CEF); **Phenicol**, Chloramphenicol (C) and Florfenicol (FF); **Aminoglycocide;** Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); **Tetracycline,** Tetracycline (T); **Quinolone,** Nalidixic Acid (N); **Sulpha Drug,** Sulfamethoxazole (SU); **Sulpha drug Combo,**

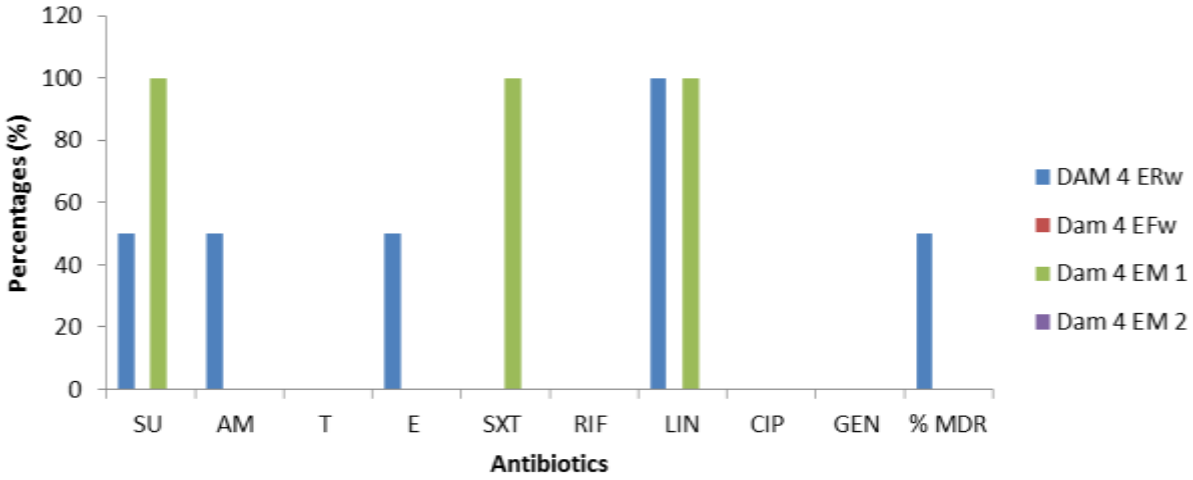
Sulfamethoxazole/ Trimethoprim (SXT); **Beta lactam + Inhibitor Combo,** Amoxicillin/Clavulanic Acid (AMC); **Macrolide,** Erythromycin (E); **Rifamycin,** Riframprim (RIF); **Lincosamides,** Lincomycin (LIN); **Fluroquinolone,** Ciprofloxacin (CIP)

A



(EM1= Eleyele Tap 1, EM2= Eleyele Tap 2)
(Rw= 17, Fw= 2, M1= 1, M2= 0, Total no of Bacteria= 20)

B

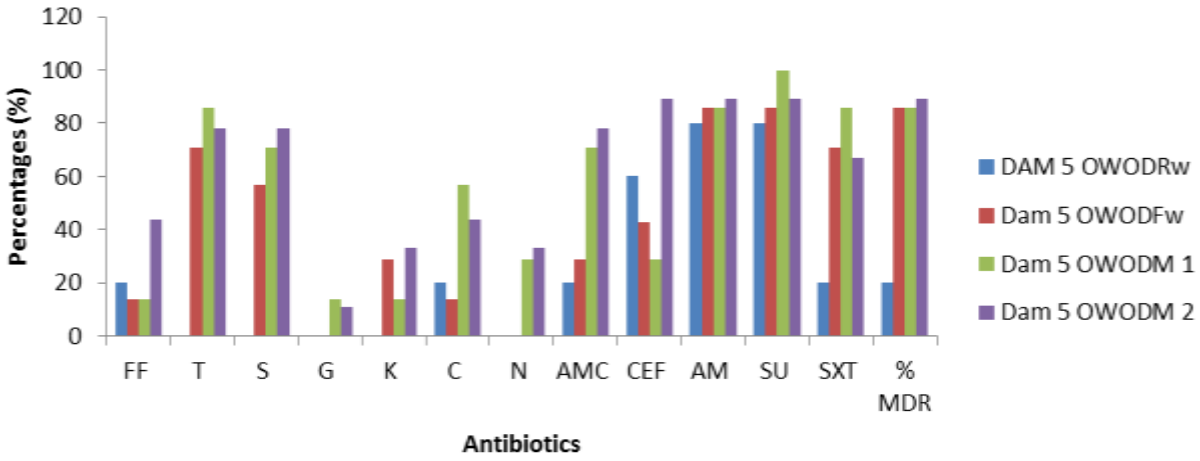


(EM1= Eleyele Tap 1, EM2= Eleyele Tap 2)
(Rw= 2, Fw= 0, M1= 1, M2= 0, Total no of Bacteria= 3)

Fig 4.8: Bacteria that were resistant to antibiotics in Eleyele water samples
A= Gram negative and B= Gram positive

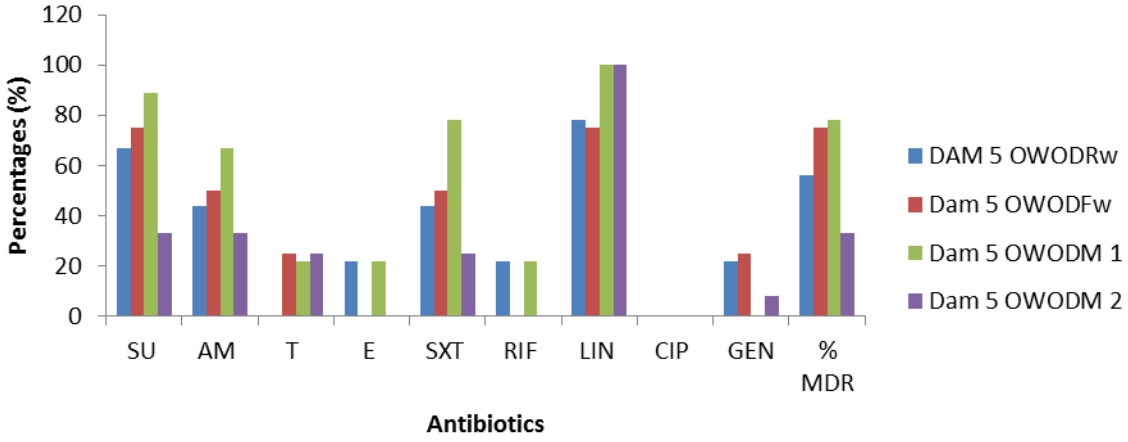
Class and codes of Antibiotics: Beta-lactam, Ampicillin (AM); **Cephalosporin**, Ceftiofur (CEF); **Phenicol**, Chloramphenicol (C) and Florfenicol (FF); **Aminoglycocide;** Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); **Tetracycline,** Tetracycline (T); **Quinolone,** Nalidixic Acid (N); **Sulpha Drug,** Sulfamethoxazole (SU); **Sulpha drug Combo,** Sulfamethoxazole/ Trimethoprim (SXT); **Beta lactam + Inhibitor Combo,** Amoxicillin/Clavulanic Acid (AMC); **Macrolide,** Erythromycin (E); **Rifamycin,** Riframprim (RIF); **Lincosamides,** Lincomycin (LIN); **Fluroquinolone,** Ciprofloxacin (CIP)

A



(OWODM1= NEPA, Akure OWODM2= Arakale, Akure)
(Rw= 4, Fw= 7, M1= 11, M2=12, Total no of Bacteria= 33)

B

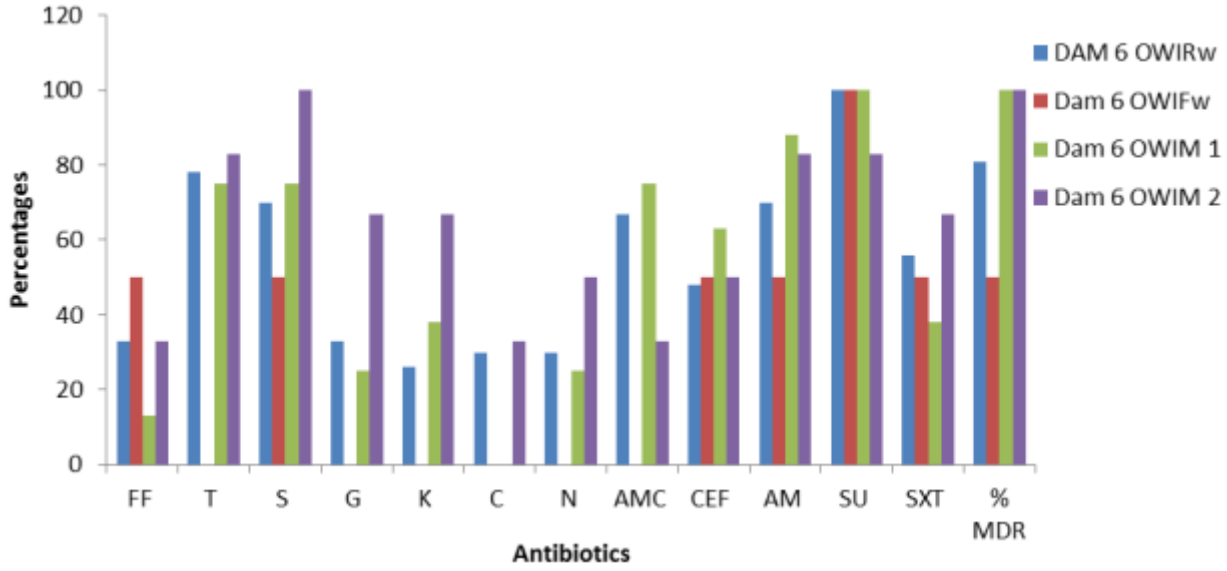


(OWODM1= NEPA, Akure, OWODM2= Arakale, Akure)
(Rw= 9, Fw= 4, M1= 11, M2= 15 Total no of Bacteria= 39)

Fig 4.9: Bacteria that were resistant to antibiotics in Owena-ondo water samples A= Gram negative and B= Gram positive

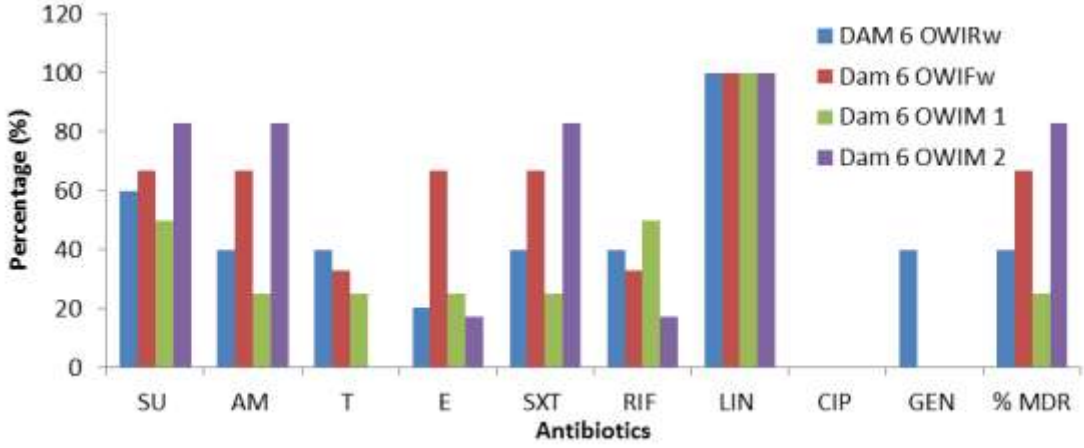
Class and codes of Antibiotics: Beta-lactam, Ampicillin (AM); **Cephalosporin**, Ceftiofur (CEF); **Phenicol**, Chloramphenicol (C) and Florfenicol (FF); **Aminoglycocide;** Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); **Tetracycline,** Tetracycline (T); **Quinolone,** Nalidixic Acid (N); **Sulpha Drug,** Sulfamethoxazole (SU); **Sulpha drug Combo,** Sulfamethoxazole/ Trimethoprim (SXT); **Beta lactam + Inhibitor Combo,** Amoxicillin/Clavulanic Acid (AMC); **Macrolide,** Erythromycin (E); **Rifamycin,** Riframprim (RIF); **Lincosamides,** Lincomycin (LIN); **Fluroquinolone,** Ciprofloxacin (CIP)

A



(OWIM1= Owena-Igbara oke Tap, OWIM2= Owena-Ijesha Tap).
(Rw= 28, Fw= 2, M1= 11, M2= 3, Total no of Bacteria= 44)

B



(OWIM1= Owena-Igbara Oke Tap, OWODM2= Owena-Ijesha Tap)
(Rw= 4, Fw= 3, M1= 4, M2= 6, Total no of Bacteria= 17)

Fig 4.10: Bacteria that were resistant to antibiotics in Owena-Idanre water samples A= Gram negative and B= Gram positive

Class and codes of Antibiotics: Beta-lactam, Ampicillin (AM); **Cephalosporin**, Ceftiofur (CEF); **Phenicol**, Chloramphenicol (C) and Florfenicol (FF); **Aminoglycocide;** Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); **Tetracycline,** Tetracycline (T); **Quinolone,** Nalidixic Acid (N); **Sulpha Drug,** Sulfamethoxazole (SU); **Sulpha drug Combo,** Sulfamethoxazole/ Trimethoprim (SXT); **Beta lactam + Inhibitor Combo,** Amoxicillin/Clavulanic Acid (AMC); **Macrolide,** Erythromycin (E); **Rifamycin,** Riframprim (RIF); **Lincosamides,** Lincomycin (LIN); **Fluroquinolone,** Ciprofloxacin (CIP)

4.6. Distribution of multi-drug Resistant bacteria in water samples from the water distribution systems of dams in southwestern Nigeria

In this study, 191 MDR bacteria were selected from all the sampling points. The distributions of these bacteria across these sample points are shown in Table 4.7. It was observed that the highest percentage (12.6%) was found in Owena-Idanre raw water followed by 8.7% in Eleyele raw water. Significant difference was observed in the percentage of MDR bacteria across the dams at p=0.05. It was observed that 6.3% of the MDR bacteria were isolated in Ife treated water while no MDR bacteria was isolated in the Asejire second municipal tap and Eleyele second municipal tap.

4.7. Percentage resistance to various antibiotics of MDR bacteria selected for PCR genotyping from water distribution systems of dams in southwestern Nigeria

The results of MDR bacteria selected for PCR genotyping in this study is shown in Table 4.8. A total of 191 bacteria were found to be MDR out of which 133 were gram negative and 58 were gram positive. It was observed that out of the total 191 MDR bacteria 120 (62.8%), 179 (93.7%), 172 (90.0), 141 (73.8%) and 101 (52.8%) were resistant to tetracycline, sulfamethoxazole, ampicillin, combination of sulfamethoxazole and trimethoprim and streptomycin respectively. It was observed that out of a total of 133 gram negative bacteria, 40 (30.1%), 41 (30.8%) and 32 (24.1%) were resistant to Kanamycin, chloramphenicol and Nalidixic acid respectively while out of a total of 58 gram positive bacteria 19 (32.8%), 18 (31.0%) and 2 (3.4%) were resistant to erythromycin, rifamprim and ciprofloxacin respectively.

4.8. Antibiotic resistant genes using microarray

The genes on the microarray are illustrated in Table 4.9 and Plate 4.1 showed some of the bacteria carrying these genes. Microarray slides used in these studies were constructed with 125 oligonucleotide probes coding for various antibiotic resistant genes. After the construction, 22 multi-drug resistant bacteria were randomly selected from bacteria isolates across all the sampling sites of the selected water samples. In Table 4.6, it was observed that *Aeromonas caviae* with strain ID 321B from raw water of Ife (Dam 1) showed resistant phenotype to tetracycline, ampicillin, sulfamethoxazole, combination of sulfamethoxazole and trimethoprim, as well as streptomycin and chloramphenicol. It also showed the presence of the following genes in the microarray *tetk* gene coding resistant to tetracycline: *cat 1*, *cat B2*, *cat 4* coding resistant

Table 4.7: Distribution of bacteria in water samples from the water distribution systems in selected dams in southwestern Nigeria

Source	Dam 1 (Ife)		Dam 2 (Ede)		Dam 3 (Asejire)		Dam 4 (Eleyele)		Dam 5 (Owena-Ondo)		Dam 6 (Owena-Idanre)	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Rw	8	4.2^{a}	11	5.8^{b}	10	5.2^{b}	17	8.9^{c}	6	3.1^{d}	24	12.6^{e}
Fw	12	6.3^{a}	3	1.6^{b}	10	5.2^{c}	1	0.5^{d}	9	4.7^{e}	3	1.6^{b}
M1	4	2.1^{a}	8	4.2^{b}	5	2.6^{a}	1	0.5^{c}	8	4.2^{b}	9	4.7^{b}
M2	3	1.6^{a}	2	1.0^{a}	0	0	0	0	14	7.3^{b}	11	5.8^{c}

Total MDR bacteria= 191
Code: Rw= Raw water, Fw= Treated water, M1, M2= Municipal Taps
A= Number of MDR bacteria, B= Percentage Total
Values carry different letters were significantly different at P=0

Table 4.8: Percentage resistance to various antibiotics in MDR bacteria selected for PCR genotyping from the water distribution systems of dams in southwestern Nigeria

Antibiotics	T	SU	AMP	SXT	S	GEN	FF	K	C	N	AMC	CEF	E	RIF	LIN	CIP
Total MDR	191	191	191	191	191	191	133	133	133	133	133	133	133	133	133	133
No of resistance to tested antibiotics	120	179	172	141	101	50	37	40	41	32	69	74	19	18	50	2
% resistance	62.8	93.7	90.0	73.8	52.8	26.2	27.8	30.1	30.8	24.1	51.9	55.6	32.8	31.0	86.2	3.4

Code: T= Tetracycline**, SU=** Sulfamethoxazole**, AMP=** Ampicillin**, SXT=** Sulfamethoxazole/Trimethoprim**, S=** Streptomycin**, GEN=** Gentamycin**, FF=** Florfenicol**, K=** Kanamycin**, C=** Chloramphenicol**, N=** Nalidixic acid**, AMC=** Amoxicillin/Clavulanic Acid**, CEF=** Ceftiofur**, E=** Erythromycin**, RIF=** Rifamycin**, LIN=** Lincomycin**, CIP=** Ciprofloxacin

Antibiotic tested on Gram positive and Gram negative bacteria: Tetracycline, Sulfamethoxazole, Ampicillin, Sulfamethoxazole/Trimethoprim, and Gentamycin

Antibiotic tested only on Gram negative bacteria: Florfenicol, Kanamycin, Chloramphenicol, Nalidixic acid, Amoxicillin/Clavulanic Acid, Ceftiofur

Antibiotic tested only on Gram positive bacteria: Erythromycin, Rifamycin, Lincomycin and Ciprofloxacin

Table 4.9: Microarray analysis of selected MDR bacteria isolated from selected water samples in southwestern Nigeria

Source	Bacteria/Strain ID	Resistance Phenotypes	Genes detected on Array	Genes detected by PCR Genotyping
DAM 1 IFRW	*Aeromonas caviae* (321B)	T, AM, SU, SXT, AMC, S, C	*mpha, cat4, acc(3)-II, tetK, catB2, catI, Sul 1*	*Sul 1, ant(3'')*[b]
DAM 1 IFRW	*Aeromonas hydrophila* (321A)	T, AM, SU, SXT, AMC, S, C	*tetH, sul1, strA, floR, aafA, strB, tetJ, mpha, tetX, tetE*	*tetE, sul 1, ant(3'')*[b]
DAM 2 EDRW	*Chromobacterium violaceum* (382)	AM, SU, SXT, AMC, S, GEN, CEF	*mpha, catB2*	*intI 1, ant (3'')*[b]
DAM 2 EDRW	*Bordetella sp* (51)	T, SU, SXT, AMC, S, C, N, CEF, FF	*aadE, aphD, cat4, catI, dfrA1, sul2, tetE, aadA1, aadA2, aphE, aadA21, bla*$_{PSE-4}$*,strA*	*Sul 2, Bla*$_{TEM}$*, aph (3'')*[c]*, aph (6)1*[dd]
DAM 2 EDFW	*Alcaligenes faecalis* (28A)	T, AM, SU, SXT, S, GEN, K, N, CEF	*qnrA1, mpha,sul1*	*Tet A, tet 30, sul 1*
DAM 3 ARW	*Proteus vulgaris* (14B)	T, AM , SU, SXT, S, C, N, CEF, FF	*Mpha, sul2*	*Sul 2, aph (3'')*[c]
DAM 3 AFW	*Proteus vulgaris* (43)	T, AM, SU, SXT, AMC, S, C, N, CEF, FF	*aafA, floR, strA, sul2, tetJ, tetH, strB, catIII, mpha*	*Sul 2, Bla*$_{TEM}$*, aph (3'')*[c]
DAM 3 AM2	*Klebsiella pneumoniae* (287)	AM, SU, SXT, AMC, C, CEF, FF	No resistant gene	*bla*$_{SHV}$*, bla*$_{TEM}$
DAM 4 ERW	*Alcaligenes sp.* (3A)	T, AM , SU, SXT, AMC, S, GEN, K, N, CEF	*mpha,sul1,sul2, intI1*	*Int1, sul1, sul2, ant (3'')*[b]
DAM 4 ERW	*Alcaligenes faecalis* (1A)	T, AM, SU, S, K, FF	*sul1, strA, aadB, aac(6')-Ia, mpha, floR, tetH, strB,*	*Bla*$_{TEM}$
DAM 4 ERW	*Klebsiella pneumoniae* (386A)	T, AM, SU, SXT, AMC, S, C, FF	*floR, strA, strB, sul1, tetH, tetJ, aafA*	*Sul 1, bla*$_{SHV}$*, bla*$_{TEM}$*, ant (3')*[b]

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)
Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.9 (Cont'd): Microarray analysis of selected MDR bacteria isolated from selected water samples in southwestern Nigeria

Source	Bacteria/Strain ID	Phenotypes	Antbiotic Resistant Genes detected on Microarray	Antibiotic resistance Genes detected by PCR Genotyping
DAM 4 EFW	*Proteus vulgaris* (46)	T, AM, SU, SXT, AMC, S, GEN, K, C, N, FF	*intI1, sul1, sul2*	*tetB, intI1, sul1,sul2*
DAM 5 OWODM1	*Morganella morganii* (215A)	T, AM, SU, SXT, AMC, S, CEF	*floR, dfrA1, strA, sul2, strB, mpha*	*tetA, sul2,* bla_{TEM}
DAM 5 OWODM2	*Providencia rettgeri* (209)	T, AM, SU, SXT, AMC, S, C, N, CEF	*catII, qnrA1,* bla_{DHA-1}*,sul 1, sul 2*	*intI1,sul1,sul 2, aph (6)1*^{dd}
DAM 5 OWODM1	*Morganella morganii* (215A)	T,S, CEF, AM, SXT, AMC, SU	*Sul 2, TetA,* bla_{DHA-1}	*TetA, Sul 2,* Bla_{TEM}
DAM 6 OWIRW	*Serratia marcescens* (348)	T, AM, SU, AMC, S, C, CEF	*mpha,* bla_{CMY-2}*, catB2,*	bla_{SHV}*, aph(3")*^{c}
DAM 6 OWIRW	Uncultured bacterium (205A)	AM, SU, S, GEN, K, C	*mph*a, *cat*B2, sul2	*Sul2,* bla_{TEM}
DAM 6 OWIM1	*Citrobacter murliniae* (363)	T, AM, SU, AMC, S, CEF	bla_{PSE-4}*, aph*D, *str*A, *aad*E, *otr*B, bla_{MIR}*, eae, aph(3')-Iia, tetA, , mpha*	No Gene
DAM 6 OWIM1	*Alcaligenes faecalis* (250A2)	T, AM, SU, AMC, S, GEN, K, CEF	*mpha,tet E, intI, sul1, sul2, strA*	*tetE, int 1, sul1, sul 2,* bla_{SHV}*,* bla_{TEM}*, aph(3")*^{c}*,ant(3")*^{b}*, aph(6')1*^{dd}
DAM 6 OWIM2	*Alcaligenes faecalis* (239)	T, AM, SU, SXT, S, K, CEF	*mpha,tetA, tet E, Sul 1,int 1*	*tetA, tetE, intI1, sul1, aph(3')*^{c}
DAM 6 OWIRW	*Proteus vulgaris* (179)	FF, T, S, C, CEF, SXT, N, AMC, SU	*TetC, mpha, Sul 2*	*Sul 2,* Bla_{TEM}*, aph (3")*^{c}*, ant (3")*^{b}*, aph(6")1*^{dd}
DAM 6 OWIRW	Acinetobacter junii (205B)	T, S, G, CEF, AM, SXT, AMC, SU	*Tet J, sul 2, mpha*	*Sul 2*

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)
Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

chloramphenicol, *acc(3)-II* gene coding resistant to aminoglycoside like streptomycin and *mpha* coding resistant to macrolides, and *sul1* coding resistant to sulfa drug. In comparing the genes found in this bacterium on the microarray with that detected with PCR amplification with specific primer it was observed that *sul 1* gene was also detected by PCR genotyping. Also observed from the microarray results was that *Alcaligenes feacalis* with strain ID 28A from Dam 2 tested water showed the presence of *qnr A1* gene for quilonone resistance, *mpha* for macrolide resistance and *sul 1* gene for sulfa drug resistance. *Sul 1* gene was confirmed by PCR amplification while the phenotype of the bacteria showed resistance to Nalidixic acid (quinolone) and sulfamethoxazole (sulfa drug). From Dam 3 treated water, *Proteus vulgaris* with strain ID 43 was one of the bacteria selected for the microarray genotyping. The microarray results showed the presence of *aaf A*, *strA* and *strB* gene for aminoglycoside resistance, *floR* for florfenicol resistance, *tet J* and *tet H* for tetracycline resistance, *cat III* for chloramphenicol resistance and *mpha* for macrolide resistance.

From amplification of PCR with specific primer of some antibiotics resistance gene, *sul 2* gene was confirmed from this bacterium. It was also observed that the bacterium showed resistance phenotype to all the genes detected on the microarray and with PCR ampification. *Klebsiella pneumoniae* with strain ID 287 from dam 3 municipal 2 did not show the presence of any resistance gene on the microarray while bla_{SHV} and bla_{TEM} both coding resistance for entended beta lactam were detected by PCR amplification. It should be noted that these genes were not part of the oligonucleotide probe on the microarray. *Klebsiella pneumonia* with strain ID 386A from Dam 4 raw water showed the presence of the following genes on the microarray results *floR* coding for florfenicol resistance, *strA* and *strB* coding for streptomycin resistance, *sul 1* coding for sulfa drug resistance, *tet H* and *tet J* coding for tetracycline and *aafA* coding for aminoglycoside resistance. PCR amplification confirmed the presence of sul 1 gene in this bacterium. At Dam 5 Municipal 1 *Morganella morganii* with strain ID 215A selected for microarray genotyping showed the presence of the following genes: *sul 2* for sulfa drug resistance, *tet A* for tetracycline resistance and bla_{DHA-1} for extended beta lactamase gene. Genotyping with PCR amplification with some specific primers confirmed the presence of *tet A* and *sul 2* gene. *Proteus vulgaris* with strain ID 179 was one of the bacteria also selected from dam 6 raw water and it showed the presence of *tet C* for tetracycline resistance, *mpha* for macrolide resistance and *sul 2* gene for sulfa drug resistance. Amplification with PCR with some specific primers showed the presence of *sul 2* gene.

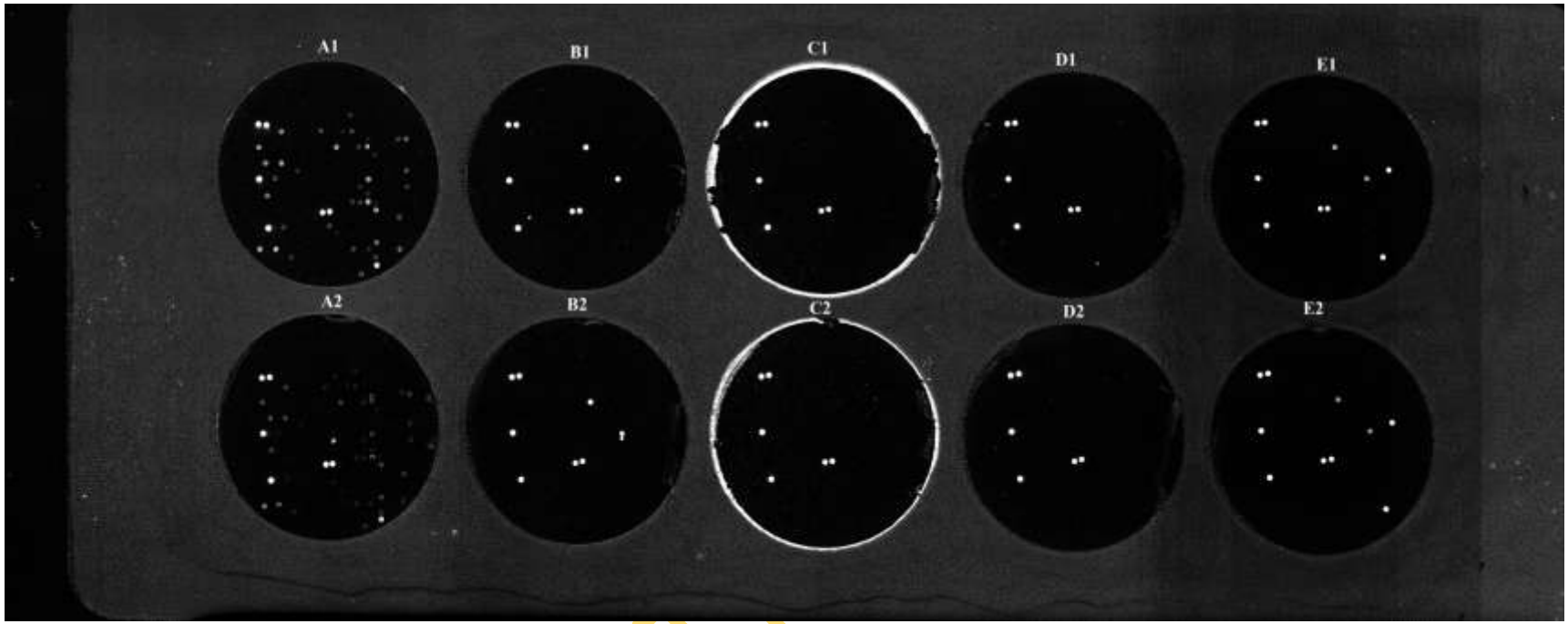


Plate 4.1: Hybridization of resistant genes from selected multi-drug resistant bacteria in water samples in southwestern Nigeria.

Codes: A1 and A2: *E. coli* strain H4H; **B1 and B2:** *E. coli* strain K12; **C1 and C2:** *Proteus vulgaris* from OWIRW; **D1 and D2:** *Alcaligenes faecalis* from OWIRW; **E1 and E2:** *Acinetobacter junii* from OWIRW

4.9. Antibiotic resistant genes from bacteria isolated from selected water samples from southwestern Nigeria using PCR genotyping

The results of antibiotic resistant genes in the bacteria from Ife Dam water are shown in Table 4.10. *Tet A* gene was detected in *E. coli* from the raw water and *Psudomonas spp* (89) and *Alcaligene spp* (strain ID 87A) from the treated water. None of the bacteria carried the presence of *tet B* gene. *Brevundimonas diminuta* (strain ID 119B) from the treated water sample of Ife Dam carried bla_{SHV} *which* encode resistance to extended spectrum beta lactam. *Proteus mirabilis* (strain ID 122A) also from the treated water from this site carried five antibiotic resistance genes which were *sul 1* that encode resistance to sulfa drug, Bla_{TEM} that encode resistance to beta lactam drug and all the 3 streptomycin resistant genes tested in this study. From the first Municipal tap sampled, three MDR bacteria isolated showed the presence of at least one of the antibiotic resistant genes examined. It was observed that *Bacillus thuringiensis* from this tap had three antibiotic resistant genes which are bla_{SHV} and bla_{TEM} encoding beta lactam resistant and *aph (3")*^{b} encoding resistant to aminoglycoside antibiotics as well as streptomycin.

Table 4.10 shows bacteria from Ede Dam and its water distribution systems carrying different antibiotic resistant genes. *Bordetella spp* (strain ID 51) and *Proteus vulgaris* (strain ID 58B) both from the raw water contained the same antibiotic resistant genes; *sul 2* for sulfa drug, bla_{TEM} for beta lactam antibiotics and aminoglycoside resistant genes which were *aph (3")*^{c} *and aph (6)1d*^{d}. No MDR bacteria from the raw water tested carried any of the tetracycline resistant gene while *Bacillus altitudinus* (strain ID 248) a gram positive bacteria from the raw water tested positive for *sul 1* gene, and two aminoglycoside resistant genes which were *aph (3")*^{c} and *ant (3").* At the first water distribution tap sampled *Pseudomonas putida* (strain ID 85B) carried two antibiotic resistance gene which were *sul 2* and bla_{SHV} gene. No bacteria from this point tested positive for the presence of any of the tetracycline resistant genes even when some of the bacteria showed tetracycline resistance phenotype. Only one gram negative bacteria from Ede Municipal 2 tested positive to at least one of the antibiotic resistant genes. The bacteria was identified as *Pschrobacter spp* (strain ID 140) and it carried bla_{TEM} and *ant (3")*^{b}.

Antibiotic resistant genes found in bacteria from Asejire water distribution taps, raw water and treated water are shown in Table 4.12. *Leucobacter komagatae* (2 strain ID 6B) from the raw water sample from Asejire dam had five antibiotic resistant genes which were *tet B, sul 1* and *sul 2,*

Table 4.10: Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Ife dam (Dam 1) as detected by PCR genotying

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*Tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	*Bl* $_{TEM}$	*aph* (3")[C]	*ant* (3") [b]	*aph* (6) 1d[d]
							Raw Water							
Aeromonas caviae (321B)	T, S, C, AM, SXT, AMC, SU	-	-	-	-	-	+	-	-	-	-	-	+	-
Aeromonas hydrophila (321A)	T, S, C, AM, SXT, AMC, SU	-	-	+	-	-	+	-	-	-	-	-	+	-
Escherichia coli (319)	T, S, C, N, SXT, SU	+	-	-	-	-	-	+	-	-	+	-	+	+
Providencia vermicola (82)	T, S, GEN, CEF, AM, FF, SXT, SU	-	-	-	-	-	+	-	-	-	-	+	-	-
							Treated water							
Pseudomonas sp. (89)	T, S, GEN, K, C, AM, SXT, AMC, SU	+	-	-	-	-	+	-	+	-	+	+	+	-
Brevundimonas diminuta (119B)	S, GEN, K, N, AM, SXT, SU	-	-	-	-	-	-	-	+	-	-	+	+	+
Alcaligenes sp (87A)	T, S, AM, SXT, SU	+	-	-	-	-	+	-	-	-	-	-	+	-
Uncultured bacterium clone (144A)	FF, T, GEN, SU	-	-	-	-	-	-	-	-	-	+	-	-	-
Proteus mirabilis (122A)	FF, T, S, GEN, K, C, AMC, AM, SU, SXT	-	-	-	-	-	+	-	-	-	+	+	+	+
Bacillus thuringiensis (85A)	SU, AM, T, E, SXT, RIF, LIN, GEN	-	-	-	-	-	-	-	-	-	+	-	-	-

Codes: Present (+), Absent (-). **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.10 (Cont'd): Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Ife dam (Dam 1) as detected PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*Tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	Bla_{TEM}	*aph* (3")[C]	*ant* (3") [b]	*aph* (6) 1d[d]
						Treated water								
Bacillus pumilus (122B)	SU, T, E, SXT, RIF, LIN	-	-	+	-	-	-	+	-	-	-	-	-	-
						Municipal Tap 1								
Uncultured bacterium clone (92A)	T. GEN, AM, SU	-	-	-	-	-	-	-	-	-	-	+	+	-
Chromobacterium violaceum (129)	FF, S, GEN, K, CEF, AMC, SU	-	-	-	-	-	-	-	-	-	-	-	+	-
Bacillus thuringiensis (329)	SU, AM, E, SXT, RIF, LIN	-	-	-	-	-	-	-	+	-	+	-	+	-

Codes: Present (+), Absent (-). **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT

Table 4.11: Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Ede dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[c]	*ant* (3")[b]	*aph* (6) 1d[d]
Raw water (Dam 2)														
Stenotrophomonas maltophilia (58A)	T, S, K, CEF, AM, AMC, SU	-	-	-	-	-	-	-	+	-	+	-	-	-
Chromobacterium violaceum (382)	S, GEN, CEF, AM, SXT, AMC, SU	-	-	-	-	-	-	-	-	-	-	-	+	-
Bordetella sp (51)	FF, T, S, C, N, CEF, AM, SXT, SU	-	-	-	-	-	-	+	-	-	+	+	-	+
Proteus vulgaris (58B)	T, AM, SXT, SU	-	-	-	-	-	-	+	-	-	+	+	-	+
Pseudomonas putida (99A)	FF, AM, SXT, AMC	-	-	-	-	-	-	+	-	-	-	-	-	-
Pseudomonas sp. (299B2)	FF, CEF, AM, AMC, SU	-	-	-	-	-	-	-	-	-	-	-	+	-
Bacillus altitudinis (52B)	SU, E, RIF, LIN	-	-	-	-	-	-	-	+	-	-	-	-	-
Bacillus altitudinis (24B)	SU, AM, T, E, SXT, RIF, LIN, G	-	+	-	-	-	+	-	-	-	-	+	+	-
Staphylococcus sp. (98)	SU, T, E, SXT, RIF, LIN	-	-	-	-	-	-	-	-	-	+	-	-	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT

Table 4.11 (Cont'd): Antibiotics resistant phenotypes and genes in multi-drug resistant bacteria isolated from Ede dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[c]	*ant* (3")[b]	*aph* (6)1d[d]
Treated Water (Dam 2)														
Alcaligenes faecalis (28A)	T, S, GEN, K, CEF, AM, SXT, SU	+	-	-	-	+	+	-	-	-	-	-	-	-
Morganella sp. (U)	T, S, AM, SXT, SU	+	-	-	-	-	+	+	-	-	+	+	-	-
Bacillus cereus (137A1)	SU, AM, T, E, SXT, RIF, LIN	-	-	-	-	-	-	-	-	-	+	-	-	-
Municipal Tap 1 (Dam 2)														
Acinetobacter baumannii (107A)	FF, S, C, AM, AMC, CEF, SU	-	-	-	-	-	-	-	-	-	-	-	+	-
Pseudomonas sp (159B)	T, S, GEN, N, CEF, AM, SXT	-	-	-	-	-	-	-	-	-	+	-	-	-
Acinetobacter baumannii (109)	AM, SU, SXT, AMC, S, C, CEF, FF	-	-	-	-	-	-	-	-	-	-	+	-	-
Pseudomonas putida (85B)	AM, SXT, AMC, C, FF	-	-	-	-	-	-	+	+	-	-	-	-	-
Pseudomonas sp. (106)	T, AM, SU, SXT, AMC, S, N	-	-	-	-	-	-	-	-	-	+	-	-	-
Pseudomonas sp (306B)	SU, T, E, RIF, LIN	-	-	-	-	-	-	-	-	-	-	-	+	-
Pseudomonas sp (304A)	T, AM, SU, SXT, AMC, S, GEN, CEF, FF	-	-	-	-	-	-	-	-	-	+	-	+	-
Bacillus sp. (66A)	SU, AM, SXT, LIN	-	-	-	-	-	-	-	-	-	-	-	+	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.11 (Cont'd): Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Ede dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph* (6) 1d[d]
Municipal Tap 2 (Dam 2)														
Psychrobacter sp (140)	T, S, CEF, AM, SXT, SU	-	-	-	-	-	-	-	-	-	+	-	+	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT

Table 4.12: Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Asejire dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph*(6)1d[d]
						Raw water								
Uncultured bacterium clone (18B1)	T, GEN, K, C, N, CEF, AM, SXT, AMC, SU	-	+	-	-	-	+	+	-	-	+	-	+	+
Alcaligenes faecalis (45)	S, CEF, AM, SXT, SU	-	-	-	-	-	+	-	+	-	-	-	-	-
Leucobacter komagatae (26B)	T, S, GEN, K, SXT, N, SU	-	+	-	-	-	+	+	-	-	+	+	-	-
Proteus mirabilis (18B2)	T, S, N, SXT, SU	+	-	-	-	-	+	+	-	-	+	-	+	-
Proteus vulgaris (14B)	FF, T, S, C, N, CEF, AM, SXT, SU	-	-	-	-	-	-	+	-	-	-	+	-	-
Klebsiella pneumoniae (279)	FF, T, S, C, AMC, CEF, AM, SU, SXT	-	-	-	-	-	-	-	-	-	+	-	-	-
						Treated Water								
Proteus mirabilis (49)	T, S, CEF, AM, AMC, SU	-	-	-	-	-	-	-	-	-	-	+	-	-
Pseudomonas sp. (6B)	T, S, GEN, K, CEF, AM, SXT	-	-	-	-	-	-	+	-	-	-	-	-	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT

Table 4.12 (Cont'd): Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Asejire dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph*(6)1 d[d]
						Treated Water (Cont'd)								
Pseudomonas sp (6A)	T, S, G, K, C, CEF, AM, SXT, AMC, SU, FF	+	-	-	-	-	+	-	+	-	+	+	+	-
Proteus vulgaris (43)	FF, T, S, C, N, CEF, AM, SXT, AMC, SU	-	-	-	-	-	-	+	-	-	+	+	-	-
Alcaligenes sp (9B)	S, AM, SXT, SU	-	-	-	-	-	-	+	-	-	-	-	-	-
Klebsiella pneumoniae (286)	T, S, C, AM, SXT, SU	-	-	-	-	-	+	+	+	-	-	-	+	-
Myroides odoratus (38A)	FF, T, S, GEN, K, C, AM, SXT, AMC, SU	-	-	-	-	-	-	-	-	-	+	-	-	-
Bacillus pumilus (284)	SU, AM, T, E, SXT, RIF, LIN	-	-	-	-	-	-	-	-	-	+	-	-	-
						Municipal Tap 1								
Chromobacterium sp. (295A)	S, T, CEF, AM, SXT, AMC, SU, AMC, SU	-	-	-	-	-	-	-	-	-	+	+	+	-
Uncultured bacterium clone (41)	T, S, GEN, K, N, CEF, AM, SXT, AMC, SU	-	+	-	-	-	+	+	-	-	-	+	+	-
						Municipal Tap 2								
Acinetobacter calcoaceticus (291)	SU, GEN, C, AM, SXT, N, CEF, SU	-	-	-	-	-	-	-	+	-	+	-	-	-
Proteus vulgaris (13B)	T, S, GEN, C, AM, SXT, N, CEF, SU	-	-	-	-	-	-	+	-	-	-	+	-	-
Klebsiella pneumoniae (287)	FF, C, CEF, AM, SXT, AMC, SU, AMC, SU	-	-	-	-	-	-	-	+	-	+	-	-	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

bla$_{TEM}$ and *aph (3'')* genes. *Proteus mirabilis* (strain ID 18B2) also from this sample carried *tet A, sul 1 and sul 2, bla*$_{TEM}$ and *ant (3'')*^{b} genes. *Pseudomonas spp* from the treated water of Asejire with strain ID 6A that showed resistant phenotype to 11 antibiotics carried 6 antibiotic resistant genes which were *tet A, Sul 1, bla*$_{SHV}$, *bla*$_{TEM}$, *aph (3'')*^{C} and *ant (3'')*^{b}. Two MDR bacteria from the first Municipal tap which included *Chromobacterium sp* (strain ID 295A) and Uncultured bacterium clone (41) carried at least one antibiotic resistant gene examined by PCR. It was observed that the uncultured bacterium clone showed antibiotic resistant phenotype to 10 antibiotics and the following resistant genes were detected in it: *tet B, sul 1 and sul 2, aph (3'')* and *ant (3'')*^{b}. *Klebsiella pneumonia* (strain ID 287) from the second Municipal tap tested positive for two beta lactam genes which include *bla*$_{SHV}$ and *bla*$_{TEM}$.

Results of antibiotic resistant genes in bacteria from Eleyele (Dam 4) and its water distribution taps are illustrated in Table 4.13. *Aeromonas caviae* with strain ID 376 from the raw water sample showed resistant phenotypes to 7 antibiotics carried 5 genes which were *bla*$_{TEM}$, *aph (3''), ant (3'')*^{b}, *aph (6)1d*^{d} and *sul 1* gene. None of the bacteria isolates from the raw water samples tested positive for any of the tetracycline resistant genes, even though some of them showed tetracycline resistance phenotype. *Proteus vulgaris* (strain ID 4) from the treated water was the only isolate from the treated water carrying antibiotic resistance genes among the genes examined. The following genes were found in the bacteria: *tet B, sul 1, sul 2, bla*$_{TEM}$, *aph (3'')*^{c} and *ant (3'')*^{c}. One bacterium isolated at the Municipal tap 1 also had at least one of the genes tested. The isolate was identified by 16S rDNA as *Alcaligenes spp* and it carried *sul 1, sul 2* and *ant (3'')*^{b}.

Antibiotic resistant genes in bacteria from selected water samples from Owena-Ondo are shown in Table 4.14. *Leucobacter komagatae* with strain ID 230 from the raw water sample showed the presence of 3 antibiotic resistant genes which included *sul 1, sul 2* and *aph (3'')*^{c} though the bacteria showed antibiotic resistant phenotypes to ampicillin but did not carry the presence of any of the beta lactam resistant gene tested in this study. Three bacteria isolates which were *Alcaligenes spp* (strain ID 198), *Alcaligene feacalis* (strain ID 197) and *Morganella morganii* (strain ID 199) from the treated water tested positive for *tet A*. *Pseudomonas otitidis* from these sites showed resistant phenotype to ampicillin, sulfamethoxazole, and combination of sulfa drug and trimethoprim only tested positive to *sul 1*. *Klebsiella spp* (strain ID 386B) tested

Table 4.13: Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Eleyele dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph*(6)1d[d]
							Raw water							
Aeromonas caviae (376)	T, S, AM, SXT, N, AMC, SU	-	-	-	-	-	+	-	-	-	+	+	+	+
Aeromonas hydrophila (391A)	S, K, N, SU	-	-	-	-	-	+	+	-	-	-	-	+	-
Morganella morganii (384)	T, S, K, CEF, AM, SXT, AMC, SU	-	-	-	+	-	-	+	-	-	+	-	+	-
Alcaligenes faecalis (1B)	T, S, K, AM, SU	-	-	-	-	-	-	-	-	-	+	-	-	-
Alcaligenes sp (3A)	T, S, GEN, K, N, CEF, AM, SXT, AMC, SU	-	-	-	-	-	+	+	-	-	-	-	+	-
Alcaligenes sp (50)	S, CEF, AM, SXT, AMC, SU	-	-	-	-	-	+	-	-	-	-	-	-	-
Alcaligenes faecalis (1A)	T, S, K, AM, SU	-	-	-	-	-	-	-	-	-	+	-	-	-
Proteus vulgaris (33B)	T, C, CEF, AM	-	-	-	-	-	-	-	-	-	+	+	-	-
Proteus mirabilis (385A)	T, S, K, N, AM, SXT, AMC, SU	-	-	-	-	-	-	+	-	-	+	+	-	-
Providencia vermicola (44A)	T, GEN, AM, SU	-	-	-	-	-	-	-	-	-	+	-	-	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.13 (Cont'd): Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Eleyele dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph*(6)1d[d]
						Raw water (Cont'd)								
Klebsiella pneumoniae (378)	S, CEF, AM, SXT, AMC, SU	-	-	-	-	-	+	-	-	-	+	-	+	-
Klebsiella pneumoniae (375)	T, K, N, AM, SU	-	-	-	-	-	+	+	+	-	+	-	+	-
Klebsiella pneumoniae (386A)	FF, T, S, C, AM, SXT, AMC, SU	-	-	-	-	-	+	-	+	-	+	-	+	-
Trabulsiella guamensis (380A)	T, C, CEF, AM, SU, SXT	-	-	-	-	-	-	+	+	-	-	-	-	-
						Treated water								
Proteus vulgaris (46)	FF, T, S, GEN, K, C, AM, SXT, AMC, SU	-	+	-	-	-	+	+	-	-	+	+	+	-
						Municipal Tap 1								
Alcaligenes sp (19B1)	T, S, K, AM, SXT, AMC, SU	-	-	-	-	-	+	+	-	-	-	-	+	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.14: Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Owena-Ondo dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph*(6)1d[d]
						Raw water								
Uncultured bacterium clone (230A)	SU, AM, E, RIF, LIN, GEN	-	-	-	-	-	-	+	-	-	-	-	-	-
Leucobacter komagatae (230B)	SU, AM, SXT, GEN	-	-	-	-	-	+	+	-	-	-	+	-	-
						Treated water								
Alcaligenes sp. (198)	T, S, K, AM, SXT, SU	+	-	-	-	-	+	+	-	-	-	-	+	-
Alcaligenes faecalis (197)	T, S, K, CEF, AM, SXT, SU	+	-	-	-	-	+	-	-	-	-	-	+	-
Morganella morganii (199)	T, S, CEF, AM, AMC, SU	+	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas otitidis (350)	AM, SXT, SU	-	-	-	-	-	+	-	-	-	-	-	-	-
Klebsiella sp (386B)	FF, T, S, C, AM, SXT, AMC, SU	-	-	-	-	-	+	+	+	-	+	-	+	-
Proteus mirabilis (201)	S, CEF, AM, SU, SXT	-	-	-	-	-	-	-	-	-	-	-	+	-
Bacillus sp (202B)	SU, AM, SXT, LIN	-	-	-	-	-	-	-	-	-	-	-	+	-
						Municipal Tap 1								
Morganella morganii (215A)	T, S, CEF, AM, SXT, AMC, SU	+	-	-	-	-	-	+	-	-	+	-	-	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.14 (Cont'd): Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Owena-Ondo dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph*(6)1d[d]
							Municipal Tap 1 (Cont'd)							
Pantoea agglomerans (214)	T, S, AM, AMC, SU	-	-	-	-	-	-	+	-	-	-	-	+	-
Proteus mirabilis (273)	T, S, SXT, SU	-	-	-	-	-	-	-	-	-	-	-	+	-
Pseudomonas sp. (260)	FF, T, C, N, CEF, AM, SXT, AMC, SU	-	-	-	-	-	-	-	-	-	+	-	+	-
Bacillus cereus (215B)	SU, AM, E, SXT, LIN	-	-	-	-	-	-	+	-	-	-	-	-	-
Bacillus sp. (245A1)	SU, AM, T, RIF, LIN, CIP, GEN	-	-	-	-	-	-	-	-	-	-	-	+	-
							Municipal Tap 2							
Escherichia coli (210B)	T, AM, SXT, AMC, SU	+	-	-	-	-	-	-	-	-	+	-	-	-
Escherichia coli (210A)	T, AM, AMC, SU	+	-	-	-	-	-	-	-	-	+	-	-	-
Pseudomonas putida (251B)	T, S, C, AM, SXT, SU	+	-	-	-	-	+	+	+	-	-	+	+	+
Providencia rettgeri (209)	T, S, C, N, CEF, AM, SXT, AMC, SU	-	-	-	-	-	+	+	-	-	-	+	-	+
Alcaligenes faecalis (253A)	T, S, GEN, K, C, AM, SXT, SU	+	-	-	-	-	-	-	-	-	+	-	+	-
Providencia rettgeri (253B1)	S, SXT, AMC, SU	-	-	-	-	-	-	-	-	-	-	-	+	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.15: Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Owena-Idanre dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*tet* B	*tet* E	*tet* M	*tet* 0	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph*(6)1d[d]
						Raw water								
Alcaligenes sp (174A)	T, S, GEN, K, C, N, CEF, AM, SXT, AMC, SU	-	-	-	-	-	+	+	-	-	-	+	+	+
Alcaligenes faecalis (173B)	T, S, CEF, SXT, AMC, SU	+	-	+	+	-	+	+	-	-	-	-	+	-
Acinetobacter baumannii (222)	FF, T, S, C, AM, SXT, AMC, SU	-	-	-	-	-	-	+	-	-	+	-	-	-
Acinetobacter junii (205B)	T, S, G, CEF, AM, SXT, AMC, SU	-	-	-	-	-	-	+	-	-	-	-	-	-
Klebsiella sp. (347)	T, S, AM, SXT, SU	-	-	-	-	-	-	-	-	+	+	+	-	-
Klebsiella sp (389B2)	T, N, AM, AMC, SU	-	-	+	-	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca (175A)	K, AM, SXT, SU	-	-	-	-	-	-	-	-	+	-	-	-	-
Klebsiella pneumoniae (345)	T, S, C, AM, SXT, AMC, SU	-	-	-	-	-	-	-	+	-	+	-	+	-
Proteus vulgaris (178B)	FF, T, S, C, N, CEF, AM, SXT, AMC, SU	-	-	-	-	-	-	+	-	-	-	+	-	-
Proteus vulgaris (179)	FF, T, S, C, CEF, SXT, N, AMC, SU	-	-	-	-	-	-	+	-	-	+	+	+	+

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.15 (Cont'd): Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Owena-Idanre dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet* A	*Tet* B	*tet* E	*tet* M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph*(6)1d[d]
							Raw water (Cont'd)							
Pseudomonas sp. (175B)	T, S, G, K, CEF, SXT, AMC, SU	+	-	-	-	-	+	+	-	-	+	+	-	+
Pseudomonas sp. (343B)	FF, T, S, N, CEF, AM, AMC, SU	+	-	-	-	-	+	-	-	-	-	-	-	-
Pseudomonas sp (223)	FF, S, C, CEF, SXT, AMC, SU	-	-	-	-	-	-	-	-	-	-	+	-	-
Pseudomonas sp (343A)	FF, K, C, AM, SXT, N, CEF, AMC, SU	-	-	-	-	-	-	-	-	-	-	+	-	-
Morganella morganii (206)	T, S, AM, AMC, SU	-	-	-	+	-	-	+	-	+	-	-	-	-
Myroides odoratus (174B)	S, GEN, K, CEF, AM, SXT, AMC, SU	-	-	-	-	-	-	+	-	-	-	-	-	-
Serratia marcescens (348)	T, S, C, CEF, AM, AMC, SU	-	-	-	-	-	-	-	+	-	-	+	-	-
Uncultured bacterium (205A)	S, GEN, K, AM, SU	-	-	-	-	-	-	+	-	-	+	-	-	-
Bacillus thuringiensis (265)	SU, AM, T, SXT, RIF, LIN, CIP, GEN	-	-	-	-	-	+	+	+	-	+	+	+	+

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU);

Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)
Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.15 (Cont'd): Antibiotics phenotypes and genes in multi-drug resistant bacteria isolated from Owena-Idanre dam as detected by PCR

Bacteria /Strain ID	Phenotypes	*tet*A	*tet*B	*tet* E	*tet*M	*tet* 30	*sul* 1	*sul* 2	bla_{SHV}	bla_{CTX}	bla_{TEM}	*aph* (3")[C]	*ant* (3")[b]	*aph*(6)1d[d]
							Final water							
Proteus vulgaris (257B)	S, AM, SXT, CEF, SU	-	-	-	-	-	-	+	-	-	-	-	-	-
Bacillus altitudinis (257A)	SU, AM, T, E, SXT, RIF, LIN	-	-	-	-	-	-	-	+	-	+	-	-	-
							Municipal Tap 1							
Alcaligenes sp (250B2)	T, S, K, N, CEF, SU	+	-	-	-	-	+	+	-	-	+	-	-	-
Alcaligenes faecalis (250A2)	T, S, GEN, K, CEF, AM, AMC, SU	-	-	+	-	-	+	+	+	-	+	+	+	+
Citrobacter freundii (362)	T, S, AM, SXT, N, AMC, SU	-	-	-	-	-	-	-	-	-	+	-	-	-
Klebsiella pneumoniae (361)	T, S, CEF, AM, SU	-	-	-	-	-	-	-	+	-	-	-	+	-
Pseudomonas sp. (196)	FF, T, S, GEN, K, AM, AMC, SU	+	-	+	-	-	+	-	-	-	+	+	+	-
							Municipal Tap 2							
Alcaligenes faecalis (239)	T, S, K, CEF, AM, SXT, SU	+	-	+	-	-	+	-	-	-	-	+	-	-
Alcaligenes sp (238B)	T, S, GEN, K, N, CEF, AM, SU	+	-	+	-	+	+	+	+	-	-	-	+	-
Proteus mirabilis (385B)	T, S, C, N, AM, SXT	-	-	-	-	-	-	-	+	-	-	-	+	-
Pseudomonas sp	FF, T, S, GEN, K, C, AM, SXT,	+	-	-	-	-	+	+	+	-	+	+	+	+

(244B) AMC, SU

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

positive to *tet 30, sul 1, sul 2, bla$_{SHV}$, bla$_{TEM}$ and ant (3")^{b}*. At the municipal tap 1 sampling 6 bacteria showed the presence of the antibiotic resistant genes. *Morganella morganii* from this site tested positive for *tet A, sul 2* and *bla$_{TEM}$* . At Municipal tap 2 *Pseudomonas putida* (strain ID 251B) carried presence of 7 antibiotic resistant genes which were *tet A, sul 1, sul 2, bla$_{SHV}$, aph (3")^{c}, ant (3")^{b}* and *aph (6)1d^{d}*. Two *E. coli* isolates from municipal tap 2 had tet *A* and *bla$_{TEM}$* genes while *Pseudomonas putida* with strain ID 209 from this same point carried the presence of 7 antibiotic resistant genes which were *tet A, sul 1, sul 2, bla$_{SHV}$, aph (3")^{c}, ant (3")^{b}* and *aph (6)1d^{d}*. *Proteus vulgaris* (strain ID 171B) had 3 antibiotic resistant genes which were *sul 1, sul 2* and *aph (6)1d^{d}*.

Table 4.15 shows antibiotic resistant genes in bacteria from selected water samples from Owena-Idanre dam. Results showed that 19 bacteria from the raw water were having at least one antibiotic resistant genes. *Alcaligenes spp* with (strain ID 174A) had the presence of *sul 1, sul 2, aph (3")^{c}, ant (3")^{b}* and *aph (6)1d^{d}*. *Klebsiella spp* (strain ID 347) had the presence of *bla$_{CTX}$, bla$_{TEM}$* and *aph (3")^{c}*. The bacteria did not show the presence of any of the tetracycline resistant genes amplified in this study. *Proteus vulgaris* (strain ID 179) had 5 antibiotic resistant genes which were *sul 2, bla$_{TEM}$, aph (3")^{c}, ant (3")^{b}* and *aph (6)1d^{d}*. The treated water sample had 2 bacteria carried at least one antibiotic resistance genes among the one tested in this study. The bacteria was *Proteus vulgaris* (strain ID 257B) showing the presence of only *sul 2* gene and *Bacillus altitudinis* tested positive to *bla$_{SHV}$* and *bla$_{TEM}$*. *Alcaligene feacalis* (strain ID 250A2) from Municipal tap 1 sample tested positive to 8 antibiotic resistant genes which were *tet E, sul 1, sul 2, bla$_{SHV}$, bla$_{TEM}$, aph (3")^{c}, ant (3")^{b}* and *aph (6)1d^{d}* while *Pseudomonas spp* (strain ID 196) also from the same point showed the presence of 6 antibiotic resistant genes which were *tet A, tet E, sul 1, bla$_{TEM}$, aph (3")^{c}* and *ant (3")^{b}*. At the municipal tap 2 sample, 4 bacteria showed the presence of at least one antibiotic resistance genes in this study. *Proteus mirabilis* (strain ID 385B) from this point tested positive to *bla$_{SHV}$* and *ant (3")^{b}*. *Alcaligene spp* (strain ID 238B) also from this point showed the presence of 7 antibiotic resistant genes which were *tet A, tet B, sul 1, tet 30, sul 1, sul 2, bla$_{SHV}$* and *ant (3").*

4.10. Presence of integron and gene cassettes in bacteria from selected water samples from southwestern Nigeria.

The results obtained from the analyses of integrons and gene cassettes are shown in Tables 4.16 to 4.21 and plates 4.2 and 4.3 while those of variable regions are shown on plates 4.4 and 4.5. *Providencia vermicola* (strain ID 82) and *Bacillus pumilus* (strain ID 117) isolated from raw water sample from Ife dam (Table 4.16) had the presence of class 1 integron but no class 2 integron. Gene cassettes were not detected in the isolates from the raw water. Treated water from this dam had 3 bacteria carrying class 1 integron as shown in Table 4.16. One of the bacteria *Alcaligene sp* (strain ID 87A) had *dfr A15* and *aadA1* gene cassettes that codes resistance to trimethoprim and aminoglycosides respectively while *Proteus mirabilis* (strain ID 122A) carried *aad2* gene cassettes responsible for resistance to aminoglycoside. At the first municipal sampling point (tap 1) of the distribution channel of the same dam, *Chromobacterium violaceum* (strain ID 129) and uncultured bacterium were obtained that carried the presence of class 1 integron without gene cassettes. There were no bacteria from the second distribution channel (tap 2) with class 1 integron and gene cassettes as shown in the Table.

Presence of integrons and gene cassettes among bacteria from selected water samples from Dam 2 Ede are shown on Table 4.17. It was observed that one bacteria each from the raw water, treated water and Municipal tap 1 tested positive to the presence of class 1 integron and it was observed also that only *Morganella sp* from the treated water showed the presence of *dfr 7* gene cassettes that encodes resistance to trimethoprim among the bacteria isolates. No bacteria from all the sampling points of this dam showed the presence of class 2 integron. Table 4.18 shows the presence of integrons and gene cassettes in bacteria from selected water samples from the raw and water distribution points of Asejire Dam. It was observed that class 1 integron was present in four bacteria isolated from the raw water. The bacteria were identified by 16S rDNA sequencing as an uncultured bacterium clone (strain ID 18B1), *Alcaligenes feacalis* (strain ID 45), *Leucobacter komagatae* (strain ID 26B) and *Proteus mirabilis* (strain ID 18B2). The following gene cassettes were found in the three bacteria isolates: *aadA1, aadA2* that encodes resistance to aminoglycoside, *dfr* that encodes resistance to trimethoprim, *qnr A1* that encodes resistance to quinolone, *ampR* that encode resistance to ampicillin, *sul 1* that encode resistance to sulfa drug and gene of unknown function. It was observed from this study that the bacterium identified as an uncultured bacterium clone is the only bacterium from the raw water that carried class 2 integron and it showed the presence of the following gene cassettes *dfrA1* that encodes

Table 4.16: Bacteria carrying class 1 and 2 integron and gene cassettes isolated from Ife water sampling

Bacteria/Strain ID	Phenotypes	Class 1 integron	Gene cassettes (Class 1)/Base pair (bp)	Class 2 integron	Gene cassettes (
			Raw water		
Providencia vermicola (82)	T, S, GEN, CEF, AM, FF, SXT, SU	+	-	-	
Bacillus pumilus (117A)	SU, T, E, RIF, LIN, GEN	+	-	-	
			Treated water		
Alcaligenes sp (87A)	T, S, AM, SXT, SU	+	*dfrA15* and *aadA1* (1000bp)	-	
Proteus mirabilis (122A)	FF, T, S, GEN, K, C, AMC, AM, SU, SXT	+	*aad2* (1000bp)	-	
Bacillus pumilus (122B)	SU, T, E, SXT, RIF, LIN	+	-	-	
			Municipal Tap 1		
Uncultured bacterium clone (92A)	T, GEN, AM, SU	+	-	-	
Chromobacterium violaceum (129)	FF, S, GEN, K, CEF, AMC, SU	+	-	-	

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT

4.17: Bacteria carrying class 1 and 2 integron and gene cassettes isolated from Ede water sampling

/Strain ID	Phenotypes	Class 1 integron	Gene cassettes (Class 1)/Base pair (bp)	Class 2 integron	Gene cassettes (class 2)/Base pair (bp)	QUA
			Raw water			
obacterium violaceum (382)	S,GEN, CEF, AM, SXT, AMC, SU	+	-	-	-	
			Treated water			
nella sp (U)	T, S, AM, SXT, SU	+	*dfr7* (900bp)	-	-	
			Municipal Tap 1			
obacter baumannii (109)	FF, S, C, AM, SXT, AMC, CEF, SU	+	-	-	-	

s: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

egative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); thoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

ositive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

d 2 integrons and gene cassettes isolated from Asejire water sampling

notypes	Class 1 integron	Gene cassettes (Class 1)/ Base pair (bp)	Class 2 integron	Gene cassettes (class 2)/B
		Raw water		
GEN, K, C, N, CEF, AM, SXT, AMC, SU	+	-	+	*dfrA1, Sat 1, aadA1,*
EF, AM, SXT, SU	+	*aadA1* (1000bp)	-	
, GEN, K, SXT, N, SU	+	*dfr15, aadA2, qacEΔDelta 1, Sul 1, qnrA1, ampR* and unknown gene (1000bp)	-	
, N, SXT, SU	+	*aad1* (1000bp)	-	
		Final water		
T, S, G, K, C, CEF, AM, SXT, AMC, SU	+	*aadA2*	-	
		Municipal Tap 1		
, GEN, K, N, CEF, AM, SXT, AMC, SU	+	-	+	*dfrA1*,u (3

te: Only isolates that gave a positive signal in at least one PCR experiment were included in this table

r (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); avulanic Acid (AMC)

picillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

trimethoprim resistance, *sat 1* that encodes resistance to streptothricin, *aadA1* that encodes resistance to aminoglycoside and gene of unknown function. *Pseudomonas spp* (strain ID 6A) and an uncultured bacterium clone (strain ID 41) from treated and municipal tap 1 respectively were the only bacteria carrying class 1 integron while the formal showed the presence of *aadA2* gene that encodes aminoglycoside resistance. It was observed that the later bacterium from the the first municipal tap sampled showed the presence of class 2 integron with *dfrA1* gene cassettes and a gene cassette with unknown function.

Bacteria carrying integrons and gene cassettes from selected water samples of Dam 4 (Eleyele) are shown in Table 4.19. It was observed in the table that two bacteria from the genus *Aeromonas*, two from genus *Alcaligenes* and two from genus *klebsiella* all from the raw water carried class 1 integron while only the two *Alcaligenes* showed the absence of gene cassettes. *Proteus vulgaris* (strain ID 46) is the only bacteria from the treated water that carried class 1 integron while the bacteria did not show the presence of gene cassettes. *Alcaligene spp* from Municipal Tap 1 was also the only bacterium that had the presence of class 1 integron and it showed the presence of *aadA1* gene cassette that codes for aminoglycoside resistance. The results of integron and gene cassettes carrying bacteria from water distribution systems of Owena-Ondo dam are shown in Table 4.20. It was observed that one bacterium from the raw water carried class 1 integron. The bacterim was identified by 16S rDNA sequencing as *Leucobacter komagatae* with strain ID 230B. PCR amplification and sequencing of the variable region of the bacterium showed the presence of *dfr 15* and *aad2* gene cassettes that code for trimethoprim and aminoglycoside respectively. From the treated water of this dam, two *Alcaligene sp* with strain ID 198 and 197 respectively showed the presence of class 1 integron and *aadA1* gene cassettes respectively. *Proteus mirabilis* from this same sites showed the presence of class 2 integron and the gene cassettes *aadA1, sat 2* and *dfrA1*. The presence of class 2 integron was also detected in *Proteus mirabilis* with strain ID 273. In the second water distribution tap sampled four bacteria isolates which included *Pseudomonas putida* (strain ID 251B), *Providencia rettgeri* (strain ID 209), *Alcaligenes feacalis* (strain ID 253A) and *Providencia rettgeri* (strain ID 253B1) showed the presence of class 1 integron. Class 2 integron was also observed in *Providencia rettgeri* (strain ID 253B1) while the following gene cassettes were observed in the bacteria after amplification and sequencing of it class 2 gene cassettes: *dfrA, Sat 2, aadB, cat B2, dfr A1,* bla_{CARB-4}*, aadA1* and unknown gene.

The results of bacteria from Owena-Idanre dam carrying integron and gene cassettes are shown in Table 4.21. It was observed that two bacteria from the genus of *Alcaligenes*, two bacteria from the genus of *Pseudomonas* and one from *Bacillus* all from the raw water tested positive to class 1 integron. It was also observed that only one bacterium identified as *Proteus vulgaris* (strain ID 257B) from the treated water carried only class 2 integron. At the municipal tap 1 three bacteria identified as *Alcaligens sp, Alcaligenes feacalis* and *Pseudomonas sp* tested positive for the presence of class 1 integron and they also showed the presence of gene cassettes. It was observed from this study that three bacteria also identified as *Alcaligens sp, Alcaligenes feacalis* and *Pseudomonas sp* from the second municipal tap sampled showed the presence of class 1 integrons.

Table 4.19: Bacteria carrying class 1 and 2 integrons and gene cassettes isolated from Eleyele water sampling

Bacteria/Strain ID	Phenotypes	Class 1 integron	Gene cassettes (Class 1)/ Base pair (bp)	Class 2 integro
			Raw water	
Aeromonas caviae 376	T, S, AM, SXT, N, AMC, SU	+	*aadA1* and *dfrA1* (2000bp)	-
Aeromonas hydrophila 391A	S, K, N, SU	+	+	-
Alcaligenes sp 3A	T, S, GEN, K, N, CEF, AM, SXT, AMC, SU	+	-	-
Klebsiella pneumoniae 378	S, CEF, AM, SXT, AMC, SU	+	*dfrA15* and *aadA1*(2000bp)	-
Klebsiella pneumoniae 375	T, K, N, AM, SU	+	*dfrA15* and *aadA1*(2000bp)	-
Alcaligenes sp 50	S, CEF, AM, SXT, AMC, SU	+	-	-
			Treated water	
Proteus vulgaris 46	FF, T, S, GEN, K, C, AM, SXT, N, AMC, SU	+	-	-
			Municipal Tap 1	
Alcaligenes sp 19B1	T, S, K, AM, SXT, AMC, SU	+	*aadA1*(1000bp)	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (S

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP

Table 4.20: Bacteria carrying class 1 and class 2 integrons and gene cassettes isolated from Owena-Ondo water sampling

Bacteria/Strain ID	Phenotypes	Class 1 integron	Gene cassettes (Class 1)/ Base pair (bp)	Class 2 integron	Gene cassettes (class 2)/ Base pair (bp)
			Raw water		
Leucobacter komagatae 230B	SU, AM, SXT, GEN	+	*dfr15, aad2*	-	-
			Treated water		
Alcaligenes sp 198	T, S, K, AM, SXT, SU	+	*aadA1*	-	-
Alcaligenes faecalis 197	T, S, K, CEF, AM, SXT, SU	+	*aadA1*	-	-
Proteus mirabilis 201	S, CEF, AM, SU, SXT	-	-	+	*aadA1, Sat 2, dfrA1* (3000bp)
			Municipal Tap 1		
Proteus mirabilis 273	T, S, SXT, SU	-	-	+	+

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT

Table 4.20 (cont'd): Bacteria carrying class 1 and class 2 integrons isolated from Owena-Ondo water sampling

Bacteria/Strain ID	Phenotypes	Class 1 integron	Gene cassettes (Class 1)/ Base pair (bp)	Class 2 integron	Gene cassettes (class 2)/ Base pair (bp)	QUAC SUL1
			Municipal Tap 2			
Pseudomonas putida 251B	T, S, C, AM, SXT, SU	+	+	-	-	-
Providencia rettgeri 209	T, S, C, N, CEF, AM, SXT, AMC, SU	+	*dfrA, Orfc* of dihydrofolate reductase & unknown function Gene (1500bp)	-	-	-
Alcaligenes faecalis 253A	T, S, GEN, K, C, AM, SXT, SU	+	*aadA1* (1000bp)	-	-	-
Providencia rettgeri 253B1	S, SXT, AMC, SU. AM	+	-	+	*dfrA, Sat 2, aadB, Cat B2, dfrA1,* bla_{CARB-4}*,aadA1*, unknown gene (2000bp)	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T126); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.21: Bacteria carrying class 1 and 2 integrons and gene cassettes isolated from Owena-Idanre water sampling

Bacteria/Strain ID	Phenotypes	Class 1 integron	Gene cassettes (Class 1)/ Base pair (bp)	Class 2 Integron	Gene cassettes (class 2) Base pair (bp)	QUAC SUL1
			Raw water			
Alcaligenes faecalis (173B)	T, S, CEF, SXT, AMC, SU	+	*aadA1* (1000bp)	-	-	-
Alcaligenes sp (174A)	T, S, G, K, C, N, CEF, AM, SXT, AMC, SU	+	+	-	-	-
Pseudomonas sp (343B)	FF, T, S, N, CEF, AM, AMC, SU	+	+	-	-	-
Pseudomonas sp (175B)	T, S, G, K, CEF, SXT, AMC, SU	+	+	-	-	+
Bacillus thuringiensis (265)	SU, AM, T, SXT, RIF, LIN, CIP, GEN	+	*dfr15*, *aad2* (4000bp)	-	-	-
			Treated water			
Proteus vulgaris (257B)	S, AM, SXT, CEF, SU	-	-	+	*dhfr 1*, *Sat 1*. *aadA1* (2000bp)	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.21 (Cont'd): Bacteria carrying class 1 and 2 integrons and gene cassettes isolated from Owena-Idanre water sampling

Bacteria/Strain ID	Phenotypes	Class 1 integron	Gene cassettes (Class 1)/ Base pair (bp)	Class 2 Integron	Gene cassettes (class 2)/Base pair (bp)	QUAC SUL1
			Municipal Tap 1			
Alcaligenes sp (250B2)	T, S, K, N, CEF, SU	+	*GES-1, aac6, cm1A4,aadA2* (900bp)	-	-	-
Alcaligenes faecalis (250A2)	T, S, GEN, K, CEF, AM, AMC, SU	+	+	-	-	-
Pseudomonas sp (196)	FF, T, S, GEN, K, AM, AMC, SU	+	+	-	-	-
			Municipal Tap 2			
Alcaligenes faecalis (239)	T, S, K, CEF, AM, SXT, SU	+	*aadA2, Sul 1, bla* $_{CTX\text{-}M\text{-}59}$*, Orf 3, dfr15* (900bp)	-	-	-
Alcaligenes sp (238B)	T, S, G, K, N, CEF, AM, SU	+	*aadB, aac-u, BLApse* (1500bp)	-	-	-
Pseudomonas sp (244B)	FF, T, S, G, K, C, AM, SXT, AMC, SU	+	+	-	-	-

Codes: Present (+), Absent (-) **Note:** Only isolates that gave a positive signal in at least one PCR experiment were included in this table
Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)
Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

4.11. Presence of *sul 1* and *qac Δ 1* gene at the 3' conserve region of class 1 integron

This study revealed the presence of *sul 1* gene coding for sulfa drug resistance and *qac Δ 1* gene coding for resistance to quaternary ammonium compound at the 3' conserved region of class 1 integron in six bacteria. The bacteria were *Alcaligenes sp* (strain ID 87A) from dam 1 treated water, Ife (Table 4.16), *Alcaligene faecalis* (strain ID 45) from dam 3 raw water, Asejire (Table 4.17), two bacteria from the genus *Aeromonas* (strain ID 376 and 391) obtained from raw and treated water respectively of dam 4, Eleyele (Table 4.19), *Alcaligene spp* (strain ID 198) from dam 5 treated water (Table 4.20) and *Pseudomonas spp* (strain ID 175B) from dam 6 raw water (Table 4.21).

4.12. Distribution and percentage occurrence of antibiotic resistant genes within the MDR bacteria isolated from southwestern Nigeria

4.12.1. Distribution and percentages of Tetracycline resistant genes

Six tetracycline resistant genes were tested on 120 tetracycline MDR bacteria from all the sampled points in this study. The genes were *tet A*, *tet B*, *tet E* and *tet 30* that encode for efflux pumps and *tet O* and *tet M* that encode for ribosomal protection protein. Out of the total bacteria tested 23 (19.2%), 8 (6.67%), 5 (4.17%), 4 (3.33%) and 2 (1.67%), carried *tet A*, *tet E*, *tet B*, *tet M* and *tet 30* respectively (Table 4.22). Three (13.04%) of bacteria carrying *tet A* gene were from Owena-Ondo treated water and 2 (8.70) from Ife treated water. It was observed that out of all bacteria carrying *tet E*, 1 (12.5%) each was from Ife raw water and the treated water while 2 (25%) were from each of Owena-Idanre raw water and second municipal distribution tap sampled. The detail is shown inTable 4.22.

4.12.2. Distribution and percentages of sulfa drug resistant genes

Percentages of bacteria carrying the presence of sulfa genes are shown in Table 4.23. Out of 179 sulfamethoxazole resistant bacteria amplified, 44 (24.58%) had *sul 1* while *sul 2* had the highest frequency of 51 (28.49%) among the bacteria. There was no *sul 3* detected among the bacteria. Among bacteria carrying *sul 1* gene the highest was from Eleyele raw water (15.9%) while no bacteria carried this gene at the two water distribution taps sampled at Ife dam and Ede dam. The highest number of bacteria carrying *sul 2* gene were from Owena-Idanre raw water (21.57%) followed by Eleyele raw water (11.76%).

4.12.3. Distribution and percentages of extended beta lactam resistance genes

A total of 172 ampicillin resistant bacteria DNA were analysed for these resistant genes among all the isolates. Bla_{TEM} showed the highest frequency of occurrence having been detected in 59 (34.30%) bacteria (Table 4.24). The lowest occurrence of extended beta lactamase resistant gene

was Bla_{CTX} found in 3 (1.74%) bacteria. It was observed that the highest bla_{TEM} gene 10 (16.95%) gene was found in bacteria from Eleyele raw water while the highest bla_{SHV} gene 3 (11.54%) was found in each of the bacteria from Eleyele raw water and raw and second municipal water distribution tap sampled from Owena-Idanre dam.

Table 4.22: Occurrence of tetracycline resistant genes out of 120 tetracycline resistant bacteria from water distribution systems in

southwestern Nigeria

Dam 1(Ife)			Dam 2 (Ede)			Dam 3 (Asejire)			Dam 4 (Eleyele)			Dam 5 (Owena-Ondo)			Dam 6 (Owena-Idanre)		
Tet A																	
	A	B		A	B		A	B		A	B		A	B		A	B
Rw	1	4.35	Rw	0	0	Rw	1	4.35	Rw	0	0	Rw	0	0	Rw	3	13.04
Fw	2	8.70	Fw	2	8.70	Fw	1	4.35	Fw	0	0	Fw	3	13.04	Fw	0	0
M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	1	4.35	M 1	2	8.70
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	4	17.39	M 2	3	13.04
(Total bacteria carrying *tet A*= 23, Total= 19.2%)																	
Tet B																	
	A	B		A	B		A	B		A	B		A	B		A	B
Rw	0	0	Rw	1	20	Rw	2	40	Rw	0	0	Rw	0	0	Rw	0	0
Fw	0	0	Fw	0	0	Fw	0	0	Fw	1	20	Fw	0	0	Fw	0	0
M 1	0	0	M 1	0	0	M 1	1	20	M 1	0	0	M 1	0	0	M 1	0	0
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0
(Total bacteria carrying *tet B*=5 , Percentage of Total: 4.17)																	
Tet 30																	
	A	B		A	B		A	B		A	B		A	B		A	B
Rw	0	0	Rw	0	0	Rw	0	0	Rw	0	0	Rw	0	0	Rw	0	0
Fw	0	0	Fw	1	50	Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0
M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	1	50
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0
(Total bacteria carrying *tet 30*=2 , Percentage of Total: 1.67)																	

Code: A=No of bacteria from total gene, B= Percentage of bacteria carrying the gene, Rw= Raw water, Fw= Treated water, M 1= Municipal 1 tap, M 2= Municipal 2 tap

Table 4.22 (Cont'd): Occurrence of tetracycline resistant genes out of 120 tetracycline resistant bacteria from water distribution systems in southwestern Nigeria

Dam 1(Ife)			Dam 2 (Ede)			Dam 3 (Asejire)			Dam 4 (Eleyele)			Dam 5 (Owena-Ondo)			Dam 6 (Owena-Idanre)		
Tet M																	
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	0	0	Rw	0	0	Rw	0	0	Rw	1	25	Rw	0	0	Rw	2	50
Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0
M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	1	25	M 2	0	0
(Total bacteria carrying *tet M*= 4 , Total Percentage= 3.33)																	
Tet E																	
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	1	12.5	Rw	0	0	Rw	0	0	Rw	0	0	Rw	0	0	Rw	2	25
Fw	1	12.5	Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0
M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	2	25
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	2	25
(Total bacteria carrying *tet E*= 8 , Total Percentage= 6.67)																	

Code: A=No of bacteria from total gene, B= Percentage of bacteria carring the gene, Rw= Raw water, Fw= Treated water, M 1= Municipal 1 tap, M 2= Municipal 2 tap

Table 4.23: Occurrence of sulfa drug resistant genes out of 179 sulfamethoxazole resistant bacteria from water distribution systems in southwestern Nigeria

Dam 1(Ife)			Dam 2 (Ede)			Dam 3 (Asejire)			Dam 4 (Eleyele)			Dam 5 (Owena-Ondo)			Dam 6 (Owena-Idanre)		
									Sul 1								
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	3	6.81	Rw	1	2.27	Rw	4	9.09	Rw	7	15.90	Rw	1	2.27	Rw	5	11.36

Fw	3	6.81	Fw	2	4.54	Fw	2	4.54	Fw	1	2.27	Fw	4	9.09	Fw	0	0
M 1	0	0	M 1	0	0	M 1	1	2.27	M 1	1	2.27	M 2	2	4.54	M 1	3	6.81
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 3	1	2.27	M 2	3	6.81

(Total bacteria carrying *sul 1*= 44 , Total Percentage= 24.58%)

Sul 2

	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	1	1.96	Rw	3	5.88	Rw	4	7.84	Rw	6	11.76	Rw	2	3.92	Rw	11	21.57
Fw	1	1.96	Fw	1	1.96	Fw	4	7.84	Fw	1	1.96	Fw	2	3.92	Fw	1	1.96
M 1	0	0	M 1	1	1.96	M 1	1	1.96	M 1	1	1.96	M 1	3	5.88	M 1	2	3.92
M 2	0	0	M 2	0	0	M 2	1	1.96	M 2	0	0	M 2	3	5.88	M 2	2	3.92

(Total bacteria carrying *Sul 2*= 51 , Total Percentage= 28.49)

Code: A=No of bacteria from total gene, B= Percentage of bacteria carrying the gene, Rw= Raw water, Fw= Treated water, M 1= Municipal 1 tap, M 2= Municipal 2 tap

Table 4.24: Occurrence of Extended beta lactam resistant genes out of 172 ampicillin resistant bacteria from water distribution systems in southwestern Nigeria

Dam 1(Ife)			Dam 2 (Ede)			Dam 3 (Asejire)			Dam 4 (Eleyele)			Dam 5 (Owena-Ondo)			Dam 6 (Owena-Idanre)		
									Bla$_{TEM}$								
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	1	1.69	Rw	4	6.78	Rw	4	6.78	Rw	10	16.95	Rw	0	0	Rw	7	11.86
Fw	4	6.78	Fw	2	3.39	Fw	4	6.78	Fw	1	1.69	Fw	1	1.69	Fw	1	1.69
M 1	1	1.69	M 1	3	5.08	M 1	3	5.08	M 1	0	0	M 1	3	5.08	M 1	4	6.78
M 2	0	0	M 2	1	1.69	M 2	1	1.69	M 2	0	0	M 2	4	6.78	M 2	1	1.69
(Total bacteria carrying *Bla$_{TEM}$* = 59, Total Percentage= 34.30%)																	
									Bla$_{SHV}$								
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	0	0	Rw	2	7.69	Rw	1	3.85	Rw	3	11.54	Rw	0	0	Rw	3	11.54
Fw	2	7.69	Fw	0	0	Fw	2	7.69	Fw	0	0	Fw	1	3.85	Fw	1	3.85
M 1	1	3.85	M 1	1	3.85	M 1	0	0	M 1	0	0	M 1	1	3.85	M 1	2	7.69
M 2	0	0	M 2	0	0	M 2	2	7.69	M 2	0	0	M 2	1	3.85	M 2	3	11.54
(Total bacteria carrying *Bla$_{SHV}$* = 26, Total Percentage= 15.11)																	
									Bla$_{CTX}$								
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	0	0	Rw	0	0	Rw	0	0	Rw	0	0	Rw	0	0	Rw	3	100
Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0
M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0
(Total bacteria carrying *Bla$_{CTX}$* = 3, Total Percentage= 1.74)																	

Code: A=No of bacteria from total gene, B= Percentage gene, Rw= Raw water, Fw= Treated water, M 1= Municipal 1 tap, M 2= Municipal 2 tap

4.12.4. Distribution and percentages of streptomycin resistant genes

In this study, 101 streptomycin resistant bacteria were examined (Table 4.25). *Ant (3'')^{b}* was the highest occurring gene. It was found in 56 (55.45%) bacteria followed by aph (3'') which is found in 39 (38.61%) bacteria while 16 (15.84%) bacteria carried *aph (6'')^{1dd}* gene. The highest *ant (3'')^{b}* gene was found among bacteria from Eleyele raw water (12.5%) while that of *aph (3'')^{c}* was found among bacteria from Owena-Idanre raw water (23.08%).

4.12.5. Distribution and percentages of class 1 and class 2 integrons

From this study, it was observed that the highest occurring integron among the 191 bacteria tested was class 1 integron (Table 4.26). It was detected in 42 (21.99%) bacteria while class 2 was detected in 6 (3.14%) bacteria. Among class 1 carrying bacteria the highest (14.28%) was detected in Eleyele raw water followed by Owena-Idanre raw water (11.90%)

4.13. Occurrence of plasmid in bacteria from selected dams in southwestern Nigeria

It was observed in Table 4.27 that 4 (10%) bacteria identified as *E. coli* with strain ID (319A), *Alcaligenes sp* with strain ID 87A, *Pseudomonas sp* with strain ID 89 and *Proteus mirabilis* with strain ID 122A from ife water sample has at least one plasmid each ranging from 28kb to 95kb. It was also found that 6 (10.7%) bacteria from Ede water samples possess at least one plasmid each ranging from 28kb to 95kb. From Asejire water samples 3 (9.1%) bacteria isolates showed the presence of at least one plasmid while *Proteus mirabilis* (strain ID 18B2) showed the presence of two plasmids 130Kb and 38Kb respectively. It was also observed that 2 (8.7%) bacteria from Eleyele water samples carried the presence of at least one plasmid. The bacteria were both identified as *Proteus vulgaris* (strain ID 33B and 46). From Dam 5 (Owena-Ondo) water samples, 10 (13.9%) of the total bacteria showend the presence of at least one plasmid while it was observed that 9 (14.8) bacteria from Owena-Idanre showed the presence of at least one plasmid. *Alcaligenes sp* (strain ID 250B2) from Owena-Idanre municipal tap 2 showed the presence of two plasmid with size of 120kb and 22kb respectively.

Table 4.25: Occurrence of streptomycin resistant genes out of 101 streptomycin resistant bacteria from water distribution systems in southwestern Nigeria

Dam 1(Ife)			Dam 2 (Ede)			Dam 3 (Asejire)			Dam 4 (Eleyele)			Dam 5 (Owena-Ondo)			Dam 6 (Owena-Idanre)		
								ant(3'')^{b}									
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	3	5.36	Rw	3	5.36	Rw	2	3.57	Rw	7	12.5	Rw	0	0	Rw	5	8.93
Fw	4	7.14	Fw	0	0	Fw	2	3.57	Fw	1	1.79	Fw	5	8.93	Fw	0	0
M 1	3	5.36	M 1	4	7.14	M 1	2	3.57	M 1	1	1.79	M 1	4	7.14	M 1	3	5.36
M 2	0	0	M 2	1	1.79	M 2	0	0	M 2	0	0	M 2	3	5.36	M 2	3	5.36
(Total bacteria carrying *ant(3'')^{b}*= 56, Total Percentage= 55.45)																	
								Aph(3'')^{c}									
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	1	2.56	Rw	3	7.69	Rw	2	5.12	Rw	3	7.69	Rw	1	2.56	Rw	9	23.08
Fw	3	7.69	Fw	1	2.56	Fw	3	7.69	Fw	1	2.56	Fw	0	0	Fw	0	0
M 1	1	2.56	M 1	1	2.56	M 1	2	5.12	M 1	0	0	M 1	2	5.12	M 1	2	5.12
M 2	0	0	M 2	0	0	M 2	1	2.56	M 2	0	0	M 2	1	2.56	M 2	2	5.12
(Total bacteria carrying *aph(3'')^{c}*= 39, Total Percentage= 38.61)																	
								Aph(6'')^{1dd}									
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	1	6.25	Rw	2	12.5	Rw	1	6.25	Rw	1	6.25	Rw	0	0	Rw	4	25
Fw	2	12.5	Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0
M 1	0	0	M 1	0	0	M 1	0	0	M 1	0	0	M 1	2	12.5	M 1	1	6.25
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	1	6.25	M 2	1	6.25
(Total bacteria carrying *aph(6'')^{1dd}*= 16 , Total Percentage= 15.84)																	

Code: A=No of bacteria from total gene, B= Percentage gene, Rw= Raw water, Fw= Treated water, M 1= Municipal 1 tap, M 2= Municipal 2 tap

Table 4.26: Occurrence of class 1 and class 2 integrase genes out of 191 MDR bacteria from water distribution systems in southwestern Nigeria

Dam 1(Ife)			Dam 2 (Ede)			Dam 3 (Asejire)			Dam 4 (Eleyele)			Dam 5 (Owena-Ondo)			Dam 6 (Owena-Idanre)		
Class 1 integron																	
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	2	4.76	Rw	1	2.38	Rw	4	9.52	Rw	6	14.28	Rw	1	2.38	Rw	5	11.90
Fw	3	7.14	Fw	1	2.38	Fw	1	2.38	Fw	1	2.38	Fw	2	4.76	Fw	0	0
M 1	2	4.76	M 1	1	2.38	M 1	1	2.38	M 1	1	2.38	M 1	0	0	M 1	4	9.52
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	4	9.52	M 2	3	7.14
(Total bacteria carrying class 1 integrase gene= 42, Total Percentage= 21.99)																	
Class 2 integron																	
	A	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**		**A**	**B**
Rw	0	0	Rw	0	0	Rw	1	16.67	Rw	0	0	Rw	0	0	Rw	0	0
Fw	0	0	Fw	0	0	Fw	0	0	Fw	0	0	Fw	1	16.67	Fw	1	16.67
M 1	0	0	M 1	0	0	M 1	1	16.67	M 1	0	0	M 1	1	16.67	M 1	0	0
M 2	0	0	M 2	0	0	M 2	0	0	M 2	0	0	M 2	1	16.67	M 2	0	0
(Total bacteria carrying class 2 integrase gene= 6, Total Percentage= 3.14)																	

Code: A=No of bacteria from total gene, B= Percentage gene, Rw= Raw water, Fw= Treated water, M 1= Municipal 1 tap, M 2= Municipal 2 tap

Table 4.27: Plasmid carrying bacteria isolated from selected water samples from southwestern Nigeria

Source	Bacteria/Strain ID	Resistant Phenotypes	No of Plasmid and Size
		DAM 1 (IFE)	
DAM 1 IRW	*Escherichia coli* (319A)	T,S, C, N, SXT, SU	1 (95kb)
DAM 1 IFW	*Alcaligenes sp* (87A)	T,S, AM, SXT, SU	1 (28kb)
DAM 1 IFW	*Pseudomonas sp* (89)	T, S, G, K, C, AM, SXT, AMC, SU	1 (95kb)
DAM 1 IFW	*Proteus mirabilis* (122A)	FF, T, S, G, K, C, AMC, AM, SU, SXT	1 (28kb)
		DAM 2 (EDE)	
DAM 2 EDRW	*Chromobacterium violaceum* (382)	S, G, CEF, AM, SXT, AMC, SU	1 (95kb)
DAM 2 EDFW	*Morganella sp* (U)	T, S, AM, SXT, SU	1 (28kb)
DAM 2 EDFW	*Alcaligenes faecalis* (28A)	T, S, G, K, N, CEF, AM, SXT, SU	1 (28kb)
DAM 2 EDM1	*Pseudomonas sp* (306A)	FF, CEF, AM, AMC, SU	1 (95)
DAM 2 EDM1	*Klebsiella pneumoniae* (378)	S, CEF, AM, SXT, AMC, SU	1 (95)
DAM 2 EDM2	*Bacillus sp.* (110)	SU, AM, SXT, LIN	1 (120kb)
		DAM 3 (ASEJIRE)	
DAM 3 ARW	*Proteus mirabilis* (18B2)	T, S, N, SXT, SU	2 (130kb and 38kb)
DAM 3 AFW	*Pseudomonas sp* (6A)	FF, T, S, G, K, C, CEF, AM, SXT, AMC, SU	1 (95kb)
DAM 3 AFW	*Pseudomonas sp* (6)	FF, T, S, G, K, C, CEF, AM, SXT, AMC, SU	1 (95kb)
		DAM 4 (ELEYELE)	
DAM 4 ERW	*Proteus vulgaris* (33B)	T, C, CEF, AM	2 (120kb and 55kb)
DAM 4 EFW	*Proteus vulgaris* (46)	FF, T, S, G, K, C, AM, SXT, N, AMC, SU	1 (55kb)

Codes: IRW= Ife Raw water; IFW= Ife Treated Water; EDFW= Ede Treated Water; EDM1= Ede Municipal 1; Ede M2= Ede Municipal 2; ARW= Asejire Raw water; AFW= Asejire Treated water; ERW= Eleyele Raw water; EFW= Eleyele Treated water; OWODFW= Owena-Ondo Treated water

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table 4.27 (Cont'd): Plasmid carrying bacteria isolated from selected water samples from southwestern Nigeria

DAM 5 (OWENA-ONDO)			
DAM 5 OWODFW	*Alcaligenes faecalis* (197)	T, S, K, CEF, AM, SXT, SU	1 (55kb)
DAM 5 OWODFW	*Alcaligenes sp*. (198)	T, S, K, AM, SXT, SU	1 (28kb)
DAM 5 OWODFW	*Morganella morganii* (199)	S, CEF, AM, AMC, SU	1 (55kb)
DAM 5 OWODFW	*Bacillus sp* (202B)	SU, AM, T, SXT, GEN	1 (95kb)
DAM 5 OWODM1	*Morganella morganii* (215A)	T, S, CEF, AM, SXT, AMC, SU	2 (120kb and 28kb)
DAM 5 OWODM2	*Escherichia coli* (210A)	T, AM, SXT, AMC, SU	1 (55kb)
DAM 5 OWODM2	*Bacillus cereus* (245B)	SU, AM, SXT, LIN	1 (95kb)
DAM 5 OWODM2	*Alcaligenes faecalis* (253A)	T, S, G, K, C, AM, SXT, SU	1 (120kb)
DAM 5 OWODM2	*Pseudomonas putida* (251B)	T, S, C, AM, SXT, SU	1 (22kb)
DAM 5 OWODM2	*Escherichia coli* (210B)	T, AM, AMC, SU	1 (95kb)
DAM 6 (OWENA-IDANRE)			
DAM 6 OWIRW	*Pseudomonas sp* (175B)	T, S, G, K, CEF, SXT, AMC, SU	1 (55kb)
DAM 6 OWIRW	*Alcaligenes faecalis* (173B)	T, S, CEF, SXT, AMC, SU	1 (55kb)
DAM 6 OWIRW	*Pseudomonas sp* (342A)	FF, CEF, AM, AMC, CEF, SU	1 (130kb)
DAM 6 OWIRW	*Pseudomonas sp* (343B)	FF, T, S, N, CEF, AM, AMC, SU	1 (130kb)

Codes: OWODFW= Owena-Ondo treated Water, OWODM1= Owena-Ondo Municipal 1, OWODM2= Owena-Ondo municipal 2 Tap, OWIRW= Owena-Idanre Raw water

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

Table (30) (Cont'd): Plasmid carrying bacteria isolated from selected water samples from southwestern Nigeria

DAM 6 (OWENA-IDANRE)			
DAM 6 OWIM1	*Pseudomonas sp* (196)	FF, T, S, G, K, AM, AMC, SU	1 (120kb)
DAM 6 OWIM1	*Alcaligenes sp* (250B2)	T, S, K, N, CEF, SU	2 (120kb and 22kb))
DAM 6 OWIM2	*Alcaligenes sp* (238B)	T, S, G, K, N, CEF, AM, SU	1 (55kb)
DAM 6 OWIM2	*Pseudomonas sp* (244B)	FF, T, S, G, K, C, AM, SXT, AMC, SU	1 (130kb)
DAM 6 OWIM2	*Alcaligenes faecalis* (239)	T, S, K, CEF, AM, SXT, SU	1 (95kb)

Codes: OWODM1= Owena-Ondo Municipal 1; OWODM2= Owena-Ondo Municipal 2; OWIRW= Owena-Idanre Raw water; OWIM1= Owena-Idanre Municipal 1; OWIM2= Owena-Idanre Municipal 2

Gram negative Antibiotics: Ampicillin (AM); Ceftiofur (CEF); Chloramphenicol and Florfenicol (FF); Kanamycin (K), Streptomycin (S) and Gentamycin (GEN); Tetracycline (T); Nalidixic Acid (N); Sulfamethoxazole (SU); Sufamethoxazole/ Trimethoprim (SXT); Amoxicillin/Clavulanic Acid (AMC)

Gram positive Antibiotics: Sufamethoxazole (SU); Ampicillin (AM); Tetracycline (T); Gentamycin (GEN); Erythromycin; Riframprim (RIF); Lincomycin (LIN); Ciprofloxacin (CIP), Sulfamethoxazole/Trimethoprin (SXT)

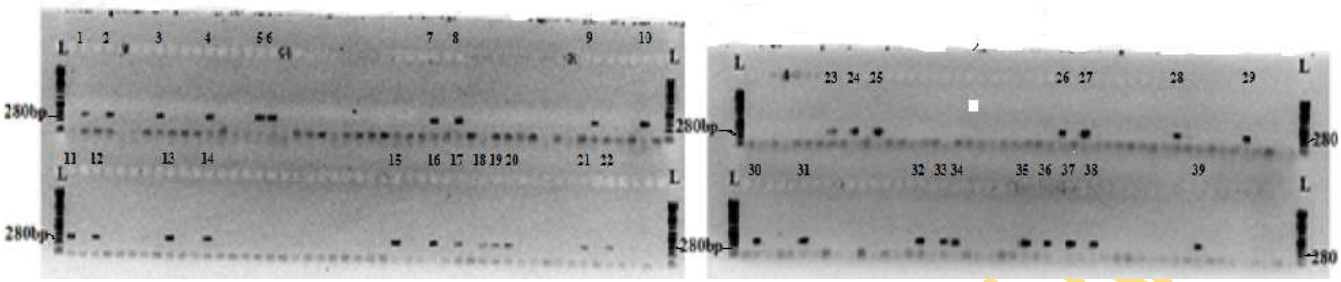


Plate 4.2: The 280bp product of *int* 1 resistance genes of class 1 integron from bacteria from water samples from southwestern Nigeria

Lane: L, 1kb ladder, **Bacteria from Ife (Dam 1)**: 1: *Providencia vermicola* (82), 2: *Bacillus pumilus* (117A), 3: *Alcaligenes sp* (87A), 4: *Proteus mirabilis* (122A), 5: *Bacillus pumilus* (122B), 6: Uncultured bacterium clone (92A), 7: *Chromobacterium violaceum* (128), 8: **Bacteria from Ede (Dam 2)** *Chromobacterium violaceum* (382), 9: *Morganella sp* (U), 10: *Acinetobacter baumannii* (109), 11: **Bacteria from Asejire (Dam 3)** *Alcaligenes faecalis* (45), 12: *Leucobacter komagatae* (26B), 13: *Proteus mirabilis* (18B2), 14: *Pseudomonas sp* (6A), 15: Uncultured bacterium clone (41), 16: **Bacteria from Eleyele (Dam 4)** *Aeromonas caviae* (376), 17: *Aeromonas hydrophila* (391A), 19: *Alcaligenes sp* (3A), 20: *Klebsiella pneumoniae* (378), 21: *Klebsiella pneumoniae* (375), 22: *Alcaligenes sp* (50), 23: *Proteus vulgaris* (46) , 24: *Alcaligenes sp* (19B1), 25: **Bacteria from Owena-Ondo (Dam 5)** *Leucobacter komagatae* (230B), 26: *Alcaligenes sp* (198), 27: *Alcaligenes faecalis* (197), 28: *Proteus mirabilis* (201), 29: *Proteus mirabilis* (273), 30: *Pseudomonas putida* (251B), 31: *Providencia rettgeri* (209), 32: *Alcaligenes faecalis* (253A), 33: *Providencia rettgeri* (253B1), 34: **Bacteria from Owena-Idanre (Dam 6)** *Alcaligenes faecalis* (173B), 35: *Alcaligenes sp* (174A), 36: *Pseudomonas sp* (343B), 37: *Pseudomonas sp* (175B), 38; *Bacillus thuringiensis* (265), 39: *Proteus vulgaris* (257B),

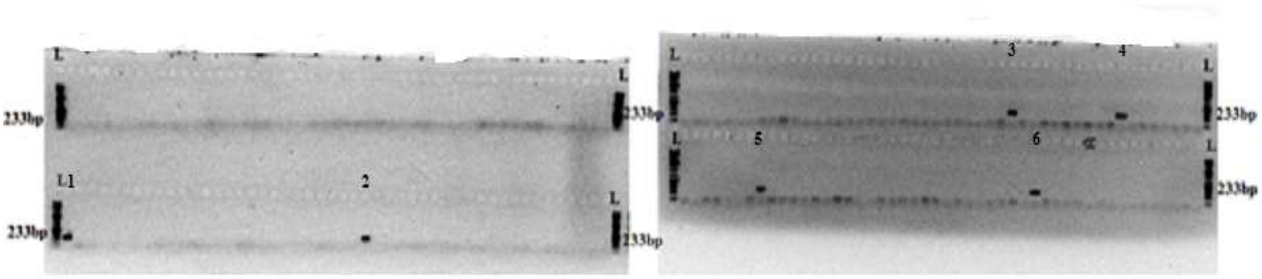


Plate 4.3: The 233bp product of *int* 2 resistance genes of class 2 integron from bacteria from water samples from southwestern Nigeria

Lane: L: 1kb ladder, **Bacteria from Arejire (Dam 3):** 1: Uncultured bacterium clone (18B1), 2: Uncultured bacterium clone (41), **Bacteria from Owena-Ondo (Dam 5),** 3: *Proteus mirabilis* (201), 4: *Proteus mirabilis* (273), 5: *Providencia rettgeri* (253B1), **Bacterium from Owena- Idanre (Dam 6)**, *Proteus vulgaris* (257B)

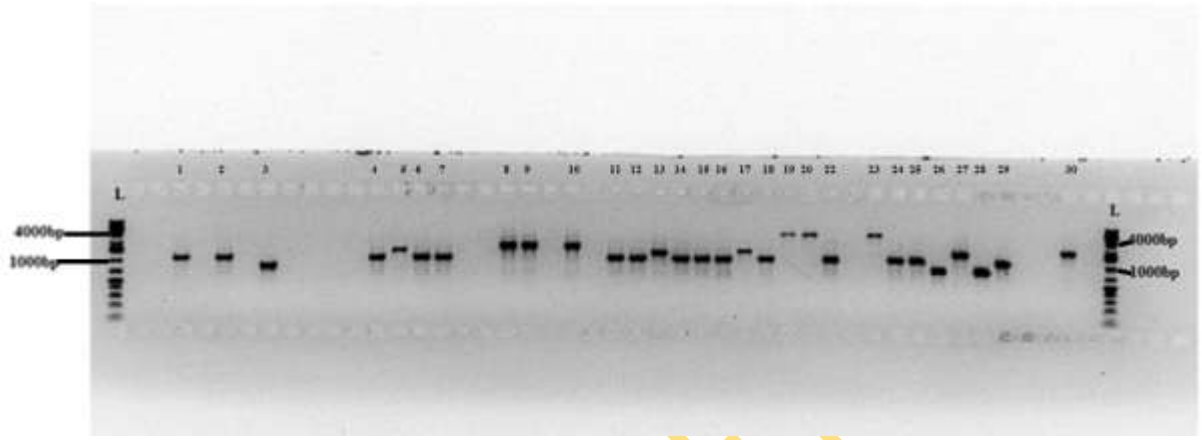


Plate 4.4: The variable regions of class 1 integrons showing gene cassettes of variable DNA base pair sizes of bacteria from water samples from southwestern Nigeria

Lane: L: 1kb ladder, **Bacteria from Ife (Dam 1):** 1**:** *Alcaligenes* spp (87A), 2: *Proteus mirabilis* (122A), **Bacteria from Ede (Dam 2),** 3: *Morganelle* spp (U), **Bacteria from Asejire (Dam 3),** 4: *Alcaligenes feacalis* (45), 5: *Leucobacter Komagatae* (26B), 6: *Proteus mirabilis* (18B2), 7: *Pseudomonas* spp (6A), **Bacteria from Eleyeye (Dam 4),** 8: *Klebsiella pneumoniae* (378), 9: *Klebsiella pneumoniae* (375), 10: *Aeromonas caviae* (376), 11: *Alcaligenes* spp (19B1), **Bacteria from Owena-Ondo (Dam 5),** 12: *Alcaligenes faecalis* (253A), 13: *Providencia rettgeri* (209), 14: *Pseudomonas putida* (251B), 15: *Alcaligenes faecalis* (197), 16: *Alcaligene* spp (198), 17: *Leucobacter Komagatae* (230B), 29: *Providencia rettgeri* (253B1), **Bacteria from Owena-Idanre (Dam 6),** 18: *Pseudomonas* spp (343B), 19: *Alcaligenes* sp (174A), 20: *Bacillus thuringiensis* (265), 21: *Pseudomonas* spp (175B), 22: *Alcaligenes faecalis* (250A2), 23: *Pseudomonas* spp (196), 24: *Pseudomonas* spp (244B), 25: *Alcaligenes faecalis* (239), 26: *Alcaligenes* sp (238B), 27: *Alcaligenes* spp (250B2), 29: *Alcaligene faecalis* (173B), *Alcaligenes* sp (174A)

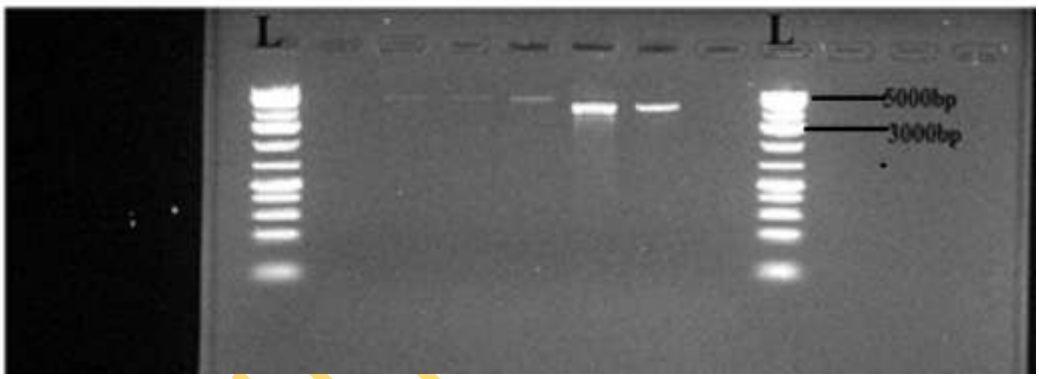


Plate 4.5: The variable regions of class 2 integrons showing gene cassettes of variable DNA base pair sizes of bacteria from water samples from southwestern Nigeria

Lane: L: 1kb ladder, **Bacteria from Arejire (Dam 3):** 1: Uncultured bacterium clone (18B1), 2: Uncultured bacterium clone (41), **Bacteria from Owena-Ondo (Dam 5),** 3: *Proteus mirabilis* (201), 4: *Proteus mirabilis* (273), 5: *Providencia rettgeri* (253B1), **Bacterium from Owena- Idanre (Dam 6)**, *Proteus vulgaris* (257B)

4.14. Bacteria with ability to transfer tetracycline resistant genes by conjugation

In this study, 17 bacteria carrying *Tet A* resistant gene were subjected to a conjugative with *E.coli* strain DH5α and it was discovered that 5 (29.4%) of the bacteria were able to transfer the *tet A* gene to the recipient bacteria. The bacteria were identified as three *Alcaligenes feacalis* with strain ID 197, 198 and 173B. Two of the *Alcaligenes* were obtained from Owena-ondo treated water while one was found from Owena-Idanre raw water. Two *E. coli* with strain ID 210A and 210B both from Owena-ondo Municipal 2 were also observed to transfer their *tet A* resistant gene to the recipient cell (E.coli strain DH5α).

It was also observed that *Proteus vulgaris* with strain ID 46 from Eleyele raw water sample was able to transfer its *tet B* resistant gene to the recipient bacteria. *Alcaligenes faecalis* with strain ID 28A from Ede raw water sample was also able to transfer its *tet 30* gene to the recipient cell. The ability to transfer the resistant plasmid to the recipient bacteria was confirmed by PCR amplification of the recipient cell DNA.

CHAPTER FIVE

Discussion

5.1. Physico-chemical, microbial properties and isolated bacteria of the water samples

5.1.1. Physico-chemical properties

The results of this study showed that the pH of all the raw water samples (Table 4.1) during both sampling periods were between 6.5 and 8.2 which were within WHO permissible standard. It was also observed (Table 4.2) that the pH of the treated water sample from Dam 3 (Asejire), Dam 6 (Owena-Idanre) during the December/January sampling period and June/July sampling period in Dam 5 (Owena-Ondo) were between 5.2 and 5.7 which are below the WHO permissible limit (6.5-8.5). This result was similar to the results of Adefemi *et al.* (2007) who carried out research on water samples from Ureje, Egbe, Ero and Hapaji dams in southwestern Nigeria. Asaolu *et al.* (1997) also obtained similar results in water samples from the coastal regions of Ondo State, southwest of Nigeria. Treated water from these studies whose pH were below 6.5, WHO permissible standard which signifies acidic water could have adverse effects on the digestive and lymphatic system of human as reported by Shalom *et al.* (2011). WHO, 1986 also report that low pH could lead to swelling of hair fibres, redness and irritation of eyes in human being. The pH range observed for the treated drinking water from this study (5.20 to 7.70) was observe not to be within the range observed by Oluyemi *et al.*, 2010, who works on various drinking water from Ife North Local Government in Osun State Nigeria. In their study, they observed a pH range of between 6.53 to 8.90.

The results obtained in this study indicated that the conductivity of all the water samples were all within the WHO limit (500µs/cm). This means the presence of low quantity of dissolved minerals and salt in the water sample. High values indicate a high amount of these materials in the water as reported by Sangpal *et al.*, 2011. This implies that this water samples may be a poor conductor of electricity, heat and sound. WHO standard for total suspended solid is 500mg/l and it was discovered from this study that water sample from Dam 2 (Ede) raw water during the June/July sampling was the only sample that showed total suspended solid value of 769 mg/l which exceed the WHO standard. This could have an adverse effect on the water clarity and

visibility of the water (Davis-colley and Smith 2000). It may also have an effect on aquatic life from fish to phytoplankton (Bash *et al.,* 2000)

Dissolved oxygen (DO) is one of the important parameters in water quality assessment. It reflects the physical and biological processes prevailing in the water. Non polluted surface water is normally saturated with DO (Sangpal *et al.,* 2011). The DO of raw water samples from this study were between 1.45 and 5.61 which was lower that the range of 3.11 and 6.82 of the treated water. The DO range of the treated water is contrary to what was reported by Chinwe *et al.,* 2010 who reported DO range of between 4.25 to 6.10 for samples from distilled water, borehole, tap water, bottle water and sachet water. The DO range for raw water was also observed to be contrary to what was reported by Muhibbu-Din *et al.,* 2011, who reported DO value of 3.2 to 11.6 in streams in Ile-Ife that have been impacted with effluent.

BOD is the amount of oxygen required by the bacteria in stabilizing the decomposable organic matter. The aim of BOD test is to determine the amount of bio-chemically oxidizable carbonaceous matter (Gupta *et al*., 2003). The BOD results for the two sampling period was found to be within the WHO limits (6 to 9 mg/l) except raw water sample from dam 4 during December/January sampling period where 12.3 mg/l was found. Higher BOD value was observed at the raw water samples compared to the final water samples and this could be as a result of a large load of sediments in the raw water samples compared to the final water samples as reported by Sangpal *et al.* (2011).

COD is the amount of oxygen consumed during the chemical oxidation of organic matter using strong oxidizing agent like acidified potassium dichromate (Sangpal *et al.,* 2011). The COD of both sampling periods in all the water samples in this study were between 11.00 and 88.00 mg/l higher than what was reported by Sangpa*l et al.* (2011) where values of between 9.18 and 46.4 mg/l were obtained during the pre-monsoon sample of ujjani reservoir in India.

5.1.2. Microbial properties of water samples

It was observed that all the treated water and municipal water samplings of both sampling periods in Eleyele met the WHO standard for portable water i.e. no coliform should be isolated per 100ml of drinking water. And it was observed from this Eleyele treated water samples that no coliform bacteria was observed. Similar results were also obtained in Asejire treated and municipal samples during the December/January samples.

However, high total heterotrophic bacteria count were obtained from some of the treated water samples from Dam 1 (Ife), Dam 2 (Ede), Dam 5 (Owena-Ondo), Dam 6 (Owena-Idanre) during the December/January period. This suggested that the treatment processes might not be functioning effectively. WHO (2004) suggested some of the reasons for this ineffectiveness as, elevated source water contamination, inefficient coagulation, inefficient filtration (e.g. failure in filtration, backwash recycling and poor maturation of filters) and poor disinfection (e.g. no free-residual disinfectant and short contact times). Payment *et al.* (1991, 1997) in their report also wrote that pathogenic microorganisms that evade treatment and enter the distribution system may survive and be the source of an important level of endemic disease in the population. As the dam is available for various uses, it could be suggested that the high microbial loads may be due to human and animal contact with these water sources. Observed activities of fishermen, farmers and feeding water birds in the study locations supported this submission. The direct washing of legs, hands, clothes and utensils like cutlasses could also lead to contamination. Also the presence of nearby bushes as reported by a similar study (Banwo, 2006) might increase the possibility of hide-outs for smaller mammals who frequently visit these water bodies to drink water and to pass waste products. Absence of coliform in the treated water compared to the raw water in some of the dams could be attributed to filtration and disinfection process which the water had been subjected to at the treatment plant. However, these appeared not to be effective enough to bring the microbial loads to levels below the acceptable WHO standard for drinking water (i.e absence of coliform in 100ml of drinking water) except in Dam 4 and Dam 3 during the dry season. At all June/July samples, it was observed that all the residual chlorine of the treated water samples were below the WHO standard i.e 0.5ml/l. This could also be the reason why microbial counts in these sample periods could not meet the WHO standard for potable water. However, for these samples whose residual chlorine are above the recommended WHO standard and yet could not meet the WHO standard for coliform count, it could probably means that these bacteria isolates are resistant to the chlorine treatment and another method of treatment could be tried. It was also observed that the total heterotrophic plate count in Dam 4 (Eleyele Dam) was also below the WHO standard of below log 1 cfu/ml. This suggested that the treatment plant of this dam is still working up to it maximum capacity and this might be because the dam had just been renovated by the sate government managing the dam as at the time this study was conducted.

An increase in the microbial loads at the consumers points (i.e. the municipal taps located around streets in the town) could also be due to leakages as observed during sample collection in the pipelines during distribution as leakages in pipe water transmission pipelines have been reported to allow contaminants into the water transmission line. This is similar to the observation of van Zyl (2004) that there is a renewed international awareness that water distribution systems world-wide are aging and deteriorating, while the demands on these systems, and thus on our natural water resources, are ever increasing. Unaccounted for water in water distribution systems are reaching alarming levels in many towns and cities throughout the world. United Nations (2002) and Hall (2006) in their studies confirmed that about half of the water in drinking water supply systems in the developing world is lost to leakage, illegal hook-ups and vandalism which eventually can lead to production of water with poor microbial quality and eventually to public health crisis. Another probable reason might be that the disinfectants used may be bacteriostatic and not effective enough to wipe out the microbes completely. This may results in the reactivation of the static microbes some days after disinfection. Another reason may also be that the absence of enough residual chlorine in some of the treated water being plumped out to the community.

The relatively high amount of bacteria isolated in some of the treated water sampled in this study was similar to the report of Ridgway and Olson (1982) who also isolated relatively high numbers of bacteria from chlorinated Irvine water system. In addition, they suggested that certain bacteria may possess mechanisms enabling them to survive in highly chlorinated environments. Afterward, they discovered that the bacteria residing in the chlorinated Irvine water distribution system were significantly more resistant to both free and organically combined forms of chlorine than bacteria found in the unchlorinated Garden Grove system in which they made comparison with. They suggested that there may be strong selection for chlorine tolerant microorganisms in chlorinated water distribution system. *Bacillus* was the highest bacteria genera obtained not only from the treated water but also in the municipal and the raw water sampled for this study. This was similar to what Ridgway and Olson observed in their study.

Experience has shown that maintenance of chlorine residual cannot be relied upon to totally prevent the occurrence of bacteria (Oliveri *et al.*, 1985 and LeChevallier *et al.*, 1987). High concentration of bacteria found in both the chlorinated treated water and municipal tap as

observed in this study may be similar to the reason adduced by LeChevallier *et al.* (1987) that in drinking water distribution systems, encapsulated bacteria attached to pipe surfaces grow under low-nutrient conditions. But it was observed in this study that there was no or very low residual chlorine in the treated water during June/July sampling period at all samples and this may be one of the contributing factors for poor microbial quality of the water flowing through the water distributing systems of these dams.

Available research has shown that increased resistance to disinfection may result from the attachment of microorganisms to or the association of microorganisms with various surfaces, including macroinvertebrates (Crustacea, Nematoda, Platyhelminthes, and Insecta) (Levy *et al.*, 1984 and Tracy *et al.*, 1966), turbidity particles (Ridgway and Olson, 1982 and Herson *et al.*, 1987), algae (Silverman *et al.*, 1983), carbon fines (LeChevallier *et al.*, 1984 and Camper *et al.*, 1986), and even glass microscope slides (Oliveri *et al.*, 1985). Geldreich *et al.* (1977) also reported that heterotrophic organisms in water supplies most often originate in the source water, survive the rigors of treatment processes, and adapt to the environment of the water distribution network and trace organic nutrients already present in the bulk water or accumulated in reservoir and pipe sediments can support a diverse population of surviving organisms (heterotrophs). Habitat sites that are successfully colonized almost always invoke the mixed growth of organisms that are attached to each other or to particles, sediments, and porous structures in the pipe tubercles or sediments.

They went further to report that the spectrum of organisms may include many gram-negative bacteria (such as coliforms and *Pseudomonas*), grampositive organisms, sporeformers, acid-fast bacilli, pigmented organisms, actinomycetes, fungi, and yeast. In addition, various protozoans and nematodes that feed on these microbial populations can be found in protected areas of low-flow sections or dead ends. This could be one of the reasons why coliforms like *Proteus, Klebsiella, Morganella, Providencia Trabulsiella, Serratia* were isolated from the distributed water in these studies. Camper *et al.* (1985) wrote in their report that coliform bacteria are chlorine sensitive, but they may be protected from destruction by associated particles originating in source water turbidity, activated carbon fines released from Granular activated carbon filtration and inorganic sediments in the contact basin, as well as by inadequate conditions for

disinfection action (contact time, water pH, and temperature). This may be another reason for the survival of these bacteria in the treated and municipal taps sampled in this study.

Allen *et al.* (1980) and Tuovinen *et al.* (1980) reported that Coliform colonization of the pipe network may occur in areas where porous sediments accumulate and these sediments develop from the action of corrosion or are the accumulation of particles that passed through the treatment works because of inadequate processing and then settled in slow-flow and dead-end areas. Such sites are attractive to bacterial colonization because these deposits adsorb trace nutrients from the passing water and provide numerous surface areas for bacterial attachment against the flow of water. Among the coliform bacteria they reported were the most successful colonizers like *Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter cloacae,* and *Citrobacter freundii* which were similar to the genera of bacteria observed in this study.

The high prevalence of coliforms in water samples in this study is of public health concern because several studies have reported a prevalence of hospital acquired infections between 5 and 10% (Somwang *et al.,* 2007 and Olawale *et al.,* 2011). *Escherichia, Klebsiella, Enterobacter, Serratia, Proteus* and *Citrobacter* genera are obligatory and opportunistic pathogens responsible for infections ranging from urinary tract, surgical wounds and lower respiratory tract infections (Mouton *et al.,* 2001) among hospital acquired infections. Many species of these general are members of the normal intestinal flora. *Escherichia coli* are the most common isolates reported from many hospital laboratories (Bello *et al.,* 2005). This means before the water flowing from the distribution pipes in this study were to be used in hospitals for washing equipments or for bathing by patients it would be of high value if it was re-disinfected in order to prevent the spread of nocosomial infections.

Bacteremia is caused by *Klebsiella, Enterobacter,* and *Serratia* species that are also frequently involved in infections with respiratory tract, such as tracheostomy and manipulations using contaminated inhalation therapy equipment (Bello *et al.,* 2005). Other organisms that are occasionally encountered in urinary tract infections are *Klebsiella, Enterobacter* and *Serratia* species (Shah *et al.,* 2002). *Proteus mirabilis* is believed to be the most common cause of infection – related to kidney stones, which is one of the most serious complications of recurrent bacteriuria (Alli *et al.,* 1998). *Citrobacter freundii* and *C. diversus* have been isolated

predominantly as super infecting agents from urinary and respiratory tract infections. *Citrobacter* septicaemia may occur in patients with multiple predisposing factors; *Citrobacter* species also cause meningitis, septicemia and pulmonary infection in neonates and young children (Shah *et al.*, 2002). Hence, prevalence of coliform bacteria found in this water samples could lead to community acquired infection if used for domestic purposes like drinking, water, bathing e.t.c.

Most of the bacteria isolated from the treated and municipal taps in this study were bacteria that are potential opportunistic pathogen. Zagainova *et al*. (2010) defined opportunistic pathogens as those organisms which exist as a part of the normal body microflora but under certain conditions, they cause diseases in compromised hosts. They ordinarily are not invasive but dependent on opportunities through breakdown in the natural body barriers, such as wounds, burns, depressed gastric acidity. The exposure risk includes persons who have become debilitated by some other bacterial, viral or other diseases (e.g., gastrointestinal or hepatic disease, hypo-or achlorhydria, diabetes), usually focusing on senior citizens in rest homes or newborns and infants without established natural immunity systems. The exposure risk also includes patients of all ages receiving antibiotics or chemotherapy and particularly of concern are AIDS victims. Opportunistic pathogen infections are a serious public health problem in any areas where large numbers of people are in close confinement, particularly hospitals and senior care facilities (Zagainova *et al*., 2010). For instance, *Pseudomonas* which was isolated in the treated water and municipal taps of some of the sampled water has been described as a major cause of skin inflammation and infection (dermatitis and folliculitis) which spread through water. Bacteria from the water attach to the skin and enter in the hair follicles. In the follicles, the bacteria multiply and their waste products cause inflammation. The affected area may appear as a red, itchy rash or like a burn (Levy, 1998). This means using water containing this bacterium for recreational activity like swimming is of great public health significance.

Bergogne-Berezin and Towner (1996) described *Acinetobacter sp* which was also encountered in the municipal water in this study, as a commensal organism which occasionally cause infections, predominantly in susceptible patients in hospitals. They are opportunistic pathogens that may also cause urinary tract infections, pneumonia, bacteraemia, secondary meningitis and wound infections. These diseases are predisposed by factors such as malignancy, burns, major surgery and weakened immune systems, such as in neonates and elderly individuals. Though in the report

of Rusin *et al.* (1997), there is no evidence of gastrointestinal infection through ingestion of *Acinetobacter* spp. in drinking-water among the general population. However, transmission of non-gastrointestinal infections in drinking water may be possible in susceptible individuals, particularly in settings such as health care facilities and hospitals. This means tap water sampled in this study containing this bacterium will not be good for use any hospital environment.

Although most *Bacillus spp* are harmless, a few are pathogenic to humans and animals. *Bacillus cereus* causes food poisoning similar to staphylococcal food poisoning. Some strains produce heat-stable toxin in food that are associated with spore germination and give rise to a syndrome of vomiting within one to five hours of ingestion. Other strains produce a heat-labile enterotoxin after ingestion which causes diarrhoea within 10 to 15 hours. *Bacillus cereus* is known to cause bacteraemia in immunocompromised patients as well as symptoms such as vomiting and diarrhoea. *Bacillus anthracis* causes anthrax in humans and animals (Bartram, *et al.,* 2003). *Bacillus* spp is often detected in drinking water supplies, even supplies treated and disinfected by acceptable procedures. This is largely due to the resistance of spores to disinfection processes. Owing to a lack of evidence that waterborne *Bacillus* spp is clinically significant, specific management strategies are not required (Bartram, *et al.,* 2003). This may be the reason why *Bacillus* was the highest percentage of bacteria isolated from all the sample sites of the raw, treated and municipal points sampled in this study. But it presence might not really be of public health significance in the water distribution system of the dams.

The isolation of *Aeromonas spp* only from the raw water of Ife, Ede, Eleyele and Owena-Idanre support the findings of Kannan *et al.* (2001) and it is in agreement with many reports which suggest that *A. caviae* is one of the dominating species in the human faeces and consequently, in raw municipal wastewater. It has been found elsewhere that *A. caviae* abundances correlate with those of faecal coliforms (Araujo *et al.,* 1991). In addition, many authors have reported that the stabilization ponds select *A. sobria* strains, leading to its domination in the effluent (Monfort and Baleux, 1990; Stecchini and Domenis, 1994). It was observed during this study that there are feaces around the farmlands located very close to these dams because most of the houses in the villages where these dams are constructed do not have toilets. Hence, most of them defecate around the farmland which will eventually be washed into the water bodies where these dams are located. There are reports that in middle- income and wealthy areas of Nigeria, most houses have

pit lactrines or septic tanks (Onibokun and Kumuyi, 1999). Sangodoyin (1991) and Olaseha and Sridhar (2004) also reported that in most low-income settlements, most households do not have toilets and open defecation on refuse dumps and wasteland, or in open drains, are reported to be widespread. In addition, toilets discharging directly to open drains are reported to be common.

5.2. Antibiotics resistance profiles and multi-drug antibiotic resistant (MAR) bacteria

The relatively high level of resistance to antimicrobial agents in bacteria from this study could be a reflection of misuse or abuse of these agents in the environment and clinical settings (Silva and Hoffer, 1993; Malik and Ahmad, 1994). Antibiotic prescriptions in hospitals are given without clear evidence of infection or adequate medical indication. Toxic broad-spectrum antibiotics are sometimes given in place of narrow-spectrum drugs as substitute for culture and sensitivity testing, with the consequent risk of dangerous side effects, super infections, and the selection of drug-resistant mutants (Prescott *et al*., 1999). In developing countries, drugs are available to the public and thus people may practice self-administration of antibiotics and further increase the prevalence of drug-resistant strains. Some of the antibiotics used in this study are antibiotics that are commonly prescribed and used. Yah *et al.* (2008) reported that ampicillin, gentamicin, tetracycline, penicillin erythromycin, chloramphenicol were the most commonly prescribed antibiotics while pefloxacin, ciprofloxacin and cefotaxime were the least prescribed in Benin city of Nigeria. Olayemi *et al.* (2010) also reported that most of the antibiotics that were reportedly used for self-medication were ampicillin, tetracycline, co-trimoxazole and ciprofloxacin. The high percentage resistance observed in this study to some of the antibiotics like tetracycline, streptomycin, sulfamethoxazole, ampicillin, combination of amoxillin/clavulanic acid, combination of sulfamethoxazole and trimethoprim, lincosamide in both gram negative and gram positive bacteria (as shown in Fig 4.5 to 4.10) could be as a result of the abuse of these antibiotics. Resistance to gentamicin in gram positive bacteria from Owena-Idanre Dam 6 ranges from 0 to 40% while resistance in gram positive bacteria from Owena-Ondo Dam 5 ranges from 8 to 25%. However, numerous studies have also shown a direct relationship between the use of antibiotics and the spread of antibiotic-resistant bacteria (Moller, 1989 and Mouton *et al.*, 1990). Studies also indicated that reducing the use of antibiotics may lower the frequency of antibiotic-resistant bacteria (Ballow and Schentag, 1992). The focus of reducing antibiotics' usage has been on reducing inappropriate uses of antibiotics in human therapy and animal husbandary. There are however, reports that residues of antibiotics administered to humans and animals entered the

sewage systems through urine or faeces, in the form of either parent compound or degraded metabolites depending on the pharmacology of the specific antibiotics. Furthermore, an unknown amount of antibiotics enter the sewers by waste derived from antibiotic production and disposal of a surplus of drugs. Indeed, various antibiotics have been found in municipal sewage, including fluoroquinolones, sulfonamides and erythromicin metabolites (Raloff, 1998; Hartmann *et al.*, 1998; Hirsch *et al.*, 1999 and Hartig *et al.*, 1999). Hence, since there are reports that most households in Nigeria do not have toilets and defecate in wasteland and even houses that have toilet discharge directly into open drain (Sangodoyin, 1991), there are tendencies that antibiotic residues from human wastes could have got into the rivers in that fed these dams with water. This could have led to selective pressure on the bacteria in these rivers as reported by Guardabassi and Dalsgaard (2002) that occurrence of such antibiotics concentrations in sewage has the potential to select for antibiotic resistance among bacteria population of the water body. Thus, it is likely that a large proportion of the antibiotic residues introduced into the sewage system can reach surface waters through municipal sewage effluents. Consequently, resistance genes existing in bacteria of human and animal origin can be transferred to environmental bacteria, contributing to the formation of an environmental pool of resistant bacteria and resistance genes.

In this study, it was observed that there were lower resistances in the bacteria isolated, to antibiotics like ciprofloxacin, gentamycin and nalidixic acid which was similar to the report of Atif *et al.* (2010); Aman *et al.* (2012) and Saiful *et al.* (2010). Lower resistance to these antibiotics could be as a result of the fact that the antibiotics are very expensive, scarce and potent as reported by Yah *et al.* (2008). Therefore, as suggested by Calva *et al.* (1993) one would expect that drugs most commonly affected by bacterial resistance in developing countries are generally inexpensive antibiotic that are popular broad-spectrum agents. Hence, according to Lau *et al.* (2004) resistance of pathogens to these available, cheap, older and commonly abused drugs would definitely result in high cost of treatment, longer hospital stay and therapeutic failure, which might lead to life threatening cases and deaths.

This study demonstrated the occurrence of antibiotics resistance bacteria not only in raw water but also in treated water and municipal water distribution system which was similar to what was reported by Schwartz *et al.* (2003); Pavlov *et al.* (2004) and Zhang *et al.* (2009) that antibiotic

resistant organisms can be found in treated water. This study also demonstrated that multi-drug resistance (MDR) bacteria can be isolated from untreated and treated water samples which are similar to the report of Ram and Shanker (2005); Hamner *et al.* (2007) and Ram *et al.* (2007). Multi-drug resistant bacteria isolated from this study included genera of *Alcaligene, Aeromonas, Pseudomonas, E. coli, Klebsiella, Providencia, Bacillus, Lysinibacillus, Acinetobacter, Brevundimonas, Chromobacterium, Citrobacter, Enterobacter, Leucobacter, Morganella, Myroides, Pantoes, Pseudochrobactrum, Psychrobacter, Serratia, Staphylococcus, Stenotrophomonas, Trabulsiella* and *Proteus* e.t.c. They are members of alpha proteobacteria, beta proteobacteria, gamma proteobacteria, bacteroides, firmicutes and actinobacteria. Most of these bacteria are opportunistic pathogens. This was similar to the report of Franklin and Cockerill (1999) who reported that opportunistic pathogens presenting broad-spectrum antibiotic resistance have emerged extensively, causing serious infections in immunocompromised hosts in hospital environments. However, the prevalence of multiple antibiotic resistance bacteria in drinking water is of public health concern because of the danger of promoting multiple antibiotic resistant organisms in humans through possible colonization of the gastrointestinal tract and conjugal transfer of antibiotic resistant genes in the normal flora leading to more multiple antibiotic resistant organisms within the human gut and the environment (Mckeon *et al.*, 1995). The prevalence of drug resistance organisms poses a great challenge to clinicians and the consumers of water containing these antibiotic resistant organisms because it may prolong the treatment of water borne diseases. This implies that treatment of water- borne diseases with these antibiotics may be inappropriate and will require new and most expensive antibiotics (Tagoe *et al.*, 2011).

Occurrence of resistance *E. coli* in some of the sample points was similar to the report of Oyagade and Fasuan (2004) who also observed the presence of antibiotic resistant strains of *E. coli* in water used for domestic purposes in Nigerian communities. The authors reported that these signals associated dangers with the use of water stored for a long period of time. In this study, the existence of multiple antibiotic resistant (MAR) *Pseudomonas spp* that were resistant to 11 antibiotics distributed across 8 classes of antibiotics was similar to the findings of Odjadjare *et al.* (2012) who reported that *Pseudomonas* was resistant to five to 11 antibiotics distributed among three to seven classes of antibiotics. Also consistent with this was the observation of Paul *et al*. (1997) who reported MAR *Pseudomonas* strains with resistant patterns

varying between five and eight antibiotics; while Lateef (2004) documented MAR *Pseudomonas* with resistant patterns of two to seven antibiotics. Moreover, environmental or non-pathogenic forms of the bacteria may serve as a storehouse for genetic determinants which, if transferred to other bacterial strains, may confer not only antibiotic resistance but also novel virulence capabilities (Sharma *et al.,* 2003). Recently, studies showed that prevalence of multiple antibiotics resistant (MAR) *Pseudomonas* strains is on the increase, whereas few antibacterial agents are being developed in parallel (Alaoui *et al.,* 2007). Therefore, the occurrence of *Pseudomonas spp* in the water sample is of major public health importance especially in hospital environments where there have been reports of the isolation of the MAR bacteria from hospital taps which eventually contaminated the hands of nurses, especially during the washing of soiled utensils which eventually led to the spread of the MAR bacteria to patients. The direct transmission of the strain from the contaminated water to patients via enteral nutrition situation was also reported in two instances by the scholars (Bert *et al.*, 1998). Tsakris *et al.* (2011) also reported that because of the long duration of colonization, hospitalization is also likely to be a risk factor for community-onset infection with multi-drug resistant *K. oxytoca*, as has recently been described in Athens, Greece in 2011. Hence, the occurrence of coliforms and other opportunistic bacteria which were not just resistant to antibiotics but show resistance to multiple antibiotics from the water distribution sytems in this study can lead to the spread of MDR bacteria in consumers of these water samples and can be a serious threat if this water is supplied to hospitals to be used by hospital staff for washing and also by patients for drinking and bathing.

5.3. Presence of plasmids in the bacteria isolate.

It is well known that plasmid is one of the most important mediators facilitating the fast spreading of antibiotic resistance in bacteria (Dale and Park, 2004). In order to determine if there is any plasmid in the bacteria isolates in this study extraction with alkaline lysis was carried out. From the results of the plasmid extraction experiment, bacteria gave large plasmids (1-2 plasmid per strain) with molecular weights ranging from 22kb to 130kb. The plasmids were bigger in size than those reported by Li *et al.* (1999), Aja *et al.* (2002), Shafiani and Malik (2003) and Wang *et al.* (2006). However as observed in this study a large number of bacteria isolates were devoid of plasmid but still showed resistance to more than three classes of antibiotics. Hence, the need for determination of other mechanisms of transfer of antibiotic resistant genes like integron and gene cassette.

5.4. Tetracyline resistant gene and conjugation experiment

In this study, *tet A, tet B, tet E* and *tet 30* encoding resistant to tetracycline by efflux pump mechanism and *tet O* and *tet M* encoding resistance by protection of the ribosome was detected in multi-drug resistant bacteria isolated from various water samples. This study showed that *tet A* gene showed the highest prevalence in the tetracycline resistant gene detected. This was similar to the report by Henriques *et al.* (2006) who reported highest *tet A* in multi resistant isolates genotypes by PCR from estuarine water compared to *tet D, tet E* and *tet M*. Soge *et al.* (2005) also reported in their study on rural southwestern Uganda ground water that *tet A* was the most common tetracycline resistant gene discovered in their isolates. This study showed the presence of *tet A* gene in bacteria from the following genera *Escherichia, Pseudomonas, Alcaligenes, Morganella* and *Proteus.* Occurrence of *tet A* gene in *E. coli, Proteus* and *Morganella* in this study have been reported by Soge *et al.* (2005). The presence of t*et A* gene in *Alcaligene* and *Pseudomonas* in this study was similar to the observation of Agersø and Sandvang (2005) who also reported the presence of *tet A* gene in *Alcaligenes* and *Pseudomonas* in soil environment.

This study discovered the presence of *tet B* in both gram positive and gram negative bacteria of genera *Leucobacter, Proteus and Bacillus*. This was similar to the observation of Chopra and Roberts (2001) who reported that efflux pump mechanism of tetracycline resistance are now prevalent in both gram positive and gram negative bacteria. The resistance mechanisms Chopra and Roberts (2001) reported are widely distributed in the bacteria due to their association with mobile genetic elements such as plasmid, transposon and integron which have facilitated the spread of more than 50 genera and are often coupled with multi-drug resistance. In this study 5 bacteria showed the presence of *tet B* (Table 4.22) and they included gram positive bacteria *Leucobacter Komagatae* (Strain ID 26B) from Dam 3 raw water (Table 4.12) and *Bacillus altitudinis* isolated from Dam 2 raw water (Table 4.11), while gram negative species *Proteus vulgaris* (Strain ID 46) from Dam 4 treated water and two uncultured bacteria clone from raw water and Municipal tap 1 of Dam 3 respectively (Table 4.18). It was found out that 8 bacteria isolates carrying *tet E* was obtained with, only one gram positive bacteria identified as *Bacillus pumilus* (strain ID 122B) from Dam 1 treated water (Table 4.10) while the others were gram negative bacteria from b genera *Aeromonas* from Dam 1 raw water (Table 4.10) and *Klebsiella,*

Alcaligene and *Pseudomonas* from Dam 6 (Table 4.15). This implies that from the isolates genotyped from southwestern Nigeria water, *tet B* and *tet E* efflux pump genes for tetracycline resistance were more prevalent in gram positive and gram negative bacteria than other efflux pump genes tested for in this study. Nevertheless, it was observed inTable 4.22 that none of the MDR bacteria from Eleyele Dam 4 had *tet A.*

In the ribosomal protection protein genes examined, *tet O* was not detected in any of the bacteria isolates while *tet M* was detected in 3.3% of the bacteria meaning that there is a lower incidence of ribosomal protection protein genes in the tetracycline resistant bacteria genotyped by PCR in this study than efflux pump resistant mechanisms. This was contrary to the report that *tet* M is one of the most widely distributed tetracycline resistance determinants (Zhang *et al*., 2009a) and that the host range for the *tet* M was 42 genera while this gene continues to have the widest host range of any *tet* genes (Roberts, 2005). Previous reports showed that the *tet* M was distributed in coastal aquaculture areas and sediments in Mekong River, Vietnam (Suzuki *et al*., 2008). The absence of *tet O* in this study was also in contradiction to the report of Nikolich *et al.* (1992); Olsvik *et al.* (1994); Olsvik *et al.* (1995) who reported the presence of *tet*O and *tet*Q from gram-positive and gram-negative oral species, respectively. However, *tet*O gene has been found in different species (Chopra and Roberts, 2001)**,** but it has been isolated only from gram-positive species (*Lactobacillus, Enterococcus, Staphylococcus* and *Streptococcus spp*) from the oral and respiratory tracts (Robert, 1998). Bacteria showing the presence of *tet M* gene were *Morganella morganii* from Dam 4 raw water (Table 4.13), Dam 5 Municipal 3 (Table 4.14), Dam 6 raw water (Table 4.15), *Alcaligene faecalis* from Dam 6 raw water (Table 4.15) and an uncultured bacterium clone from Ife treated water (Table 4.10). In the two ribosomal protection protein genes examined, *tet M* showed the highest frequency of occurrence which was similar to the report of Chopra and Roberts (2001) and Roberts (2005) who reported that in the ribosomal protection protein encoding genes examined in their studies, *tet M* was the most common and was present in a diverse range of Gram-positive and Gram-negative bacteria and mycoplasma. This could probably be the reason why it had the highest frequency of occurrence in the bacteria genotyped but it was not observed in any of the gram positive bacteria tested for tetracycline resistant gene in this study. However, Kim *et al.* (2004) reported *tet M* in *Vibrio spp, Lactococcus garviae* and *Photobacterium damselae.*

The presence of more than two tetracycline resistant genes in one bacteria was observed in some of the bacteria like *Alcaligene feacalis* (strain ID 173B) isolated from Dam 6 raw water (Table 4.15) and *Pseudomonas spp* (Strain ID 196) isolated from Dam 6 Municipal tap 1. This was similar to the observation of Villedieu *et al.* (2003) who found out that carriage of more than one tetracycline resistance gene was common with oral bacteria isolates. According to their report, this could be because some of the *tet* genes were contained within conjugative transposons. The possession of one conjugative transposon was not a barrier to that same cell being able to receive other related or unrelated conjugative transposons (Chopra and Roberts, 2001). It has also been shown that some bacteria (*Neisseria*, *Haemophilus*, and *Streptococcus spp*) are naturally competent, which could help with the further dissemination of the tetracycline resistant genes (Roberts, 1998). The absence of tetracycline resistant gene in some of the bacteria showing phenotype resistance to tetracycline could probably be due to resistance mediated by another tetracycline resistant gene which was not tested for in this study. This is because as observed earlier, there have been reports that there were over 38 tetracycline resistant determinants (Levy *et al*. 1999 and Roberts 2005).

In conjugation experiment carried out in this study, *tet A* transferred to *E.coli* strain DH5α from *Alcaligene sp* and *E.coli*. *Proteus sp* and *Alcaligene sp* were also able to transfer their *tet B* and *tet 30* respectively. The transfer of the *tet A* gene to *E.coli* strain DH5α from *Alcaligene* and *Escherichia* was partly similar to the report of L'Abee-Lund and Sorum (2001) who observed horizontal transfer of *tet A* and *Int1* gene in *Aeromonas* species from freshwater. This, according to Agersø and Sandvang (2005) demonstrated how a common gene pool can be shared among organisms belonging to different environments. They then went further to report that the presence of *Arthrobacter* or other indigenous soil bacteria with multiple resistances within a pigsty environment may lead to the transfer of resistance genes from soil bacteria to bacteria of animal origin. *Alcaligenes*, *Pseudomonas*, and *Arthrobacter spp.* are very common bacteria in soil and water and are therefore likely to be in close contact with humans and animals via crops and drinking water. The fact that these bacteria are soil bacteria means that they can survive and grow in the environment for a long period of time. *E.coli* strain DH5α used as the recipient strain was from cattle while the donor strains were isolated from water environment. This then implies that the multi-drug resistant bacteria strain from the treated municipal water can transfer this *tet A* resistant gene to bacteria in the gastrointestinal tract of consumer of this water which

can eventually be transferred to pathogens if they were infected and thereby, leads to prolonged time and high cost of treatment. Hence, this treated water can be an important determinant for horizontal transfer of tetracycline resistant gene in the environment as well as the human and animal population. *Alcaligenes* species that can exist for a long period in both soil and water environment (Agersø and Sandvang, 2005) can be an important reservoir for tetracycline resistant gene. Agersø and Sandvang (2005) also demonstrated the transfer of *tet A* resistance gene from the *Alcaligene* species they isolated from Pigsties and Manured Soil to *E.coli* and *Pseudomonas putida*.

There are reports also that *tet A, tet C,* and *tet D* genes are located in conjugative plasmids of different incompatibility groups (Jones *et al.,* 1992), whereas *tet E* determinant is located in the chromosome of some isolates and has also been associated with large, nonconjugative, nonmobile plasmids (Roberts, 1994). This probably could be the reason why none of the bacteria were able to transfer their *tet E* resistance genes in this study i.e. they could have been located in the large, nonconjugative and nonmobile plasmids.

5.5. Extended spectrum β –lactamase (ESBLs) genes

In this study, ESBLs genes of bla_{TEM}, bla_{CTX}, bla_{SHV} in 172 ampicillin resistant MDR bacteria were genotyped by PCR ampification (Table 4.24). It was noticed that the gene with highest frequency of occurrence was bla_{TEM}, found in 34.30% of the bacteria genotyped followed by bla_{SHV} having 15.11% of occurrence in the total bacteria genotyped while $bla_{CTX\text{-}M}$ showed the lowest occurrence (1.74%). This study found that bla_{TEM} and bla_{SHV} was distributed among the following gram negative bacteria genera which included *Myroides, Chromobacterium, Pseudomonas, Stenophomonas, Bordetella, Acinetobacter, Proteus, Providencia, Klebsiella, Morganella, Escherichia, Citrobacter* and *Alcaligenes, Serratia and Trabulsiella* and also among gram positive bacteria which included *Bacillus* and *Staphylococcus*. In Nigeria, there were reports on the occurrence of ESBLs genes in both clinical and environmental samples. Akinduti *et al.* (2011) reported the occurrence of bla_{TEM} in *E.coli* and *Klebsiella* isolates from feacal samples of consented residents and hospital patients in Abeokuta, southwestern Nigeria. Soge *et al.* (2006) in their work on MDR *Klebsiella pneumonia* strains isolated from patients with community acquired urinary tract infection from southwestern Nigeria also reported the presence of $bla_{CTX\text{-}M}$, bla_{TEM} and bla_{SHV} in the isolates. Similarly,

Adelowo and Fagade (2011) reported the presence of bla_{TEM} isolated from *Enterobacter hormachei* from poultry waste polluted river. Olowe *et al.* (2010) reported the presence of *CTX-M-15* in *E. coli* from hospital patients from Osogbo in southwestern Nigeria. But no report has been found on the presence of ESBLs genes in other environmental bacteria like those described in this study especially from treated and untreated water from southwestern Nigeria. This high prevalence of bla_{TEM} and bla_{SHV} was similar to the report of Jacoby (2005) that a majority of the ESBLs belonged to the *TEM* and *SHV* families while Mojtaba and Behnaz (2012) reported that most ESBLs in their study were *TEM*-type and *SHV*-type enzymes. From the two genes tested in this study, it was observed that more bacteria showed the presence of bla_{TEM} gene than bla_{SHV} gene, which can be compared to the report of Tasli and Bahar (2005) and Hosseini-Mazinani *et al.* (2007) regarding the *E.coli* isolates tested. Mojtaba and Bahnaz (2012) also reported more of bla_{TEM} than bla_{SHV} in the isolates they worked with. In their study, they discovered 15% occurrence of bla_{SHV} in the bacteria they genotyped which was similar to what was observed in this study. But they observed 65.5% of the bacteria they genotyped to possess bla_{TEM} which was higher than 37.57% observed in this study. In this study, only *Morganella* (Strain ID 206), and 2 *klebsiella* (Strain ID 347 and 175A respectively) isolated from Dam 6 raw water (Table 4.15) possesed $bla_{CTX\text{-}M}$. This was contrary to what was reported by Mojtaba and Behnaz (2012) who did not observe any of the bacteria isolates they worked on to be carrying the presence of $bla_{CTX\text{-}M}$. $Bla_{CTX\text{-}M}$ was the least occurred within the ESBLs gene tested for in this study which is similar to the report of De Champs *et al.* (2000) that *CTX-M* type showed the least rate of 1.2% in the *Enterobacteriaceae* isolates. However, there were reports in some studies that *CTX-M* types were the most frequent ESBLs (65%) observed (Ben-Ami *et al.*, 2009). Also in the studies of Ihoha *et al.* (2012) on Nigerian *E.coli* clinical isolates, it was reported that $bla_{CTX\text{-}1}$ was a common *CTX* gene in the isolates. A misuse of antibiotics might have contributed to the emergency of ESBL producing isolates. The presence of more than one ESBLs gene in one bacterium was observed in this study especially in bacteria like *Pseudomonas sp* (strain ID 89) from Dam 1 treated water (Table 4.10), *Klebsiella pneumonia* (strain ID 287) from Dam 3 Municipal tap 2 (Table 4.13), *Alcaligene faecalis* (strain ID 250A2) from Dam 6 Municipal tap 1 (Table 4.15). This is similar to the report by Mojtaba and Behnaz (2012) who observed the presence of both bla_{TEM} and bla_{SHV} in more than 14 of the bacteria isolates tested for the presence of ESBLs genes in their studies. The alaming issue in this study was the detection of these genes (bla_{TEM} and bla_{SHV}) from

the treated and municipal taps which were points that were very close to the consumers of the water. This means the water could be a major reservoir of these genes which could be easily transferred to the populace if these genes were present in mobile genetic elements like plasmid and trasposoons. Hawkey and Jones (2009) reported that ESBL genes were located in plasmids that could be easily transferred within bacterial species. Some ESBL genes are mutant derivatives of established plasmid-mediated β-lactamases (e.g., $bla_{\text{TEM/SHV}}$), and others are mobilized from environmental bacteria (e.g., $bla_{CTX\text{-}M}$). In the 1990s, most reports on ESBL genes concerned $bla_{\text{TEM/SHV}}$ types, which were related to cross-infections in hospitals. Hence, the occurrence of ESBL gene in both gram negative and gram positive bacteria in this study is of serious public health concern.

5.6. Sulfonamide resistant genes

In this study, out of the 3 *sul* resistant genes which included *sul 1*, *sul 2* and *sul 3* amplified by PCR, it was observed that *sul 2* showed the highest prevalence (27.93%) in a total of 179 sulfamethoxazole MDR bacteria tested followed by *sul 1* (22.90%). This is in contradiction to the report of Hoa *et al.* (2010) that *sul 1* was more prevalent than *sul 2* in bacteria isolated from a sulfonamides contaminated aquatic environment, but similar to the report of Su *et al.* (2011) that, in their studies with Enterobacteriaceae isolated from integrated fish farms in south China, *sul 2* gene was the most frequently detected *sul* gene (89.4% out of 66 isolates), followed by *sul 1* (50%), and *sul 3* (3%). This study did not observe the presence of *sul 3* in any of the bacteria isolates. This is in contradiction to the report of Su *et al.* (2011) who reported the presence of *sul 3* but in low prevalence i.e., 3% in the Enterobacteriaceae isolates. The two genes present were found to be more prevalent in gram negative bacteria compared to gram positive bacteria (Table 4.10 to 4.15. Bacteria isolates that carried the presence of either *sul 1* or *sul 2* (as shown in Table 4.10 to 4.15) included gram negative bacteria genera of *Escherichia, Bordetella, Proteus, Pseudomonas, Morganella, Alcaligenes, Klebsiella, Aeromonas, Pantoea, Providencia, Myroides, Trabulsiella* while *Bacillus* and *Leucobacter* represented gram positive bacteria. In Nigeria, Adelowo and Fagade (2011) reported the presence of *Sul 1* and *Sul 2* in *Citrobacter freundii* isolated from poultry waste polluted river in southwestern Nigeria. Ojo *et al.* (2002) reported that sulphonamides and trimethoprim were among the most frequently used antimicrobial drugs in Nigeria, where the combination of both drugs were used preferentially for the treatment of urinary tract infections. However, sulphonamides and trimethoprim are

commonly available, alone or in combination, over the counter in pharmacy stores in Nigeria without a doctor's prescription. Self-medication and misuse of these drugs favour the development of resistance to sulphonamides and trimethoprim in various bacterial pathogens. Most literatures considered in this study concentrated on phenotypic expression of these antibiotic-resistances among the bacteria population they studied. Also, this study found very high phenotypic expression of resistance among bacteria examined but low molecular bases of resistance in bacteria. Hence, this study is likely to be the first study to report that sulfa drug resistant bacteria are not only distributed among different bacteria genera but also that *sul* resistant genes (*sul 1* and *sul 2*) are also widely distributed among both gram positive and gram negative bacteria from dams in southwestern Nigeria. Yi *et al.* (2010) reported a widespread distribution of *sul1* and *sul2* genes in the Haihe River and its tributaries. However, the wide distribution of these genes among the bacteria from treated and untreated water from southwestern Nigeria could also be as a result of exposure of this aquatic system to sulfa drug either from sewage systems or from run off from agricultural environment.

Vinue *et al.* (2010) reported two kinds of class 1 integrons. Classic class 1 integrons usually have an integrase gene (*intI*) in their 5'-conserved segment (5'-CS) and *qacEΔ1* genes encoding resistance to quaternary ammonium compounds and sulfonamides, respectively, in their 3'-conserved segment (3'-CS). However, non-classic class 1 integrons lack the 3'-CS region. This was confirmed in this study because all the isolates that carried *intI* gene of class 1 integron as detected by PCR also carried *sul 1* gene as detected by PCR amplification. The bacteria were *Alcaligenes spp* (strain ID 87A) from Dam 1 treated water (Table 4.10), *Aeromonas caviae* (strain ID 375) and *Aeromonas hydrophila* (strain ID 391A) both from Dam 4 raw water (Table 4.12), *Alcaligene faecalis* (strain ID 45) from Dam 3 raw water (Table 4.12), *Alcaligenes spp* (strain ID 198) from Dam 5 treated water (Table 4.14) and *Pseudomonas spp* (strain ID 175B) from Dam 6 raw water (Table 4.15). This impies that *sul 1* gene in these bacteria was present in the integron which is a mobile genetic element which can help the spread of this gene to other bacteria genera either in the environment or in the GIT of animals or humans.

5.7. Streptomycin resistant genes

In this study, three streptomycin resistant genes which are *aph (3'')*^{c} and *aph (6')1d*^{d} both coding for streptomycin phosphotransferases, and *ant (3'')*^{b} coding for streptomycin adenylases were

amplified in 101 streptomycin resistant gram negative bacteria that were MDR. It was observed (Table 4.25) that *ant (3')*^{b} showed the highest percentage of occurrence (48.5%), followed by *aph (3')*^{c} (38.61%) while *aph (6)1d*^{d} was observed to be the least occurred among the gram negative bacteria genotyped with percentage value of 15.84% of the total bacteria genotyped. This report was similar to what was reported by van Overbeek *et al.* (2002) in which they verified *ant (3')*^{b} and *aph (3')*^{c} genes as the most frequently occurring streptomycin modifying genes followed by *aph (6')1d*^{d} in the European habitats they studied. All the three genes have been reported to reside in mobile genetic elements in isolates from clinical environments (Tauch *et al.*, 2000), plants (Palmer *et al.*, 1997) and soil environment (Magrini *et al.*, 1998), or other habitats (Sundin and Bender, 1996). This illustrates their ubiquity in aquatic environment. Gene encoding streptomycin adenylylating enzymes *ant (3')* and *ant (6')* are found in high amount among gram negative MDR bacteria tested for in this research. They had been detected in both gram positive and gram negative bacteria. *Ant (6')* was first detected in plasmid pS194 in *Staphylococcus aureus* (Projan *et al.*, 1988) and in pK214 of *Lactococcus lactis* (Perreten *et al.*, 1997). The *ant (3')* family is the best described streptomycin resistant gene family, as observed in many different habitats (Bass *et al.*, 1999; Rosser and Young, 1999 and Daly and Fanning, 2000). This could be the reason why this gene has a wide range of distribution and dissemination among the bacteria from all the sample sites in this study thereby affirming an earlier observation by van Overbeek *et al.* (1997).

However, many scholars have reported the occurrence of other streptomycin resistant genes in gram negative bacteria. Sundin and Bender (1996) reported that *strA-StrB* streptomycin resistance genes were notable because these genes are distributed among commensal and pathogenic bacteria isolated from humans, animals, and plants. For example Hyo *et al.* (2004) reported the detection of *strA-strB* gene in *Peseudomonas sp* isolated from Kiwi plants. These genes were reported to code for the streptomycin-modifying enzyme aminoglycoside-3-phosphotransferase (*aph(3")-1b*) and aminoglycoside-6-phosphotransferase (*aph(6)-1d* (Scholz *et al.*, 1989). These could be the genes that are responsible for streptomycin resistance in bacteria that does not show the presence of any of the three resistant genes amplified in this study. Tolba *et al.* (2002) in their report observed that streptomycin genes *strA* and *strB1* were widely distributed in isolates recovered from all soil samples sites in their study, however, the percentage containing *strA* was higher in the streptomycin treated soil isolates compared with the

control. According to them, a selection for streptomycin resistance had occurred and the resistance genes recovered from the *Streptomycete griseus* streptomycin biosynthesis cluster was predominant. However, from this study the high occurrence of *ant (3')*^{b} could also be that the bacteria are selecting for streptomycin resistance as a result of the presence of this antibiotics and the gene in the environment. However there have been reports of the use of streptomycin in the control of bacterial canker disease that cause serious damage to kiwi fruit plant in Japan and Korea (Hyo *et al.*, 2004) which could eventually get into aquatic system from runoff, whereas in Nigeria streptomycin is commonly used in livestock (Dina and Arowolo, 1991). Dipeolu and Alonge (2002) attested that some of these drugs were quickly excreted by the animals because they were not readily metabolized and so, their residues will persist in the animal tissues and enter the human food chain constituting health risks to the consumers. Also, persisting for a long period of time in the environment, leading to selection of streptomycin resistant bacteria and genes in the aquatic and other environment. However, Dipeolu and Alonge (2002) reported the presence of residues of streptomycin antibiotics in meat sold for human consumption in some states of southwestern Nigeria. It should therefore, be noted that some of these streptomycin antibiotics residues will eventually get into the water bodies either through washing of the meat or from slaughter slabs where a lot of animal feacal materials are washed into the water bodies. This can also eventually lead to a selection of streptomycin resistant bacteria and genes in the environment.

Alteration of the ribosomal subunit, which is a binding site of many aminoglycoside antibiotics caused significant resistance to streptomycin (Mingeot-Leclercq *et al*., 1999). Reduced uptake of antibiotics is likely to be due to membrane impermeabilization, but the underlying molecular mechanisms are largely unknown (Hyo *et al.*, 2004). This probably could be the other mechanisms those bacteria without any of the three streptomycin resistance gene amplified in this study is using as it streptomycin resistance mechanism which should be looked into in future studies.

5.8. Class 1 and Class 2 integrons and their resistant gene cassettes

In this study, integrons were found among Enterobacteriaceae *Providencia, Morganella, Proteus, Klebsiella* and *Citrobacter* and other gram negative genera which include *Alcaligene, Chromobacterium, Aeromonas* and *Pseudomonas*. Integrons have also been found in a few

gram-positive bacteria (Agersø and Sandvang, 2005) while *Leucobacte*r and *Bacillus* were the two gram positive bacteria in which integron was found in this study. Agersø and Sandvang (2005) reported the presence of integron in an unidentified bacterium which is 96% sequence homology with 16S rDNA of *Leucobacter komagatae* while Byrne-Bailey *et al.* (2011) observed the presence of class 1 intergron in *Bacillus sp*. Martin *et al.* (1990) were the first to report integrons in gram-positive bacteria; these authors found transposon-borne integrons in *Mycobacterium fortuitum*. In this study, Class 1 and Class 2 intergrons were found in multi-drug resistant bacteria isolated from dam water in southwestern Nigeria selected for PCR genotyping. It was observed that class 1 integron was more prevalent among bacteria genotyped than class 2 integron (Table 4.26). The result showed 21.99% of the total bacteria to be carrying class 1 integron compared to 3.14% found to be carrying class 2 integron. This was similar to what was reported by Gassama-Sow *et al.* (2010) in their work with *Shigella spp* isolated from Senegal, where they found that class 1 integron was more prevalent, being present in 92.8% of the 32 isolates compared to 57.1% of the isolates showing the presence of class 2 integrons. Also Rezaee *et al.* (2012) worked with MDR *Klebsiella pneumonia* isolates from northwest Iran found that out of 149 isolates genotyped, class 1 integrase gene (*intI 1*) showed a dominant presence; it was reported in their study to be present in 117 isolates (78.5%) and only 20 isolates (13.4%) were reported to possess class 2 integrase gene (*intI 2*). Sunde (2005), on his study on *E. coli* strain from meat and meat products from Norway, reported similar trend between class 1 and class 2 integron. He reported that 29 (12%) strains possessed class 1 integron while 14 (6%) had the class 2 integron. However, class 1 integron is the most frequently detected integron in clinical isolates and is observed to be strongly associated with multiple antibiotic resistances observed in hospital environments (Martinez-Freijo *et al.*, 1998).

This study also observed a greater prevalence of class 1 integron in gram negative bacteria than gram positive bacteria while few gram positive bacteria possessed class 1 integron. This was in consonance with the reported of Agersø and Sandvang (2005) that integron have been found in a few gram positive bacteria. Nevertheless, Nandi *et al.* (2004) reported more prevalence of class 1 integron in gram positive bacteria unlike gram negative Enterobacteriaceae which have been reported to be a higher carrier of this gene. They reported 85% of their class 1 integron carrying bacteria as gram positive bacteria which include *Staphylococcus, Aerococcus, Corynebacterium* and *Brevibacterium.* In this study, three bacteria (1.57%) which are two uncultured bacteria

clone with strain ID 18B1 and 41 from Dam 3 raw water (Table 4.18) and Municipal tap 1 respectively and one bacterium identified as *Providencia rettgeri* with strain ID 253B1 from Dam 5 Municipal 2 tap (Table 4.20) were observed to carry both class 1 and class 2 integrase genes together. This percentage frequency is lower than what was observed by Rezaee *et al.* (2012) who reported 10.7% of *Klebsiella pneumoniae* isolates from northwest Iran carrying both class 1 and 2 integrase genes. Byrne-Bailey *et al.* (2011) noted that 1.1% of 500 bacteria isolates they genotyped from manure soil possessed both classes of integrase genes which was similar in agreement to what was observed in this study. Only six bacteria (3.14%) carried three- conserved region of the *qacEΔ1-sul1* gene which were only associated with class 1 integron. Xu *et al.* (2009) affirmed this in their study on *Pseudomonas aureginosa* from clinical isolates. On the contrary, the frequency of isolates observed to carry this gene in their study was higher than what was observed in this study. They reported 51 (94%) out of their 54 isolates to be carriers of this gene compared to 3.14% observed in this study. This could be because quaternary ammonium compound is greatly used as disinfectant in clinical environments which made their isolates liable to selection pressure of the studied quaternary ammonium compound and the resistant genes. However, there are reports that quaternary ammonium compound (QAC) resistance may cause coselection for antibiotic resistance. This provides a potential reservoir of antibiotic-resistant bacteria in QAC-polluted environments. In staphylococci, the *qacA/B* genes possessing multi resistant plasmids confer a low-level resistance to chlorhexidine and QACs, and it has been suggested that the introduction of chlorhexidine into clinical environments has resulted in the selection of staphylococci containing *qacA* carried on multi resistant plasmids (Russell, 2000). Hence, the low prevalence of these genes in bacteria from this study may be because bacteria genotyped from treated and untreated water have not been exposed to quaternary ammonium compounds. However, from field observation it was observed that all the dams that were sampled used chorine gas as disinfectant in their treatment plants.

It was also observed in this study that out of the 42 (21.99%) bacteria showing the presence of class 1 integron 29 tested positive for the variable region of the class 1 integron. The sequence analysis of some of the variable regions showed they harboured gene cassette *dfr* encoding resistance to trimethoprim, aad and *aac* encoding resistance to aminoglycoside, *qnr* encoding resistance to quinolone, *ampR* encoding resistance to ampicillin, *sul 1* encoding resistance to sulfa drug and *bla* encoding resistance to beta lactamase antibiotics. This study observed that

those gene cassettes encoding resistance to aminoglycoside were frequently found in class 1 integron (Table 4.16 to 4.21). This was similar to what was observed by other authors (White and Mciver, 2001, Maguire *et al.*, 2001 and Mciver *et al.*, 2002). The *aadA* type gene, that encode resistance to aminoglycosides, such as streptomycin and spectinomycin, was most commonly found in the variable region of the bacteria which was similar to the observation of Yan *et al.* (2010) in their study with clinical bacteria isolates from South China. Therapeutic uses of streptomycin were excluded for a long period, but they continued to be used in agriculture and food animals, particularly in livestock (Yan *et al.*, 2010). However, this study observed that all organisms showing the presence of integron with this aminoglycoside resistant gene showed phenotypic resistance to streptomycin. *Dfr* gene cassettes (*dfrA15* and *dfr 7*) that conferred resistance to trimethoprim were also detected frequently in the class 1 integron in this study. Interestingly, they were found sometimes combined with *aadA* gene cassettes similar to the observation of Yan *et al.* (2010). Some of the *dfr* genes found in some bacteria in this study (e.g. *Alcaligene spp* (Strain ID 87A) from dam 1 raw water (Table 4.16), *Leucobacter komagatae* (strain ID 26B) from Dam 3 raw water (Table 4.18) and *Aeromonas caviae* (strain ID 376) from Dam 4 raw water (Table 4.19) were followed by *aadA* gene cassette downstream. Moreover, the presence of this *dfr* gene cassette directly behind the 5' conserved segment, which is the closest to the promoter, provided more expression of the gene phenotypically (Yan *et al.*, 2010).

In this study, it was observed that all the six bacteria that carried class 2 integron all carried variable region while the most common gene cassettes observed to be present in the class 2 were *dfrA* for trimethoprim resistance, *sat 1* for streptothricin resistance and *aad A* for spectinomycin resistance. This was also similar to what Yan *et al.* (2010) reported that all class 2 integrons found in bacteria genotyped in their study carried gene cassettes as those found in Tn 7, *dfrA-sat2-aadA1* gene. However, there are reports that class 2 integrons carry three specific gene cassettes, *dfrA1*, *sat1* and *aadA1*, which conferred resistance on trimethoprim, streptothricin and streptomycin/spectinomycin, respectively (Hansson *et al.*, 2002 and Labbate *et al.*, 2009). Thus, class 2 integron has been regarded as a contributor to the antibiotic resistance issue, and commonly observed in some species of gram negative organisms such as *Acinetobacter*, *Salmonella* and *Psuedomonas* (Ramirez *et al.*, 2005; Nemergut *et al.*, 2008; and Xu *et al.*, 2009. This may probably be the reason why class 2 integron was not found in gram positive bacteria in this study. Bacteria that were carriers of class 2 integron in this study were 2 gram negative

uncultured bacteria clone with strain ID 18B1 and 41 respectively both from Dam 3, 2 *Proteus* with strain ID 201 and 273 respectively both from Dam 5 Municipal tap 1 and 2 respectively and also 1 *Proteus* with strain ID 273 from Dam 6 and 1 *Providencia rettgeri* from Dam 5 Municipal tap 2. This study, revealed that *cat b* and bla_{CARB} gene cassettes for chloramphenicol and ESBLs resistance in the class 2 intgrase gene of *Providencia rettgeri* with strain ID 253B from Dam 5 Municipal 2 tap. This is different from gene cassette commonly encountered in literature. However, antibiotic resistant genes previously unassociated with class 2 integrons such as *ereA9* and *estX* (GenBank accession no. AB161462) have been shown to be associated with Tn7-related class 2 integrons (Barlow and Gobius, 2006). Furthermore, Ramirez *et al.* (2005) described a novel rearrangement of a class 2 integron from nonepidemiologically related *Acinetobacter baumannii* isolates. This class 2 integron has the genes *sat2*, *aadB* and *catB2* inserted upstream of the three conventional antibiotic resistance genes of Tn7 class 2 integrons. Hence, it is possible to occasionally observe other gene cassettes associated with class 2 integron hence this may be a novel arrangement. Therefore, integrons observed in this study appear to have an essential role in facilitating the dissemination of the resistance genes and contributing to the creation of multi-drug resistant phenotypes. This is because it was observed that more antibiotic resistant gene cassettes can be found in the integrase sequence.

5.9. Antibiotic resistant genes detected by DNA microarray technology

Microarray employed in this study detected *sul1* and *sulII*, *aadA*, *cat I*, *floR*, *tet A* (Table 4.9) in multi-drug resistant bacteria. This was also reported by Ma *et al.* (2007) in various species of *Salmonella* from swine. Call (2001) constructed a microarray to detect 17 tetracycline resistant genes in which they detected more of *tet A* in the bacteria isolates they genotyped in their studies. Several studies have demonstrated that DNA microarrays can be used to detect resistant genes as effectively as standard techniques such as polymerase chain reaction, sequencing, conjugation, and southern hybridization (Call *et al.*, 2003; Ma *et al.*, 2007; Batchelor *et al.*, 2008; and Zou *et al.*, 2009). However, in this study, comparison of the results of the microarray analysis with that of the PCR genotyping showed that all the genes observed from the use of specific primer for antibiotic resistant genes were all detected with the array. This means the constructed microarray is effective in the detection of antibiotic resistant genes from the selected bacteria genotyped. Another advantage of microarray as reported by Call (2001) is that it is possible to detect many antibiotic resistant genes on a single bacterium. This was observed in this study. For example

Bordetella sp (stain ID 51) showed the presence of 14 antibiotic resistant genes in the array which signifies that using convectional PCR will require running the test 10 times before those genes can be detected but with a single assay those genes were detected simultaneously. Genes like *cat B2* that codes for chloramphenicol acetyltransferanse, as well as (CAT) that detoxifies the antibiotics chloramphenicol and which is responsible for chloramphenicol resistant in bacteria (Shaw, 1975) were detected in *Chromobacterium violeceum* (strain ID 382) from Ede raw raw using microarray. But it was observed that this bacterium was not resistant to chloramphenicol phenotypically.

Other tetracycline resistant genes were also detected in this study apart from those detected by PCR amplification. *Tet J* and *tet H* which have been reported to be coding for efflux pump (Marilyn and Stefan, 2009) were detected in *Proteus vulgaris* (strain ID 43) from Asejire raw water. Guillaume *et al.* (2004) also reported *tet J* in *Proteus mirabilis* while *tet H* was first reported in *Pasteurella multocida* (Hansen *et al.,* 1993) using PCR amplification. Miranda *et al.* (2003) have also reported the presence of *tet H* in *Moraxella sp* by the use of PCR amplification. *Tet X* was observed to be present in *Aeromonas hydrophila* (strain ID 321A) from Ife raw water. However, this gene has been reported to encode an enzyme, which modifies and inactivates the tetracycline molecule. It does not seem to have much clinical relevance since it requires oxygen to function and is found only in a strict anaerobe, *Bacteroides*, where oxygen is excluded (Speer *et al.,* 1991). However, *tetX* gene has now been found in an aerobic gram negative *Sphingobacterium sp.,* isolated from agricultural soil (Ghosh *et al.,* 2009). Thus, it is unlikely that the *tet X* gene functions in its natural host (*Bacteroides*). Hence, this means this gene might also not be functional in *Aeromonas hydrophila*. However, from the result of the microarray study *tetH* and *tetE* which code for efflux pump were also observed in the bacteria. These could then be the mechanism the bacterium was using for the tetracycline resistance and not *tetX*. *TetX* is a cytoplasmic protein that chemically modifies tetracycline in the presence of both oxygen and NADPH, and semi-synthetic drug tigecycline (Moore *et al.,* 2005).

Presence of *qnrA1* which encode resistance to quinolone was observed in *Providencia rettgeri* (strain ID 209) from Dam 5 municipal sampling point and *Alcaligenes faecalis* (strain ID 28A) from Dam 2 treated water. Guillard *et al.* (2011) have also reported the presence of *qnrD* in two strains of *Providencia rettgeri* from stool samples. However, the plasmid-mediated quinolone

resistance associated with *qnr* (now named qnrA1) in *Klebsiella pneumoniae* was first found from the United States in 1998 (Martinez-Martinez *et al.*, 1998) and described as a plasmid-mediated quinolone resistance gene (PMQR). Since then, five types of *qnr* genes have been reported: *qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS* (Guillard *et al.*, 2011). All these organisms in which *qnrA1* gene was detected was observed to be phenotypically resistant to Nalidixic acid. The qnr gene encodes a 218-amino-acid protein. The purified *qnr* protein can protect DNA gyrase and topoisomerase IV activity *in vitro* (Martinez-Martinez *et al.*, 1998). The presence of this gene in these bacteria therefore, signifies the possibility of being transferred to other bacteria leading to a high rate of resistance between different genotypes of bacteria of both clinical and environmental consequences. The *Qnr* genes have been reported in populated continent except south America (Robicsek *et al.*, 2006). However, in different geographical areas, the prevalence of *qnr* gene type was different. In Japan, *qnrS* gene was found in *Shigella flexneri* which showed 59% amino acid identity with the *qnr* gene (Hata *et al.*, 2005). In India, *qnrB* was found in *Citrobacter koseri* with 40% amino acid identity with *qnr* (Jacoby *et al.*, 2006).

Chapter 6

Conclusion

This study was carried out to investigate the occurrence and public health significance of multi-drug resistant bacteria isolated from selected dams from southwestern Nigeria and to understand the molecular bases to which bacteria confers resistance to antibiotics. At the end of this study, the results revealed that:

Bacteria of public health significance capable of causing opportunistic infection could not only be isolated from the untreated water samples but also from treated water of the selected dams as well from the municipal taps at the point of consumption. This could be as a result of leakage of pipes due to old age, road construction and inadequate residual chlorine in the treated water from the treatment plants.

Bacteria isolates from these dams also showed high level of resistance to antibiotics that are commonly prescribed in Nigeria, especially those used without doctor's prescription. The antibiotics include tetracycline; aminoglycoside (e.g. streptomycin); sulfa drug (e.g. sulfamethoxazole); sulfa drug combination (e.g. sulfamethoxazole/ trimethoprime); beta lactam (e.g. amoxillin/clavulanic acid and ampicillin) and lincosamides (e.g. lincomycin).

Multi-drug resistant bacteria were also found among the antibiotic resistant bacteria isolated from the selected water samples of this study.

Mobile genetic elements (i.e. integron and gene cassettes) which are capable of conferring multiple resistance to antibiotics and also capable of transferring antibiotic resistant genes to other bacteria of clinical and environmental significance, thereby leading to high cost and prolong treatment of infectious diseases were detected from bacteria isolates in this study. These make the consumers of such water to be at high risk of emerging infectious diseases.

Presence of QAC gene which encodes resistance to quaternary ammonium compounds was also detected at the 3' end of some of the class 1 integrons found in bacteria isolated in this study. This shows that these bacteria can also select for resistance to disinfectant made from quaternary ammonium compound.

Other antibiotic resistant genes like *tet A, tet B, tet E, tet M* encoding resistant to tetracycline; Bla_{SHV}, Bla_{CTX}, Bla_{TEM} encoding resistant to beta lactam; *aph (3')*^{c}, *ant (3')*^{b}, *aph (6')1*^{dd} encoding

resistant to streptomycin and *sul 1* and *sul 2* encoding resistant to sulphonamide were also detected by PCR amplification. These were found among wide diversity of bacteria genera from the water samples making the water samples a reservoir for antibiotic resistant genes that have the potential to be transferred to other bacteria genera. However, the presence of plamids in some of the bacteria genera indicated that these genes could be present on plamid and hence, fercilitating the spread of these resistant genes.

The use of microarray to genotype some selected multi-drug resistant bacteria showed that more antibiotic resistant genes such as *catB2, floR, qnr, tet J, tet H* were detected from these bacteria simultenously in a single assay within a short period of time. The microarray technology was also able to detect genes that are present in some of the bacteria which were not express i.e silence genes.

6.1. Recommendations

With the observed presence of bacteria of public health importance and widespread of multi-drug resistant bacteria and genes from these selected water samples, the following are therefore recommended:

i. Proper maintenance of water distribution systems by regular flushing so as to distribute the residual chlorine to all portions of the system.
ii. More aggressive cleaning using cable-draw or water-propelled devices (pigging) should be periodically performed in order to remove the corrosion and sediment buildups that provide habitats for bacteria.
iii. It is also recommended that reservoirs of treated water should be covered to avoid dissipation of disinfectant residuals and to guard against contamination by wildlife, air pollution, accidental roadway spills and surface water runoff.
iv. Regular testing of the treated water for microbial contamination is also recommended to the Nigeria Water Cooperation.
v. Human activities like farming and defecating in the water should be discouraged around the constructed dams. Bushes that attract rodents to the dams should be cleared in order to prevent the animals from defecating or dying in the raw water. These can release antibiotic resistant bacteria into the water and their death can also release antibiotic resistant genes into the aquatic environment.

vi. Good sewage systems for residential buildings and good disposal of wastewater by abattoirs should also be encouraged.

vii. Policies should be formulated to regulate the use of antibiotics in the agriculture and health care deliveries. If possible some antibiotics should be banned and antibiotics should heneforth, not be sold again to Nigerian except by doctor's prescription.

viii. Relevant regulatory agencies should be strengthened to enforce the regulatory policies.

ix. The general public should be enlightened on the effect of indiscriminate use of antibiotics.

6.2. Contribution to Knowledge

This study has been able to determine some molecular bases by which multi-drug resistant bacteria isolated from selected water samples from southwestern Nigeria used to confer resistance to antibiotics through PCR amplification and microarray hybridization.

Extended spectrum β-lactamase (ESBL) genes which include bla_{TEM}, bla_{CTX} and bla_{SHV} were detected for the first time in bacteria like *Pseudomonas*, *Klebsiella*, *Alcaligenes*, *Acinetobacter*, *Stenotrophomonas*, *Bacillus* etc., in the drinking water distribution systems within the southwest Nigeria.

In these bacteria, *sul 1* and *sul 2* were also reported. Many works have focused on the phenotypic expression of these genes and not the molecular bases.

This study revealed that class 1 and class 2 integrons which are mobile genetic elements found in bacteria from these dams harbour gene cassettes of various sizes which make them to confer multiple resistance to antibiotics like trimethoprim, aminoglycoside, beta-lactam.

This study was able to discover that QAC gene coding for quaternary ammonium compound can be found on the 3' conserved region of class 1 integron found in bacteria genera of *Alcaligene*, *Aeromonas* and *Pseudomonas*.

The use of microarray in this study allowed rapid and simultaneous detection of other antibiotic resistance genes in a single bacterium.

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APPENDIX 1

MEDIA

EOSIN METHYLENE BLUE (EMB) AGAR

Formula in g/l

Peptone	**10,000**
Lactose	**10,000**
Dipotassium hydrogen phosphate	**2,000**
Yellowish eosine	**400**
Methylene blue	**65**
Agar	**65**

Final pH 6.8 ± 0.2 at 25^{o}C

LURIA BERTANI BROTH

Formula in g/l

Peptone	**10.0**
Yeast Extract	**5.0**
NaCl	**5.0**

Agar Agar 15.0 (For Luria Bertani agar)

NUTRIENT AGAR

Formula in g/l

Peptone	**5.0**
Meats extract	**3.0**
Agar	**15.0**

Final pH 7.0 ± 0.2

APPENDIX 2

Buffers for Microarray analysis

1. Preparation of 20X Saline Sodium Citrate (SSC)

Using a 500ml beaker with a stir bar, dissolve the following (while stirring) in 450ml nanopure de-ionized water:

87.7g Sodium Chloride

44.1 g Trisodium Citrate Dihydrate

Adjust the pH to 7.0 with approximately two drops of concentrated HCL.

Transfer to a 500ml volumetric flask and bring the volume up to 500ml with nanopure de-ionized water.

Transfer to a 500ml bottle and autoclave.

2. Preparation of 1% Bovine serum Albumin (BSA) Blocking Solution

i. Combine the follow in 100ml nanopure de-ionized water:
   a. 5g BSA
   b. 75ml 20X SSC

ii. Add nanopure de-ionized water to a final volume of 500ml.

Filter sterilize.

3. Preparation of Saline Sodium Citrate (SSC) Stringent Array Washes

a. High Stringency: 0.1X SSC

Make 0.1X SSC by placing 2.5ml 20X SSC in a 500ml graduated cylinder and adjusting the volume with nanopure de-ionized water to a final volume of 500ml.

Filter sterilize by using a bottle top filter and pour into a 500ml bottle. (Save the filter for the next reagent)

b. Medium Stringency: 0.1X SSC, 0.2% Sodium dodecyl Sulphate (SDS)

Combine the following with approximately 250ml nanopure de-ionized water:

2.5ml 20X SSC

5ml 20% SDS

Add additional nanopure de-ionized water for a final volume of 500ml.

Filter sterilize by using the bottle top filter from high stringency filtration and pour into a 500bottle.

c. Low Stringency: 1X SSC, 0.2% Sodium dodecyl sulfate (SDS)

Combine the following with approximately 250ml nanopure de-ionized water:

25ml 20X SSC

5ml 20% SDS

Add additional nanopure de-ionized water for a final volume of 500ml.

Filter sterilize by using the bottle top filter from previous filtrations and pour into a 500ml bottle.

Do not autoclave.

4. Preparation of Tris- Nacl Tween (TNT) Buffer

For a final volume of 1liter:

Measure 850 ml de-ionized nanopure water in a graduated cylinder or volumetric flask.

Add the following reagents:

100ml 1M Tris-HCl, pH 7.5

30ml 5M NaCL

500µl Tween-20

Bring volume to 1litre using de-ionized nanopure water, seal cylinder with parafilm or cap flask mix by inverting several times.

Filter sterilized using a bottle top 0.2µl filter into a bottle.

5. Preparation of TSA Detection Recipes (make 100µl per sample (two wells) and use 45 µl aliquots per well)

i. For 1:100 SA-HRP, TNB: add 1µl SA-HRP to 99µl TNB
ii. For 10% fetal equine serum (FES) in 2X SSC: add 10µl FES and 10µl 20X SSC to 80µl PCR water.
iii. For 1:50 biotinyl tyramide (BioT), 1X amplification diluent (AmpDil): add 2 µl BioT (thaw before using) to 98 µl AmpDil.
iv. For 1:50 SA-Alexa 555, 1X SSC, 5X Denhardt's solution: add 0.8µl SA-Alexa, 20.0 µl 20X SSC, and 40µl Denhardt's solution to 339.2µl PCR water.

6. Preparation of 0.5X Tris- Borate EDTA (TBE) pH 8.5

For a final volume of 10 litres:

i. In a 1litre beaker, combine a stir bar and approximately 800ml Nanopure Deionized water.

ii. Weigh out 54g Tris base and 27.5g boric acid.

iii. Add to water while stirring.

iv. Once all solid material dissolves, add 20ml 0.5M EDTA pH 8.0.

v. Bring volume up to 1 litres with deionized water.

vi. Combine above 1 litre from step 4 (5X concentration) with 9 litre deionized water in a Nalgene Carboy for a final volume of 10 litres with final concentration of 0.5X (pH of 0.5X TBE should be 8.5)

vii. Mix by shaking and rolling the 10 litres carboy vigorously.

For a final volume of 20ml:

viii. In a 2 litres beaker, combine a stir bar and approximately 1800ml Nanopure water.

ix. Weigh out 108g Tris base and 55g boric acid.

x. Add to water while stirring.

xi. Once all solid material dissolves, add 40ml 0.5 M EDTA pH 8.0

xii. Bring volume up to 2 litres with deionized water (5X concentration).

xiii. Combine above 2 litres from step 8 (5X concentration) with 18 litres deionized water for a final volume of 20 litres and a final concentration of 0.5X (pH of 0.5X TBE should be 8.5).

Mix by shaking and rolling the 20 litres carboy vigorously.

7a. Preparation of 50X TAE

For a final volume of 100ml

i. Weigh out 24.2g Tris base, and add with a stir bar to a 100ml volumetric flask.

ii. Bring volume up to approximately 60ml with deionized water. Stir to dissolve.

iii. Add 20ml 0.5M EDTA pH 8, and 5.71 ml glacial acetic acid.

iv. Bring final volume up to 100ml with deionized water.

7b. Preparation of 1X TAE

To make 1X TAE, combine 100ml 50X TAE with 4.9 liters deionized water for a final volume of 5 Liters

APPENDIX 3

Nucleotide sequence of Bacteria

Nucleotide sequence of Bacteria from Dam 1 Ife (Code: IRW= Ife raw water, IFFW= Ife treated water, IFM1 and IFM2= Ife Municipal 1 and 2 taps)

Organism: ***Acinetobacter junii*** **Accession no: AM184279.1** **Source: Dam 1 IFRW** **Strain ID (317)**
TCTGNGAGTACGTCNCTATCCAGTAGTATTAATACTAGCTAGCCTCCTCCTCGCTTAAAGTGCTTTACANCCTTNAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTCCGCCCATTGTCCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACTT
AGGCTCATCTATTAGCGCAAGGNCCGAAGATCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCATTCCTTTCGGAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGTCC
GCCGCTAAGCTAAGGTGCAAGCACCCTCGCTCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAAT

Organism: ***Aeromonas hydrophila*** **Accession no: FR870443.1** **Source: Dam 1 IFRW** **Strain ID: 321A**
GGCTTTTGGAGTACGTCACAGCCAGCAGATATTAGCTACTGACCTTTCCTCCTCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGGTTTCCCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCC
ACCTGGGCATATCCAATCGCGCAAGGCCCGAAGGTCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTTATCCCCCTCGACTGGGCAATTTCCCAGGCATTACTCACCC
GTCCGCCGCTCGCCGGCAAAAGTAGCAAGCTACTTTCCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Aeromonas caviae*** **Accession no: AB626132.1** **Source: Dam 1 IFRW** **Strain ID: 321B**
GNTCTCNCGAGTACGTCCAGCCAGCAGATATTAGCTACTGACCTTTCCTCCTCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGGTTTCCCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGGCCATTACCTCACCAACTAGCTAATCCCACC
TGGGCATATCCAATCGCGCAAGGCCCGAAGGTCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTTATCCCCCTCGACTGGGCAATTTCCCAGGCATTACTCACCCGTC
CGCCGCTCGCCGGCAAAAGTAGCAAGCTACTTTCCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Comamonas sp*** **Accession no: EF547998.1** **Source: Dam 1 IFRW** **Strain ID: 318B**
GCTTTCTTCGGTACGTCATGACCCGGGGATATTAGCCCCAGGCTTTTCGTTCCGTACAAAAGCAGTTTACAACCCGAGGGCCTTCATCCTGCACGCGGCATTGCTGGATCAGGCTTTCGCCCATTGTCC
AAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAGACCAGCTACAGATCGCAGGCTTGGTAAGCCTTTACCCCACCAACTACCTAATCTG
CCATCAGCCGCTCTAGTAGCACAAGGCCCGAAGGTCCCCTGCTTTCATCCGTAGATCTCATGCGGTATTAGCTACTCTTTCGAGTAGTTATCCCCCACTACTAGGCACGTTCCGATGTATTACTCACCCG
TTCGCCACTCGTCAGCATCCGAAGACCTGTTACCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAATC

Organism: ***Comamonas testosteroni*** **Accession no: GU428961.1** **Source: Dam 1 IFRW** **Strain ID: 318A2**
GGCTTCTTCGGTACGTCATGGACCCGGGGATATTAGCCCCAGGCTTTTCGTTCCGTACAAAAGCAGTTTACAACCCGAGGGCCTTCATCCTGCACGCGGCATTGCTGGATCAGGCTTTCGCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAGACCAGCTACAGATCGCAGGCTTGGTAAGCCTTTACCCCACCAACTACCTAATCT
GCCATCAGCCGCTCTAGTAGCACAAGGCCCGAAGGTCCCCTGCTTTCATCCGTAGATCTCATGCGGTATTAGCTACTCTTTCGAGTAGTTATCCCCCACTACTAGGCACGTTCCGATGTATTACTCACCC
GTTCGCCACTCGTCAGCATCCGAAGACCTGTTACCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAAT

Organism: ***Escherichia coli*** **Accession no: JN644604.1** **Source: Dam 1 IFRW** **Strain ID: 319B**
TGNGGGTACGTCATGAGNAAAGGAATTAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCNTAGGGCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATT
CCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGNTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGG
GCACATCCGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCC
ACTCGTCAGCAAAGAAGCAAGCTTCTTCCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGAGCCAT

Organism: ***Escherichia coli*** **Accession no: JN162446.1** **Source: Dam 1 IFRW** **Strain ID: 82B**
GGCTTTTGGGGTACGTCATGAGCAAAGGTATTAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCA
TCTGGGCACATCCGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGT
CCGCCACTCGTCAGCGAAGCAGCAAGCTGCTTCCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Klebsiella pneumoniae*** **Accession no: HQ288920.1** **Source: Dam 1 IFRW** **Strain ID: 330**
GGCGGCTTTTTGGGGNAAGTCAATNGNTGAGGTTATTAACCTNACCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCTTNAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCAT
TGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTA
ATCCCATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTC
ACCCGTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTG

Organism: ***Klebsiella pneumoniae*** **Accession no: JF919921.1** **Source: Dam 1 IFRW** **Strain ID: 320**
TCGNGGGTACGTCAATCGANAAGGTTATTAACCTTATCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCNAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTG
GGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACNTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGC
CGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Escherichia coli*** **Accession no: AP010960.1** **Source: Dam 1 IFRW** **Strain ID: 319**
GCTTTTGGGGTACGTCAATGAGTNAAGGTATTAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCA
TCTGGGCACATCCGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGT
CCGCCACTCGTCAGCAAAGAAGCAAGCTTCTTCCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Proteus mirabilis*** **Accession no: AB626123.1** **Source: Dam 1 IFRW** **Strain ID: 117**
TAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCT
GGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATATGGGTTCATCCGATAGTGCAAGGTCCGAAG
AGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCT
CCTGTTACCGCTCGACTTGCATGTGT

Organism: ***Providencia vermicola*** **IFRW** **Accession no: NR_042415.1** **Source: Dam 1 IFRW** **Strain ID: 82**
TTNTGNGGTACGTCATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTNNGGNCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCNCACCTACTAGCTAATCCCATAT
GGGTTCATCCGATAGCGCAAGGACCGAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCCGC
CGCTCGTCAGCGAGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCT

Organism: ***Pseudomonas sp*** **Accession no: FM161423.1** **Source: Dam 1 IFRW** **Strain ID: 96B**
GGCTNTTGTGGTACGTCAAACAGCAAGGTATTCGCTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAANACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGA
CCTAGGCTCATCTGATAGCGCAAGGNCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCG
TCCGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Bacillus thuringiensis*** **Accession no: EU513392.1** **Source: Dam 1 IFRW** **Strain ID: 116**
TTTGGNTGGTNCGGTCAGGTGCCAGCTTATTCAACTAGCACTTGCTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGA
AGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGA
CGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGT
TACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organism: ***Bacillus sp*** **IFRW** **Accession no: JF766690.1** **Source: Dam 1 IFRW** **Strain ID: (96A)**GGTTTTGGNTGGTACGTCAAGGGTACCGCCCTATTCGAACGGTACTTGCTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCC
ATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGC
TAATGCGCCGCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTAC
CCACGTGTTACTCACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus subtilis*** **Accession no: HE582781.1** **Source: Dam 1 IFRW** **Strain ID: 84**
GGTNCGTCNAGGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTC
CCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGG
TCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAAGCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCA
CCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Bacillus pumilus*** **Accession no: EF010673.1** **Source: Dam 1 IFRW** **Strain ID: 117**TTTGGTNGGTACGGTCATGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGG
AAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCG
CCGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTG
TTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Bacillus stratosphericus*** **Accession no: FM992660.1** **Source: Dam 1 IFRW** **Strain ID: 95A**
GGTTTGGTTGGTNNGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAA
GATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCC
GCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTT
ACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTG

Organism: ***Stenotrophomonas sp*** **Accession no: JN208914.1** **Source: Dam 1 IFRW** **Strain ID: 152A**
GNTTTNTTGGGTCCGGTCATCCCAACCGGGGTATTAACCAGCTGGATTTCTTTCCCAACAAAAGGGCTTTACAACCCNTTAGGNCTTCTTCACCCACGCGGTATGGCTGGATCAGGCTTGCGCCCATTG
TCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTACGGATCGTCGCCTTGGTGGGCCTTTACCCCGCCAACTAGCTAATC
CGACATCGGCTCATTCAATCGCGCAAGGTCCGAAGATCCCCTGCTTTCACCCGTAGGTCGTATGCGGTATTAGCGTAAGTTTCCCTACGTTATCCCCCACGAAAAAGTAGATTCCGATGTATTCCTCAC
CCGTCCGCCACTCGCCACCCAGAGAGCAAGCTCTCCTGTGCTGCCGTTCGACTTGCATGTGTTAGGCCTACCGCCAGCGTTCACTC

Organism: ***Alcaligenes sp*** **Accession no: JN019025.1** **Source: Dam 1 IFFW** **Strain ID: 87A**
TNTCTGCGATCCGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCA
AAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGA
TATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGT
CCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Alcaligenes faecalis*** **Accession no: HQ161777.1** **Source: Dam 1 IFFW** **Strain ID: 119A**
TNGCGATNCGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATC
GGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTCCGC
CACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Brevundimonas diminuta*** **Accession no: EU545397.1** **Source: Dam 1 IFFW** **Strain ID: 119B**
TCGGGTCGTCATTATCGTCCCCGGTGAAAGAATTTTACAATCCTAAGACCTTCATCATTCACGCGGCATGGCTGCGTCAGGCTTTCGCCCATTGCGCAAGATTCCCCACTGCTGCCTCCCGTAGGAGTCT
GGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTACTGATCGTCGCCTTGGTGAGCTTTTACCTCACCAACTAGCTAATCAGACGCGGGCCGCTCTAATGGCGATAAATCTTTCC
CCCGAAGGGCACATTCGGTATTAGCACAAGTTTCCCTGAGTTATTCCGAACCAAAAGGCACGTTCCCACGTGTTACTCACCCGTCCGCCACTAACTCCGAAGAGTTCGTTCGACTTGCATGTGTTAGGC
CTGCCGCCAGCGTTCGCTC

Organism: ***Chromobacterium violaceum*** **Accession no: AE016825.1** **Source: Dam 1 IFFW** **Strain ID: 86**
GTNTCTTNGGTACTGTCATCCCCGCCAGGGTATTAACCAGCGGGATTTCCTCCCTGACAAAAGTCCTTTACAACCCGNAGGCCTTCTTCAGACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACTGATCGTCGCCTTGGTGGGCTCTTACCCCACCAACTAGCTAATCA
GACATCGGCTGCTCGTATAACGCGAGGTCTTTCGATCCCCCGCTTTCCCCCTCAGGGCGTATGCGGTATTAATCCGGCTTTCGCCGAGCTATCCCCCATTACACGGTACATTCCGATGCATTACTCACCC
GTTCGCCACTCGCCACCAGGAGCAAGCTCCCGTGCTGCCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAATC

Organism: ***Proteus mirabilis*** **Accession no: AB626123.1** **Source: Dam 1 IFFW** **Strain ID: 122A**
TTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTC
TGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATATGGGTTCATCCGATAGTGCAAGGTCCGAA
GAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTT
CTCCTGTTACCGCTCGACTTGCATGTGTNA

Organism: ***Proteus mirabilis*** **Accession no: GU420970.1** **Source: Dam 1 IFFW** **Strain ID: 151B**
GCTNTTNGCGGGTACGTCATTGATAAGGGTATTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTTNAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCC
ATATGGGTTCATCCGATAGTGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCC
GTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Proteus mirabilis*** **Accession no: GU420970.1** **Source: Dam 1 IFFW** **Strain ID: 316**
CGGCTNTCGNGGGTACGTCAATTGATAAGGGTATTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTNAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGT
GCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATC
CCATATGGGTTCATCCGATAGTGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCAC
CCGTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Pseudomonas sp*** **IFFW** **Accession no: HM352366.1** **Source: Dam 1 IFFW** **Strain ID: 89**
CTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGA
CCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGNCCGAAGGNN
CCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCC
GCTCGACTTGCATGTGT

Organism: ***Pseudomonas sp*** **Accession no: HM352366.1** **Source: Dam 1 IFFW** **Strain ID: 89**
GCTTCTGTCGGTACGTCAAACAGCAAGGTATTCGCTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGTNAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTCGCCCATTGTCCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCT
AGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCGTCC
GCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Bacillus subtilis*** **Accession no: HQ622347.1** **Source: Dam 1 IFFW** **Strain ID: 146A**
ATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAG
TCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGGTAGCCGAA
GCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCAGGG
AGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTAT

Organism: ***Bacillus pumilus*** **Accession no: EF010673.1** **Source: Dam 1 IFFW** **Strain ID: 122B**
TTTGGTNGGTNCGTCAAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAAAGAGCTTTACGANCNTTAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCG
GGTCCATCTGTAAGTGACAGCCGAAACGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCAC
CCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus thuringiensis*** **Accession no: EU513392.1** **Source: Dam 1 IFFW** **Strain ID: 120**
TGGNTGGTNCGGTCAAGGTGCCAGCTTATTCAACTAGCACTTGCTTCTTCCCTAACAACAGCAGATTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCG
GAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGC
GACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCNGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGT
GTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organism: ***Bacillus pumilus*** **Accession no: EU741068.1** **Source: Dam 1 IFFW** **Strain ID: 146**
TTTGGTTGGTACGTCATGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus sp*** **Accession no: AB678447.1** **Source: Dam 1 IFFW** **Strain ID: 85B**
CTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGNTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTG
GGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGACAGCCGAAACC
GTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCCGGGAGC
AAGCTCCCTTCTGTCCGCTCGACTTGCATGTATNA

Organism: ***Bacillus thuringiensis*** **Accession no: JN377782.1** **Source: Dam 1 IFFW** **Strain ID: 85A**
GGCTNTGGNTGGGACGTCAAGGTGCCAGCTTATTCAACTAGCACTTGCTTCTTCCCTAACAACAGAGATTTTACGACCCGTAAAGCCTTCATCACTCACGCGGCGGTTGCTCCGTCAGACTTTCGTCCA
TTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCT
AATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACC
CACGTGTTACTCACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCC

Organism: ***Lysinibacillus xylanilyticus*** **Accession no: JN644597.1** **Source: Dam 1 IFFW** **Strain ID: 88**
TAGGTACGTCAGGTACAGCCAGTTACTACTGTACTTGTTCTTCCCTTACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTGGAAGATTCCC
TACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGCC
CATCCTATAGCGACAGCCGAAACCGTCTTTCAGTCTTTNACCATGAAGCAAAAGAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAGGGTAGGTTGCCCACGTGTTACTCACC
CGTCCGCCGCTAACGTCAAAGGAGCAAGCTCCTTTTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: Uncultured bacterium clone **Accession no: JN595762.1** **Source: Dam 1 IFM1** **Strain ID: 92A**
GGCTCTCTGNGGTACGTCATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTAGGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCCCACCTACTAGCTAATCCC
ATATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCC
GTCCGCCGCTCGTCAGCGAGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Chromobacterium violaceum **Accession no: HM449690.1** **Source: Dam 1 IFM1** **Strain ID: 129**
GGCTNTCTCGGTCTCTCAGCCCCAGTAGGTATTAACCACTGGGATTTGCTCCCGGACAAAAGTCCTTTACAACCCGAAGGCCTTCTTCAGACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCC
AAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACTGATCGAAGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCAG
ACATCGGCTGCTCGTATAACGTGAGGCCTTTCGGTCCCCCACTTTCCCCCTCAGGGCGTATGCGGTATTAATCCGGCTTTCGCCGAGCTATCCCCCATTACACGGTACATTCCGATGCATTACTCACCCG
TTCGCCACTCGTCAGCGGTGCAAGCACCCTGTTACCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAAT

Organism: ***Bacillus pumilus*** **IFM1** **Accession no: HQ432808.1** **Source: Dam 1 IFM1** **Strain ID: (124)**
TTTGGTTGGTACGGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Bacillus thuringiensis*** **Accession no: JN377782.1** **Source: Dam 1 IFM1** **Strain ID: 329**
TNGTNCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGCTTCTTCCCTAACAACAGCAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGA
TTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGTCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCG
GGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACT
CACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organism: ***Bacillus stratosphericus*** **Accession no: FM992660.1** **Source: Dam 1 IFM1** **Strain ID: 154B**
TTTGGTTGGTNCGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGA
TTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGC
GGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTAC
TCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Bacillus sp.*** **Accession no: JF683264.1** **Source: Dam 1 IFM2** **Strain ID: 322**
GCTTTGGNTGGACGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAA
GATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCC
GCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTT
ACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Nucleotide sequence of Bacteria from Dam 2 Ede (Code: EDRW= Ede raw water, EDFW= Ede treated water, EDM1 and EDM2= Ede Municipal 1 and 2 taps)

Organism: ***Acinetobacter baumannii*** **Accession no: JN162444.1** **Source: Dam 2 EDRW** **Strain ID: (54)**
GNGAGTACGTCCACTATCCCTAGGTATTAACTAGAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTGTCCAATAT
TCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACTTA
GGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATCCCTTTCGAGATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGTCCGC
CGCTAGGTCCGGTAGCAAGCTACCTTCCCCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATC

Organism: ***Acinetobacter sp*** **Accession no: GU726181.1** **Source: Dam 2 EDRW** **Strain ID: (390)**
GNTNTCTGCGAGTACGTCCACTATCCTAGAGTATTAATCTAAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCAANAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTCCGCCCCATTGT
CCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATC
CGACTTAGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCATTCCTTTCGGAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACC
CGTCCGCCGCTAAGCTAAGGTGCAAGCACCCTCGCTCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCGCGTTCAATC

Organism: ***Comamonas sp*** **Accession no: JN544144.1** **Source: Dam 2 EDRW** **Strain ID: (94B)**
TTGGCTNTCTNGGTACGTCATGACCCCAGGGTATTAACCCAGGGCTTTTCGTTCCGACAAAAGCAGTTTACAACCCTNAGGCCTTCATCCTGCACGCGGCATTGCTGGATCAGGCTTGCGCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTTCTCAGTCCCAGTGTGGCTGATCGTCCTCTCAGACCAGCTACAGATCGTCGGCTTGGAAGCTTTTATCCCACCAACTACCTAATCTGCC
ATCGGCCGCTCTAGTAGCACAAGGTCTTGCGATCCCCTGCTTTCATCCGTAGATCTCATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCACTACTAGGCACGTTCCGATGTATTACTCACCCGTT
CGCCACTCGTCAGCATCCGAAGACCTGTTACCGTTCGACTTGCATGTGTTAAGGCATGCCGCCAGCGTTCAAT

Organism: ***Pseudomonas putida*** **Accession no: EU196391.1** **Source: Dam 2 EDRW** **Strain ID: (99A)**
GCGGTTTCTGTNGGTACGTCAAACAGCAAGGTATTCGCTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGNAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTC
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCC
GACCTAGGCTCATCTGATAGCGCAAGGNCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACC
CGTCCGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAA

Organism: ***Pseudomonas sp*** **Accession no: HQ832880.1** **Source: Dam 2 EDRW** **Strain ID: (299A)**
GGCTTTTGTGGTACGTCAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCNNANACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGGGCCATTACCCCACCAACTAGCTAATCCGA
CCTAGGCTCATCTGATAGCGTGAGGNCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCG
TCCGCCGCTGAATCATGNAGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Stenotrophomonas maltophilia*** **Accession no: JN703732.1** **Source: Dam 2 EDRW** **Strain ID: (58A)**
GCTNTCTGAGATCCGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCC
GATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCC
GTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Sphingobacterium sp*** **Accession no: HM352374.1** **Source: Dam 2 EDRW** **Strain ID: (99B)**
TGGTNGGTNCGTCAAGGTGCAAGCAGTTACTCTTGCACTTGCTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGT

Organism: ***Stenotrophomonas maltophilia*** **Accession no: JN703732.1** **Source: Dam 2 EDRW** **Strain ID: (58A)**
GNTNTTTGGGTACGGTCATCCCAACCGGGTATTAGCCAGCTGGATTTCTTTCCCAACAAAAGGGCTTTACAACCCGAAGGCCTTCTTCACCCACGCGGTATGGCTGGATCAGGCTTGCGCCCATTGTC
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTACGGATCGTCGCCTTGGTGGGCCTTTACCCCGCCAACTAGCTAATCCG
ACATCGGCTCATTCAATCGCGCAAGGTCCGAAGATCCCCTGCTTTCACCCGTAGGTCGTATGCGGTATTAGCGTAAGTTTCCCTACGTTATCCCCCACGAAAAAGTAGATTCCGATGTATTCCTCACCC
GTCCGCCACTCGCCACCCAGAGAGCAAGCTCTCCTGTGCTGCCGTTCGACTTGCATGTGTTAGGCCTACCGCCAGCGTTCACT

Organism: ***Morganella morganii*** **Accession no: FJ971868.1** **Source: Dam 2 EDRW** **Strain ID: (384)**
GCTTTTGNGGTACGTCAATTGNTAAGGTTATTAACCTTGACACCTTCCTCCCGACTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTAAGCCGTTACCTTACCTACTAGCTAATCCCAT
ATGGGTTCATCTGATGGCGCGAGGCCCGGAGGTCCCCCGCTTTGGTCCGAAGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCGCCATCAGGCAGATCCCCATACATTACTCACCCGT
CCGCCGCTCGTCAGCAGAGAAGCAAGCTTCTCCCTGTTACCGCCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTG

Organism: ***Bacillus thuringiensis*** **Accession no: JN084031.1** **Source: Dam 2 EDRW** **Strain ID: (55)**
TTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGG
AGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCCGGGTCCATCCATAAGTGACAGCCG
AAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCTTG
AGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATT

Organism: ***Bacillus sp*** **Accession no: JF766690.1** **Source: Dam 2 EDRW** **Strain ID: (132)**
TTNTGGTTGGTACCGTCAAGGGTACCGCCCTATTCGAACGGTACTTGTTTCTTCCCTAACAACAGAGACTTTACGATCTTGAAAACCTTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCAT
TGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGCTA
ATGCGCCGCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCC
ACGTGTTACTCACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus altitudinis*** **Accession no: HQ432811.1** **Source: Dam 2 EDRW** **Strain ID: (52B)**
GTNCGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTAC
TGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCAT
CTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGT
CCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Bacillus sp*** **Accession no: JF766690.1** **Source: Dam 2 EDRW** **Strain ID: (131)**
GGTTGGTCCGTCAGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAACAACAGCAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGA
TTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATGCGCCGC
GGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTAC
TCACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus pumilus*** **Accession no: HQ432808.1** **Source: Dam 2 EDRW** **Strain ID: (52A)**
TTTGGTNGGTNCGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGAAGAT
TCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCG
GGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACT
CACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus sp.*** **Accession no: AB678448.1** **Source: Dam 2 EDRW** **Strain ID: (57)**
GTCTTCCCTAACAACAGAGTTTTACGNCNTTNAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTC
AGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTT
CGAACCATGCNGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAAT
CCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organism: ***Bacillus sp*** **Accession no: GU566359.1** **Source: Dam 2 EDRW** **Strain ID: (133)**
GTTGGTCCGTCAGGTACCGGCAGTTACTCCGGTACTTGTTCTTCCCTAACAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCC
CTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGC
CCATCTGTAAGTGATAGCAGAACCATCTTTCAATCTAATTATATGTATAAATAGATATTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTGCCCACGTGTTACTCACCC
GTCCGCCGCTAATCTTTGGGAGCAAGCTCCCTCAGATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus sp*** **Accession no: HE613375.1** **Source: Dam 2 EDRW** **Strain ID: (52B)**
AAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGT
AGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGACAG
CCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATC
CGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATG

Organism: ***Bacillus sp*** **Accession no: GU566359.1** **Source: Dam 2 EDRW** **Strain ID: (133)**
GGTNTGGTGGTNCGTCAGGTACCGGCAGTTACTCCGGTACTTGTTCTTCCCTAACAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAA
GATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATCCGCC
GCGGGCCCATCTGTAAGTGATAGCAGAACCATCTTTCAATCTAATTATATGTATAAATAGATATTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTGCCCACGTGTTA
CTCACCCGTCCGCCGCTAATCTTTGGGAGCAAGCTCCCTCAGATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Bordetella sp*** **Accession no: HQ840720.1** **Source: Dam 2 EDRW** **Strain ID: (51)**
TNGCGGTNCGTCATTAGCGCCAGGTATTAGCTGGCGCCGCTTTCTTTCCTGCCAAAAGTGCTTTACAACCCTTAAGGCCTTCATCGCACACGCGGGATGGCTGGATCAGGGTTGCCCCCATTGTCCAAA
ATTCCCCACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTCGCCTTGGTGAGCCGTTACCCCACCAACTAGCTAATCCGATA
TCGGCCGCTCCAATAGTGCGAGGCCCGAAGGTCCCCCGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACGCTTTCGCGTAGTTATCCCCCGCTACTGGGCACGTTCCGATACATTACTCACCCGTT
CGCCACTCGNCACCAGACCGAAGTCCGTGCTGNCGTCCGACTTGCATGTGTAAAGCATCCCGCCAGCGTTCAA

Organism: ***Chromobacterium violaceum*** **Accession no: HM449690.1** **Source: Dam 2 EDRW** **Strain ID: (382)**
CCNTTTAGGGNTCTTCAGACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGCGGATCATCCTCTCAG
ACCCGCTACTGATCGAAGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCAGACATCGGCTGCTCGTATAACGTGAGGCCTTACGGTCCCCCACTTCCCCCTCAGGGCGTATGCGGTATTAATCCG
GCTTTCGCCGAGCTATCCCCCATTACCGGTACATTCCGATGCATTACTCACCCGTTCGCCACTCGTCAGCGGTGCAAGCACCCGTTACCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAAT
C

Organism: ***Proteus vulgaris*** **Accession no: JN630888.1** **Source: Dam 2 EDRW** **Strain ID: (58B)**
GGCTTTTGGGGTACGTCATTGCTNAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGNATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCAT
ATGGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATNGGGCAGATCCCCATACATTACTCACCCGT
CCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAA

Organism: ***Pseudomonas sp*** **Accession no: HQ832880.1** **Source: Dam 2 EDRW** **Strain ID: (299B2)**
TGTTTACAATCCTTTAGACCTTCTTCACACACGCGGNATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGAT
CATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGGGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGTGAGGTCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGC
GGTATTAGCGTTCCTTTCGAAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGAATCATGNAGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTAGGCCTGC
CGCCAGCGTTCAATCTGAGCCA

Organism: ***Staphylococcus sp*** **Accession no: JN695710.1** **Source: Dam 2 EDRW** **Strain ID: (98)**
CACAGTTACTTACACATTTGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAGACCTTCATCACTCACGCGGCGTTGCTCCGTCAGNTTTTCGCCCATTGCGGAAGATTCCCTCTGCTGCCTCCCGTAG
GAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTAGGTCGGCTACGTATCGTTGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATACGGCGCGGGTCCATCTATAAGTGATAGCAAAA
CCATCTTTCACTTTAGAACCATGCGGTTCTAAATGTTATCCGGCATTAGCCCCGGTTTCCCGGAGTTATTCCAGTCTTATAGGTAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACGTCAAAGGA
GCAAGCTCCTTATCTGTTCGCTCGACTTGCATGTA

Organism: ***Bacillus cereus*** **Accession no: AP007209.1** **Source: Dam 2 EDFW** **Strain ID: (137A1)**
CTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTATGCTGCCTCCCGTAGG
AGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGNGGGTCCATCCATAAGTGACAGCCG
AAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATA
AGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTA

Organism: ***Alcaligenes faecalis*** **Accession no: HM145896.** **Source: Dam 2 EDFW** **Strain ID: (28A)**
GGCTTTCTGAGATACGGTCAGCAGTATCTCGTATTAGGAGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCNTAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCC
GATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCC
GTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Alcaligenes sp*** **Accession no: JN019025.1** **Source: Dam 2 EDFW** **Strain ID: (137B)**
GCTNTCTGAGATCCGGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGT
CCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATC
CGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACC
CGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCAT
CCCGCTAGCGTTCAAT

Organism: ***Morganella sp.*** **Accession no: GQ179706.1** **Source: Dam 2 EDFW** **Strain ID: (u)**
GGCTCTCTGTGGTACGTCATTGATAAGGTTATTAACCTTACCACCTTCCTCCCGACTGAAAGTACTTTACAACCCCTTTANGNCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCTNACCTACTAGCTAATCC
CATATGGGTTCATCTGATGGCGCGAGGCCCGAAGGTCCCCCGCTTTGGTCCGTAGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCGCCATCAGGCAGATCCCCATACATTACTCACC
CGTCCGCCGCTCGTCAGCAGAGAAGCAAGCTTCTCCCTGTTACCGCCCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTG

Organism: ***Bacillus sp*** **Accession no: JF766690.1** **Source: Dam 2 EDFW** **Strain ID: (104)**
GGTGGACGTCAGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGCTTTACGATCTNAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTC
CCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATGCGCCGCGGG
TCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCA
CCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus sp*** **Accession no: HM234004.1** **Source: Dam 2 EDFW** **Strain ID: (60)**
TAGGTACGTCAAGGGTACAGCCAGTTACTACTGTACTTGTTCTTCCCTTACAACAGAGTTTTACGAACCGAAATCCTTCTTCACTCACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTGGAAGATTC
CCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGG
CCCATCCTATAGCGACAGCCGAAACCGTCTTTNAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCACGTGTTACTCA
CCCGTCCGCCGCTAACGTCNAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Lysinibacillus sp*** **Accession no: JN695724.1** **Source: Dam 2 EDFW** **Strain ID: (59)**
TGGCTTTATAGGTACGCTCAGGGTACAGCCAGTTACTACTGTACTTGCTTCTTCCCTTACAACAGCAGATTTTACGAACCGAAATCCTTCTTCACTCACGCGGCGATTGCTCCATCAGGCTTTCGCCCAT
TGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTA
ATGCGCCGCGGGCCCATCCTATAGCGACAGCCGAAACCGTCTTTNAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCC
ACGTGTTACTCACCCGTCCGCCGCTAACGTCNAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCNTG

Organism: ***Acinetobacter baumannii*** **Accession no: JF919865.1** **Source: Dam 2 EDM1** **Strain ID: (107A)**
GGCTNTCTGCGAGTACGTCCACTATCTCTAGGTATTAACTAAAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTGT
CCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATC
CGACTTAGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATCCCTTTCGAGATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACC
CGTCCGCCGCTAGGTCCGGTAGCAAGCTACCTTCCCCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATC

Organism: ***Acinetobacter baumannii*** **Accession no: JN162444.1** **Source: Dam 2 EDM1** **Strain ID: (139A)**
TATCNCTAGGTATTAACTAGAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCNTAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTGTCCAATATTCCCCACTGCTGCCT
CCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACTTAGGCTCATCTATTAGC
GCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATCCCTTTCGAGATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGTCCGCCGCTAGGTCCGGTA
GCAAGCTACCTTCCCCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATCT

Organism: ***Acinetobacter baumannii*** **Accession no: JF919865.1** **Source: Dam 2 EDM1** **Strain ID: (109)**
GTTTTCTGCGAGTACGTCCACTATCTCTAGGTATTAACTAAAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCNTAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTGTC
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCC
GACTTAGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATCCCTTTCGAGATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCC
GTCCGCCGCTAGGTCCGGTAGCAAGCTACCTTCCCCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATCTG

Organism: ***Pseudomonas putida*** **Accession no: EU196391.1** **Source: Dam 2 EDM1** **Strain ID: (85B)**
GCTTTTGGNGGTACGTCAAACAGCAAGGTATTCGCTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGA
CCTAGGCTCATCTGATAGCGCAAGGNCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCG
TCCGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Pseudomonas sp*** **Accession no: HQ832880.1** **Source: Dam 2 EDM1** **Strain ID: (304A)**
GGCTNTNGGTGGTACGGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCNNAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGT
CCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCC
GACCTAGGCTCATCTGATAGCGTGAGGNCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACC
CGTCCGCCGCTGAATCATGGAGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Pseudomonas sp*** **Accession no: HM352366.1** **Source: Dam 2 EDM1** **Strain ID: (106)**
TTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGNTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGAC
CGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGNCC
CCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCG
CTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGT

Organism: ***Pseudomonas sp*** **Accession no: HQ832880.1** **Source: Dam 2 EDM1** **Strain ID: (306A)**
GGCTTCTGTGGTACGTCAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGGGCCATTACCCCACCAACTAGCTAATCCGA
CCTAGGCTCATCTGATAGCGTGAGGTCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCG
TCCGCCGCTGAATCATGGAGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Bacillus sp*** **Accession no: JF683264.1** **Source: Dam 2 EDM1** **Strain ID: (66A)**
GGTNGGTNCGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATT
CCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCGCGG
GTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTC
ACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTG

Organism: ***Bacillus sp.*** **Accession no: JF683264.1** **Source: Dam 2 EDM1** **Strain ID: (66B)**
GNTTNGGTGGTACGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAA
GATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCC
GCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTT
ACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTG

Organism: ***Bacillus pumilus*** **Accession no: AY792029.1** **Source: Dam 2 EDM1** **Strain ID: (139B2)**
TTTGGTNGGTCCGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus amyloliquefaciens*** **Accession no: JF802170.1** **Source: Dam 2 EDM1** **Strain ID: (67B)**
GNTTNGGTGGTACGTCAAGGTGCCGCCCTATTTGAACGGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGG
AAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCG
CCGCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTCTGAACCATGCGGTTCAAACAANCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTG
TTACTCACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGT

Organism: ***Bacillus firmus*** **Accession no: JN215489.1** **Source: Dam 2 EDM1** **Strain ID: (107B)**
GGCTNTGGTTGGTACGNTCAAGGATACCGGCAGNTACTCCGTGTACTTGCTTCTTCCCTAACAACAGCAGATTTTACGATCCTTAAAACCTTCATCACGTCACGCGGCGGTTGCTCCGTCAGACTTTCG
TCCATTGCGGAAGATTCCCTACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGNTGCNTTGGTGAGCCGTTACCTCACCAACT
AGCTAATGCGCCGCGGGCCCATCTGTAAGTGATAGCCGAAACCATCTTTCAGCTTTCCCTCATGTGANGGAAAGAATTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGT
TGCCCACGTGTTACTCACCCGTCCGCCGCTGACTTCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGT

Organism: ***Bacillus sp*** **Accession no: AF169546.1** **Source: Dam 2 EDM1** **Strain ID: (139B1)**
CNTTATAGGTACGNTCAAGGGTACAGCCAGGTTACTACTGTACTTGCTTCTTCCCTTACAACAGAGATTTTACGAACCNTAAATCCTTCTTCACTCACGCGGCGATTGCTCCATCAGGCTTTCGCCCATT
GTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAA
TGCGCCGCGGGCCCATCCTATAGCGACAGCCGAAACCGTCTTTNAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCA
CGTGTTACTCACCCGTCCGCCGCTAACGTCNAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGT

Organism: ***Pseudomonas sp*** **Accession no: JF683300.1** **Source: Dam 2 EDM1** **Strain ID: (159B)**
GNGGTACGTCAAATTGCAGAGTATTAATCTACAACCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCC
CCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGGC
TCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCG
CTGAATCCAGGAGCAAGCTCCTTTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Pseudomonas sp*** **Accession no: FM161425.1** **Source: Dam 2 EDM1** **Strain ID: (159A)**
GNTTNTGTGGTACGTCAAATTGCAGAGTATTAATCTACAACCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGACC
TAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCCGAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTC
CGCCGCTCTCAAGAGAAGCAAGCTTCTCTCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Alcaligenes faecalis*** **Accession no: HQ161777.1** **Source: Dam 2 EDM2** **Strain ID: (140B)**
GCTTCTGAGATCCGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCC
AAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCG
ATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCG
TCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Chromobacterium sp*** **Accession no: JF734316.1** **Source: Dam 2 EDM2** **Strain ID: (78)**
GNTTTCTTAGGTNTGGTCATCCCCGCCAGGGTATTAACCAGCGGGATTTCCTCCCTGACAAAAGTCCTTTACAACCCGTAAGGCCTTCTTCAGACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTG
TCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACTGATCGTCGCCTTGGTGGGCTCTTACCCCACCAACTAGCTAATC
AGACATCGGCTGCTCGTATAACGCGAGGTCTTTCGATCCCCCGCTTTCCCCCTCAGGGCGTATGCGGTATTAATCCGGCTTTCGCCGAGCTATCCCCCATTACACGGTACATTCCGATGCATTACTCACC
CGTTCGCCACTCGCCACCAGGAGCAAGCTCCCGTGCTGCCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAAT

Organism: ***Chromobacterium sp*** **Accession no: FJ668944.1** **Source: Dam 2 EDM2** **Strain ID: (76)**
GGCTATCTNGGTCTGTCATCCCCGCCAGGTATTAACCAGCGGGATTTCCTCCCTGACAAAAGTCCTTTACAACCCTTTAGGCCTTCTTCAGACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACTGATCGTCGCCTTGGTGGGCCTCTTACCCCACCAACTAGCTAATCA
GACATCGGCTGCTCGTATAACGCGAGGTCTTTCGATCCCCCGCTTTCCCCCTCAGGGCGTATGCGGTATTAATCCGGCTTTCGCCGAGCTATCCCCCATTACACGGTACATTCCGATGCATTACTCACCC
GTTCGCCACTCGCCACCAGGAGCAAGCTCCCGTGCTGCCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAA

Organism: ***Psychrobacter sp*** **Accession no: HQ730697.1** **Source: Dam 2 EDM2** **Strain ID: (140)**
GGGGGTNTCTGCGCTATGTCATCGTCCATGGGTATTAACCATGGAGTCTTCTTCACTGCTTAAAGTGCTTTACAACCAATAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTGCCCCCATTG
TCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCCGGGCCGTGTCTCAGTCCCGGTGTGGCTGATCATCCTCTCAGACCAGCTACAGATCGTCGCCATGGTAGGCCTTTACCCCACCATCTAGCTAATC
CGACTTAGGCTCATCTAATAGCGAGAGCAACAAGTTGCCCCCTTTCTCCCGTAGGTCGTATGCGGTATTAATTCGAGTTTCCCCGAGCTATCCCCCACTACTAGGTAGATTCCTAAGTATTACTCACCCG
TCCGCCGCTCGTCAGCGAGAAGCAAGCTTCTCCTGTTACCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATC

Organism: ***Providencia vermicola*** **Accession no: JN092797.1** **Source: Dam 2 EDM2** **Strain ID: (140A)**
GTNTTTGNGGTACGTCATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCCCACCTACTAGCTAATCCCAT
ATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTC
CGCCGCTCGTCAGCGGGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism; ***Shigella flexneri*** **Accession no: AE005674.2** **Source: Dam 2 EDM2** **Strain ID: (75)**
TTTTNGGGTACGTCAATGAGNAAAGGTATTAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATC
GGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTCCGC
CACTCGCCACCAAGAGAGCAAGCTCTCTCGTGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAATC

Organism: ***Alcaligenes sp*** **Accession no: HQ730686.1** **Source: Dam 2 EDM2** **Strain ID: (44B)**
TCTGNGATNCGTCAGCAGCATCCCGTATTAGGGGATGCCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATC
GGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTCCGC
CACTCGCCACCAAGAGAGCAAGCTCTCTCGTGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAATC

Organism: ***Escherichia coli*** **Accession no: JN162446.1** **Source: Dam 2 EDM2** **Strain ID: (74)**
TCGNGGGTACGTCAATGAGNAAAGGTATTAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCNAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTG
GGCACATCCGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCG
CCACTCGTCAGCGAAGCAGCAAGCTGNTTCCTGTTACCGTTCGACTTGCATGTGTTAGG
CCTGCCGCCAGCGTTCAAT

Organism: ***Bacillus pumilus*** **Accession no: HQ432808.1** **Source: Dam 2 EDM2** **Strain ID: (310)**
TTTGGTNGGTNCGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: Bacillus sp. **Accession no: JF426151.1** **Source: Dam 2 EDM2** **Strain ID: (110)**
TTTGGNTGGTNCGGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCNAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAA
GATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGAC
GCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCNGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTT
ACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCC

Organism: ***Lysinibacillus sp*** **Accession no: JN695724.1** **Source: Dam 2 EDM2** **Strain ID: (111A)**
GGCTNTATAGGTACGTCAAGGGTACAGCCAGTTACTACGTGTACTTGCTTCTTCCCTTACAACAGCAGTTTTACGANCCNTNAATCCTTCTTCACTCACGCGGCGATTGCTCCATCAGGCTTTCGCCCAT
TGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTA
ATGCGCCGCGGGCCCATCCTATAGCGACAGCCGAAACCGTCTTTNAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCC
ACGTGTTACTCACCCGTCCGCCGCTAACGTCNAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus pumilus*** **Accession no: EF010673.1** **Source: Dam 2 EDM2** **Strain ID: (310)**
GCTNTGGTNGGTACGTCAAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGG
AAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCG
CCGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTG
TTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus sp*** **Accession no: AF169546.1** **Source: Dam 2 EDM2** **Strain ID: (111B)**
TNATAGGTACGNTCAGGGTACAGCCAGNTTACTACTGTACTTGTTCTTCCCTTACAACAGAGTTTTACGAACCGAAATCCTTCTTCACTCACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTGGAAG
ATTCCCTACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGCCCATCCTATAGCGACAGCCGAAACCGTCTTTNAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCACGTGTTA
CTCACCCGTCCGCCGCTAACGTCNAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCG

Nucleotide sequence of Bacteria from Dam 3 Asejire (Code: ARW= Asejire raw water, AFW= Asejire treated water, AM1 and AM2= Ede Municipal 1 and 2 taps)

Organism: ***Acinetobacter baumannii*** **Accession no: GU415594.1** **Source: Dam 3 ARW** **Strain ID: (278)**
GCTNTCGCGAGTACGTCCACTATCCAGAGTATTAGTCTCAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTG
TCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATC
CGACTTAGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATTCCTTTCGGAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACC
CGTCCGCCGCTAAGATAAGGTGCAAGCACCTCATCTCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAAT

Organism: ***Acinetobacter baumannii*** **Accession no: GU415594.1** **Source: Dam 3 ARW** **Strain ID: (276)**
GNGGCTTTCGCGAGTACGTCCACTATCCAAGAGTATTAGTCTCAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCTTAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATT
GTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAA
TCCGACTTAGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATTCCTTTCGGAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCA
CCCGTCCGCCGCTAAGATAAGGTGCAAGCACCTCATCTCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATC

Organism: ***Alcaligenes faecalis*** **Accession no: JN162124.1** **Source: Dam 3 ARW** **Strain ID: (5A)**
GCTNTCTGCGATCCGNTCAGCAGTATCCCGGTATTAGGGGATACCTTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTG
TCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTAGCCTTTACCCCACCAACTAGCTAATC
CGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACC
CGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Alcaligenes faecalis*** **ARW** **Accession no: JN162124.1** **Source: Dam 3 ARW** **Strain ID: (45)**
GNTTTTGCGATCCGTCAGCAGTATCCCGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGNAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCA
AAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGA
TATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGT
CCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Klebsiella pneumoniae*** **Accession no: AB675600.1** **Source: Dam 3 ARW** **Strain ID: (279)**
TATTAACCTCACCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGNCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGA
NTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGCCC
GAAGNNCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGTCACCCGAGAGCAAGCT
CTCTGTGCTACCGCTCGACTTGCATGT

Organism: ***Proteus vulgaris*** **Accession no: JN630888.1** **Source: Dam 3 ARW** **Strain ID: (14B)**
GGCTNTTGGGGTACGTCATTGCTAAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCAT
ATGGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGT
CCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAA

Organism: ***Proteus mirabilis*** **Accession no: GU420970.1** **Source: Dam 3 ARW** **Strain ID: (25)**
GNTNTCGCGGGTACGTCATTGATAGGGTATTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATA
TGGGTTCATCCGATAGTGCAAGGTCCGAAGAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCC
GCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Proteus mirabilis*** **Accession no: AB626123.1** **Source: Dam 3 ARW** **Strain ID: (18B2)**
TTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTC
TGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATATGGGTTCATCCGATAGTGCAAGGTCCGAA
GAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTT
CTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCT

Organism: ***Pseudochrobactrum glaciei*** **Accession no: AB369864.1** **Source: Dam 3 ARW** **Strain ID: (278) (26A2)**
TCTCTCGGTNCGTCATTATCTTCACCGGGTGAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGG
AGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACGCGGGCTCATCTATCTCCGATAAATC
TTTCTCCTTACGGACGTATAGGGTATTAGCACAAGTTTCCCTGAGTTATTCCCTAGAGATAGGTAGATTCCCACGCGTTACTCACCCGTCTGCCGCTCCCTCCGAAAAGGGCGCTCGACTTGCATGTGTT
AAGCCTGCCGCCAGCGTTCGTT

Organism: Uncultured bacterium clone **Accession no: JN595783.1** **Source: Dam 3 ARW** **Strain ID: (18B1)**
GNTTTTGNGGTACGTCAATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTANGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCCCACCTACTAGCTAATCCCA
TATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGT
CCGCCGCTCGTCAGCGNGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCT

Organism: ***Lysinibacillus sp*** **Accession no: JN695724.1** **Source: Dam 3 ARW** **Strain ID: (26A1)**
TAGGTACGNTCAGGTACAGCCAGTTACTACTGTACTTGTTCTTCCCTTACAACAGAGTTTTACGAACCGAAATCCTTCTTCACTCACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTGGAAGATTCC
CTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGC
CCATCCTATAGCGACAGCCGAAACCGTCTTTNAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCACGTGTTACTCAC
CCGTCCGCCGCTAACGTCNAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus pumilus*** **Accession no: EF010673.1** **Source: Dam 3 ARW** **Strain ID: (278A)**
TTTGGTAGGTNCGTCATGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus sp*** **Accession no: HM234004.1** **Source: Dam 3 ARW** **Strain ID: (18A1)**
GCTNTATAGGTACGTCAGGTACAGCCAGTTACTACTGTACTTGTTCTTCCCTTACAACAGAGTTTTACGAACCNAAATCCTTCTTCACTCACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGCCCATCCTATAGCGACAGCCGAAACCGTCTTTNAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCACGTGTTA
CTCACCCGTCCGCCGCTAACGTCNAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Leucobacter komagatae*** **Accession no: AJ746337.1** **Source: Dam 3 ARW** **Strain ID: (26B)**
GNTTTCGCGGTCGTCCTTTCGCTTCTTCCCTACTAAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTTGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCG
TAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCATGGTGAGCCATTACCTCACCATCTAGCTGATAGGCCGCGAGTCCATCCAGAACCGATA
AATCTTTCCACCTACACACCATGCGGTGATAGGTCGTATCCAGTATTAGACACCGTTTCCAGTGCTTATCCCAGAGTTCAGGGCAGGTTACTCACGTGTTACTCACCCGTTCGCCACTCTTCCACCCAGC
AAGCTGGGCTTCATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCGTCC

Organism: ***Bacillus pumilus*** **Accession no: AY792029.1** **Source: Dam 3 ARW** **Strain ID: (14A)**
GNTTTGGTNGGTACGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGA
AGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGC
CGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGT
TACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Alcaligenes sp*** **Accession no: JF303893.1** **Source: Dam 3 AFW** **Strain ID: (9B)**
GCTATCTGCGATCCGTCAGCAGTATCTCGATATTAGGAGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCNTNAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCC
GATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCC
GTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Klebsiella pneumoniae*** **Accession no: JF919909.1** **Source: Dam 3 AFW** **Strain ID: (286)**
GGCTCTCGNGGGTACGTCAATCGATNAGGTTATTAACCTTANCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCNAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCC
CATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACNTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCC
GTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Proteus vulgaris*** **Accession no: JN630888.1** **Source: Dam 3 AFW** **Strain ID: (43)**
GGCTTTNGCGGGTACGTCAATTGCTAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCC
ATATGGGTTCATCCGATAGCGCAAGGTCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCC
GTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Pseudomonas sp*.** **Accession no: FM161425.1** **Source: Dam 3 AFW** **Strain ID: (6B)**
GNTTNTGTGGTAGTCAAATTGCAGAGTATTAATCTACAACCCTTCCTCCCAACTTAAAGTGCTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGACCTA
GGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCG
CCGCTCTCAAGAGAAGCAAGCTTCTCTCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Pseudomonas sp*** **Accession no: FM161522.1** **Source: Dam 3 AFW** **Strain ID: (6B)**
GCTTTTGTGGTACGTCAAANTTGCAGAGTATTAATCTACAACCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGACC
TAGGCTCATCTGATAGCGCAAGGNCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTACNAGGCAGATTCCTAGGCATTACTCACCCGTC
CGCCGCTCTCAAGAGAAGCAAGCTTCTCTCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: Bacillus sp AFW **Accession no: FR774979.1** **Source: Dam 3 AFW** **Strain ID: (9A)**
GTTGGTNCGTCAGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTC
CCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGG
TCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCA
CCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTG

Organism: ***Bacillus pumilus*** **Accession no: EF010673.1** **Source: Dam 3 AFW** **Strain ID: (284)**
TTTGGTGGTACGTCATGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGTAGCTTTACGATCCGAAAACCTTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAA
GATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCC
GCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTT
ACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCC

Organism: ***Bacillus cereus*** **Accession no: JF907013.1** **Source: Dam 3 AFW** **Strain ID: (280)**
TTTGGTTGGTCCGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACG
CGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organism: ***Proteus mirabilis*** **Accession no: JF919934.1** **Source: Dam 3 AFW** **Strain ID: (49)**
GCTNTCGCGGGTACGTCAATTGANAAGGGTATTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCC
ATATGGGTTCATCCGATAGTGCAAGGTCCGAAGAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCC
GTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGC
CGCCAGCGTTCAAT

Organism: ***Myroides odoratus*** **Accession no: AB517709.1** **Source: Dam 3 AFW** **Strain ID: (38A)**
CTNTCTTCGGTACGTCAATAATTACACGTAATCACATTTCTTCCCGTACAAAAGCAGTTTACAATCCATAGGACCGGTCATCCTGCACGCGGCATGGCTGGTTCAGAGTTGCCTCCATTGACCAATATT
CCTCACTGCTGCCTCCCGTAGGAGTCTGGTCCGTGTCTCAGTACCAGTGTGGGGGATCTCCCTCTCAGGACCCCTAATCATCGTAGCCTTGGTATGCCGTTACCACACCAACTAGCTAATGATACGCAT
GCCCATCTTTTACCGATAAATCTTTATTAATCAAACGATGCCATTCAATTAAACCATGGAGCATTAATCCGAATTTCTCCGGGCTATTCCCCTGTAAAAGGTAGGTTGCATACGCGTTACTCACCCATCC
GCCGGTCTCTAGAAAGCAAGCTCTCTATACCCCTCGACTTGCATGTGTTAGGCCTGCCGCTAGCGTTCATC

Organism: ***Pseudomonas sp*** **Accession no: FM161423.1** **Source: Dam 3 AFW** **Strain ID: (6A)**
TTGNNGGTACGTCAAAACAGCAAGGTATTCGCTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTA
GGCTCATCTGATAGCGCAAGGNCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCGTCCG
CCGCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: Uncultured bacterium **Accession no: JN595775.1** **Source: Dam 3 AFW** **Strain ID: (8)**
GTNTNTGCGGTACGTCATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCNCACCTACTAGCTAATCCCAT
ATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTC
CGCCGCTCGTCAGCGAGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: Uncultured bacterium clone **Accession no: JN595774.1** **Source: Dam 3 AFW** **Strain ID: (41)**
GTNTCTGTGGTACGTCAATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTNGGGNCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCCCACCTACTAGCTAATCCCA
TATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGT
CCGCCGCTCGTCAGCGNGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Chromobacterium violaceum*** **Accession no: HM449690.1** **Source: Dam 3 AM1** **Strain ID: (292A2)**
TNTNTTCGGTCTCTAGCCCCAGTAGGTATTAACCACTGGGATTTGCTCCCGGACAAAAGTCCTTTACANCCNTTNAGGCCTTCTTCAGACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAA
AATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACTGATCGAAGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCAGAC
ATCGGCTGCTCGTATAACGTGAGGCCTTACGGTCCCCCACTTTCCCCCTCAGGGCGTATGCGGTATTAATCCGGCTTTCGCCGAGCTATCCCCCATTACACGGTACATTCCGATGCATTACTCACCCGTT
CGCCACTCGTCAGCGGTGCAAGCACCCTGTTACCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAATCTGAGC

Organism: ***Chromobacterium sp*** **Accession no: AB426118.1** **Source: Dam 3 AM1** **Strain ID: (295A)**
GCTTTTTCGGTNTCTCAGCCCCAGTAGGTATTAACCACTGGGATTTGCTCCCGGACAAAAGTCCTTTACAACCCGAAGGCCTTCTTCAGACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCA
AAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACTGATCGTCGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCAGAC
ATCGGCTGCTCGTATAACGTGAGGCCTTACGGTCCCCCACTTTCCCCCTCAGGGCGTATGCGGTATTAATCCGGCTTTCGCCGAGCTATCCCCCATTACACGGTACATTCCGATGCATTACTCACCCGTT
CGCCACTCGTCAGCGGTGCAAGCACCCTGTTACCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAATCTGAAT

Organism: ***Pseudochrobactrum sp*** **Accession no: FJ581024.1** **Source: Dam 3 AM1** **Strain ID: (13A)**
TNTCCGGTNCGTCATTATCTTCACCGAGTGTAAAGAGCTTTACAACCCTAGGGCCTTCATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAG
GAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCAACGCGGGCTCATCTATCTCCGATAAAT
CTTTCTCCTTACGGACGTATAGGGTATTAGCACAAGTTTCCCTGAGTTATTCCCTAGAAATAGGTAGATTCCCACGCGTTACTCACCCGTCTGCCGCTCCCTCCGAAGAGGGCGCTCGACTTGCATGTGT
TAAGCCTGCCGCCAGCGTTCGTT

Organism: ***Bacillus sp*** **Accession no: JF766690.1** **Source: Dam 3 AM1** **Strain ID: (293B)**
GGTGGTNCGTCAGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATT
CCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGG
GTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTC
ACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: Bacillus sp. **Accession no: AM403333.1** **Source: Dam 3 AM1** **Strain ID: (11B)**
TCNGTTGGTCCGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGA
TTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGC
GGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTAC
TCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organism: ***Bacillus pumilus*** **Accession no: AY792029.1** **Source: Dam 3 AM1** **Strain ID: (294)**
GNTTTGGTGGTACGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCTNAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Staphylococcus sp*** **Accession no: HQ730688.1** **Source: Dam 3 AM1** **Strain ID: (295B1)**
GATGGTACGTCAGATGTGCACAGTTACTTACACATTTGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCC
CTACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGTATCGTTGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATACGGCGCGGGT
CCATCTATAAGTGATAGCAAAACCATCTTTTACTTTAGAACCATGCGGTTCCAAATGTTATCCGGCATTAGCTCCGGTTTCCCGAAGTTATTCCAGTCTTATAGGTAGGTTACCCACGTGTTACTCACCC
GTCCGCCGCTAACTTCAAAGGAGCAAGCTCCTTATCCGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCT

Organism: ***Bacillus sp.*** **Accession no: JN210567.1** **Source: Dam 3 AM1** **Strain ID: (295B2)**
TNTGGTTGGTACCGTCAGGGTACCGGCAGTTACTCCGATACTTGCTTCTTCCCTAACAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGA
AGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGC
CGCGGGCCCATCTGTAAGTGATAGCCGAAACCATCTTTCAGCTTAAGGACATGAGTCCAAAAGCATTATCCGGTATTAGCTCCGGTTTCCCGAAGTTATCCCAGTCTTACAGGCAGGTTGCCCACGTGT
TACTCACCCGTCCGCCGCTGACTTCAGGGAGCAAGCTCCCATCCATCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus thuringiensis*** **Accession no: EU513392.1** **Source: Dam 3 AM1** **Strain ID: (30)**
CTNTGGNTGGTACGTCAAGGTGCCAGCTTATTCAACTAGTCACTTGCTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCG
GAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGC
GACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGT
GTTACTCACCCGCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCA

Organism: ***Acinetobacter calcoaceticus*** **Accession no: JN644621.1** **Source: Dam 3 AM2** **Strain ID: (291)**
TCCACTATCTCTAGGTATTAACTAAAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGC
TGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACTTAGGCTCATCTA
TTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATTCCTTTCGAAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGTCCGCCGCTAAGAT
CAGTAGCAAGCTACCTCTCTCCGCTCGACTTGCATGTGT

Organism: ***Acinetobacter baumannii*** **Accession no: GU415594.1** **Source: Dam 3 AM2** **Strain ID: (288B)**
TNTTGNGAGTACGTCCACTATCCAAGAGTATTAGTCTCAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTGTCCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGA
CTTAGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATTCCTTTCGGAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGT
CCGCCGCTAAGATAAGGTGCAAGCACCTCATCTCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAAT

Organism: ***Acinetobacter baumannii*** **Accession no: GU415594.1** **Source: Dam 3 AM2** **Strain ID: (288A)**
TNTCGNGAGTACGTCCACTATCCAAGAGTATTAGTCTCAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTGTCCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGA
CTTAGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATTCCTTTCGGAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGT
CCGCCGCTAAGATAAGGTGCAAGCACCTCATCTCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAAT

Organism: ***Klebsiella pneumoniae*** **Accession no: JF513171.1** **Source: Dam 3 AM2** **Strain ID: (287)**
GGCTCTCGCGGGTACGTCAATCGNTGAGGTTATTAACCTCACCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCNNAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCC
CATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCC
GTCCGCCGCTCGCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAA

Organism: ***Proteus vulgaris*** **AM2** **Accession no: JN630888.1** **Source: Dam 3 AM2** **Strain ID: (13B)**
GGCTTTTGGGGTACGTCATTGCTAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATAT
GGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCC
GCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Pseudomonas sp*** **Accession no: HM352366.1** **Source: Dam 3 AM2** **Strain ID: (290)**
CCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCT
CAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGNCCGAAGGNCCCCTGCTT
TCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACT
TGCATGTGTTAGGCCTGCCGCCAGCG

Organism: Staphylococcus sp **AM2** **Accession no: EU798945.1** **Source: Dam 3 AM2** **Strain ID: (10B)**
CTTTGATNGGTACGGTCAAGACGTGCACAGNTTACTTACACATTTGCTTCTTCCCTAATAACAGAGATTTTACGATCCTTAAGACCTTCATCACTCACGCGGCGGTTGCTCCGTCAGGCTTTCGCCCATT
GCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGTATCGTTGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAA
TACGGCGCGGGTCCATCTATAAGTGATAGCAAAACCATCTTTCACTTTAGAACCATGCGGTTCTAAATGTTATCCGGCATTAGCCCCGGTTTCCCGGAGTTATTCCAGTCTTATAGGTAGGTTACCCAC
GTGTTACTCACCCGTCCGCCGCTAACGTCAAAGGAGCAAGCTCCTTATCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Nucleotide sequence of Bacteria from Dam 4 Eleyele (Code: ERW= Eleyele raw water, EFW= Eleyele treated water, EM1 and EM2= Eleyele Municipal 1 and 2 taps)

Organism: ***Aeromonas punctata*** **Accession no: FM957865.1** **Source: Dam 4 ERW** **Strain ID: (379A)**
GCTNTCGCGAGTACGTCACAGTCAGCAGATATTAGCTACTGGACCTTTCCTCCTCGCTGAAAGTGCTTTACAACCCNAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGGTTTCCCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCC
CACCTGGGCATATCCAATCGCGCAAGGNCCGAAGGTCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTTATCCCCCTCGACTGGGCAATTTCCCAGGCATTACTCACC
CGTCCGCCGCTCGCCGGCAAAAGTAGCAAGCTACTTTCCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Aeromonas hydrophila*** **Accession no: FR870443.1** **Source: Dam 4 ERW** **Strain ID: (391B)**
TCNNGAGTACGTCACAGCCAGCAGATATTAGCTACTGACCTTTCCTCCTCGCTGAAAGTGCTTTACAACCCNAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGGTTTCCCCCATTGTGCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCCACCTG
GGCATATCCAATCGCGCAAGGCCCGAAGGTCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTTATCCCCCTCGACTGGGCAATTTCCCAGGCATTACTCACCCGTCCG
CCGCTCGCCGGCAAAAGTAGCAAGCTACTTTCCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Aeromonas punctata*** **Accession no: FM957865.1** **Source: Dam 4 ERW** **Strain ID: (381)**
GGCTNTCGCGAGTACGTCCAGATCAGCAGATATTAGCTACTGACCTTTCCTCCTCGCTGAAAGTGCTTTACAACCCNAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGGTTTCCCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCC
CACCTGGGCATATCCAATCGCGCAAGGNCCGAAGGTCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTTATCCCCCTCGACTGGGCAATTTCCCAGGCATTACTCACC
CGTCCGCCGCTCGCCGGCAAAAGTAGCAAGCTACTTTCCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Aeromonas caviae*** **Accession no: AB626132.1** **Source: Dam 4 ERW** **Strain ID: (376)**
GGCTNTCGCGNGTACGGTCACAGACCAGCAGAATATTAGCTACTGACCTTTCCTCCTCGCTGAAAGTGCTTTACAACCCNAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGGTTTCCCCCATT
GTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAA
TCCCACCTGGGCATATCCAATCGCGCAAGGNCCGAAGGTCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTTATCCCCCTCGACTGGGCAATTTCCCAGGCATTACTC
ACCCGTCCGCCGCTCGCCGGCAAAAGTAGCAAGCTACTTTCCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Aeromonas hydrophila*** **Accession no: FR870443.1** **Source: Dam 4 ERW** **Strain ID: (391A)**
GCTTTTGCGNGTACGTCACAGCCAGCAGAATATTAGCTACTGACCTTTCCTCCTCGCTGAAAGTGCTTTACAACCCNAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGGTTTCCCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCC
CACCTGGGCATATCCAATCGCGCAAGGCCCGAAGGTCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTTATCCCCCTCGACTGGGCAATTTCCCAGGCATTACTCACC
CGTCCGCCGCTCGCCGGCAAAAGTAGCAAGCTACTTTCCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Alcaligenes sp*** **Accession no: JF303893.1** **Source: Dam 4 ERW** **Strain ID: (3A)**
GNTTCTGCGATCCGGTCAGCAGTATCTCGTATTAGGAGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCC
AAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCG
ATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCG
TCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Alcaligenes sp*** **Accession no: HQ730686.1** **Source: Dam 4 ERW** **Strain ID: (50)**
TNTNGCGATCCGTCAGCAGCATCCCGTATTAGGGGATGCCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCNAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAA
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATAT
CGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTCCG
CCACTCGCCACCAAGAGAGCAAGCTCTCTCGTGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Alcaligenes faecalis*** **Accession no: HQ161777.1** **Source: Dam 4 ERW** **Strain ID: (1B)**
GNTTCTGCGATCCGGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGC
CAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGT
GGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGG
CGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATG
TGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Alcaligenes faecalis*** **Accession no: JN162124.1** **Source: Dam 4 ERW** **Strain ID: (1A)**
TCNCAGATCCGTCAGCAGGNATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCTTAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCA
AAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGA
TATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGT
CCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Klebsiella pneumoniae*** **Accession no: JF513172.1** **Source: Dam 4 ERW** **Strain ID: (386B)**
GNTTTGCGGGTACGTCAATCGATGAGGTTATTAACCTNANCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCTTAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCC
CATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACNTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCC
GTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Klebsiella pneumoniae*** **Accession no: JN545039.1** **Source: Dam 4 ERW** **Strain ID: (378)**
GGTNTTAACCTTATCGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCTTNAGGGCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAG
GAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGC
CCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGTCACCCGAGAGCAAGC
TCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Klebsiella pneumoniae*** **Accession no: JN644581.1** **Source: Dam 4 ERW** **Strain ID: (386A)**
ACCCCTTTAGGGNNTCTTCACACACGCGGCATGGCTGCATNAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCT
CTCAGACCAGCTAGGGATCGTCGCCTAGGNGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGNCCGAAGGTCCCCCANTTTGGTCTTGCGACGTTATGNGGNAT
TAGCTACCGTTTCCAGTANTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGTCACCCNAGAGNAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCG
CCAGNGTTCAATCTGAGCCATGTAT

Organism: ***Klebsiella pneumoniae*** **Accession no: JN545039.1** **Source: Dam 4 ERW** **Strain ID: (375)**
GGCTCTTGCGGGTACGTCAATCGACAAGGTTATTAACCTTATCGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCNAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCC
CATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACNTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCC
GTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Klebsiella pneumoniae*** **Accession no: AB675600.1** **Source: Dam 4 ERW** **Strain ID: (386B)**
CCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGNCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCT
CAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGCCCGAAGGNCCCCCACTT
TGGTCTTGCGACNTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTC
GACTTGCATGTGT

Organism: ***Providencia vermicola*** **Accession no: NR_042415.1** **Source: Dam 4 ERW** **Strain ID: (44A)**
TTGGTTTTGCGNAGTCATCGTTGATGATTTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACACCCTTTNGGGCTTCTTCATACACGCGGTGATGGCGTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGNAGGAGTCTGGGCCGTGTCTCAGTCCCAGGGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGNCTAGGTGAGCCATTACCCCACCTACTAGCTAATCNCAT
ATGGGTTCATCCGATAGAGCAAGGACCGAAGTTCCCCTGCTTTGCTCNTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGT
CCGCCGCTCGTCAGCGAGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGNCAGCGTTCAATCT

Organism: ***Proteus mirabilis*** **Accession no: GU420988.1** **Source: Dam 4 ERW** **Strain ID: (385A)**
GGCTCTNGNGGGGTACGGTCAATTGATAAGGNTATTAACCTTATCACCTTCCTCCCCGCTGAAAGTNCTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTG
TGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTNCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCNCACCTACTAGCTAAT
CCCATATGGGTNNATCCGATAGTGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACANTATGCGGTATTAGCCACCGTTTCCAGNAGTTATCCCCCTCTATNGGGCANATCCCCATACATTACTCA
CCCGTCCGCCGCTCGTCAGC

Organism: ***Proteus vulgaris*** **ERW** **Accession no: JN630888.1** **Source: Dam 4 ERW** **Strain ID: (24B)**
TTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTC
TGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCGATAGCGCAAGGTCCGAA
GAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTT
CTCCTGTTACCGCNCGACTTGCATGTGTT

Organism: ***Proteus vulgaris*** **Accession no: JN630888.1** **Source: Dam 4 ERW** **Strain ID: (33B)**
GGGCTCTCGNGGGTACGTCAATTGCTAAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTNAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGT
GCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATC
CCATATGGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTANACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCAC
CCGTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCT

Organism: ***Trabulsiella guamensis*** **Accession no: AB273737.1** **Source: Dam 4 ERW** **Strain ID: (380A)**
TAACCATTCCCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGCCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCT
GGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGCCCGAAG
GTCCCCCACTTTGGTCCGAAGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGCCGGCGGGAAAGCAAGCTTTC
CCCCGCTGCCGCTCGACTTGCATGTGT

Organism: Uncultured bacterium clone **Accession no: JN595783.1** **Source: Dam 4 ERW** **Strain ID: (44A)**
GGCTCTCTGNGGTACGTCAATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCNTNNGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCCCACCTACTAGCTAATCC
CATATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCC
GTCCGCCGCTCGTCAGCGNGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Bacillus pumilus*** **Accession no: GU808803.1** **Source: Dam 4 ERW** **Strain ID: (377)**
GGTTGGTCCGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTC
CCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCGCGGG
TCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCA
CCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Staphylococcus arlettae*** **Accession no: JN644587.1** **Source: Dam 4 ERW** **Strain ID: (379)**
TGGCTTNTGATAGGTACGTCAAGAACGTGCACAGNTTACTTACACGTTTTGCTTCTTCCCTAATAACAGAGTTTTACGAGCCGAAACCCTTCATCACTCACGCGGCGGTTGCTCCGTCAGGCTTTCGCCC
ATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGTATCGTTGCCTTGGTAAGCCATTACCTTACCAACTAGCT
AATACGGCGCGGGTCCATCTATAAGTGATAGCAAAACCATCTTTCACTTTAGAACCATGCGGTTCTAAATGTTATCCGGCATTAGCCCCGGTTTCCCGGAGTTATTCCAGTCTTATAGGTAGGTTACCC
ACGTGTTACTCACCCGTCCGCCGCTAACGTCAAAGGAGCAAGCTCCTTATCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCAT

Organism: ***Klebsiella pneumoniae*** **Accession no: JF513171.1** **Source: Dam 4 EFW** **Strain ID: (389B1)**
GNTTCTGGGGTACGTCAATCGGTGAGGTTATTAACCTCACCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATA
TCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGT
CCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Proteus vulgaris*** **Accession no: JN630888.1** **Source: Dam 4 EFW** **Strain ID: (46)**
GGCTTTTGGGGTACGTCATTGCTAAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATA
TGGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTC
CGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Staphylococcus cohnii*** **Accession no: GU339231.1** **Source: Dam 4 EM1** **Strain ID: (19A)**
GATGGTACGTCAGACGTGCATAGTTACTTACACATTTGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAGACCTTCATCACTCACGCGGCGTTGCTCCGTCAGGCTTTCGCCCATTGCGGAAGATTCC
CTACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGTATCGTTGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATACGGCGCGGGT
CCATCTATAAGTGATAGCAAAACCATCTTTCACTTTAGAACCATGCGGTTCTAAATGTTATCCGGCATTAGCCCCGGTTTCCCGGAGTTATTCCAGTCTTATAGGTAGGTTACCCACGTGTTACTCACCC
GTCCGCCGCTAACGTCAAAGGAGCAAGCTCCTTATCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCC

Organism: ***Alcaligenes sp*** **Accession no: JN019025.1** **Source: Dam 4 EM1** **Strain ID: (19B1)**
GTTTNTGCGANCCGGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGNAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCC
GATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCC
GTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAATC

E. Nucleotide sequence of Bacteria from Dam 5 Owena-Ondo (Code: OWODW= Owena-ondo raw water, OWODFW= Owena-Ondo treated water, OWODM1 and OWOD2= Owena-Ondo Municipal 1 and 2 taps)

Organism: ***Escherichia coli*** **Accession no: GU415870.1** **Source: Dam 5 OWODRW** **Strain ID: (339)**
GCTCTCTGCGGGTACGTCAATGAGCAAAGGTATTAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCNTAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCC
ATCTGGGCACATCCGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCC
GTCCGCCACTCGTCAGCAAAGAAGCAAGCTGCTTCCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: Klebsiella pneumoniae **Accession no: JF919921.1** **Source: Dam 5 OWODRW** **Strain ID: (336B)**
GGNGGNTCTCGCGGGTACGTCAATCGACAAGGTTATTAACCTTAACGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCNTAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCAT
TGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTA
ATCCCATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTC
ACCCGTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCT

Organisms: ***Klebsiella pneumoniae*** **Accession no: JN644536.1** **Source: Dam 5 OWODRW** **Strain ID: (335)**
TTAACCTTATCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGCCNTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTC
TGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGCCCGAA
GNCCCCCACTTTGGTCTTGCGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTG
TGCTACCGCTCGACTTGCATGTGT

Organisms: ***Klebsiella pneumoniae*** **Accession no: JF919921.1** **Source: Dam 5 OWODRW** **Strain ID: (336A)**
GNTTCTGGGGTACGTCAATCGACAAGGTTATTAACCTTAACGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATC
TGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCC
GCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: ***Bacillus pumilus*** **Accession no: EU741068.1** **Source: Dam 5 OWODRW** **Strain ID: (235B)**
TTGGTTGGTNCGGTCATGGTGCAAGCAGTTACTCTTGCACTTGCTTCTTCCCTAACAACAGAGCTTTACGATCCNAAAACCTTCATCACGTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGA
AGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGC
CGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGT
TACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Bacillus stratosphericus*** **Accession no: FM992660.1** **Source: Dam 5 OWODRW** **Strain ID: (340B)**
GNTTTGGTTGGNCGTCAAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCNTTAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGA
AGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGC
CGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGT
TACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Bacillus stratosphericus*** **Accession no: FM992660.1** **Source: Dam 5 OWODRW** **Strain ID: (340A)**
GGTTGGTNCGTCAGGTGCAAGCAGTTACTCTTGCACTTGCTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATT
CCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGG
GTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTC
ACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGT

Organisms: ***Bacillus sp*** **Accession no: JF426151.1** **Source: Dam 5 OWODRW** **Strain ID: (K)**
TGGTNGGTNCGGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACG
CGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organisms: ***Bacillus sp*** **Accession no: JF683298.1** **Source: Dam 5 OWODRW** **Strain ID: (333)**
GGTNGGTNCGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGAT
TCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCG
GGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGCACCATGCGGTGCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACT
CACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCC

Organisms: ***Bacillus cereus*** **Accession no: FN667584.1** **Source: Dam 5 OWODRW** **Strain ID: (232)**
CGGCTTCGGTNGGTNCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCNGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGC
GGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATG
CGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCNGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACG
TGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organisms: ***Leucobacter komagatae*** **Accession no: AJ746337.1** **Source: Dam 5 OWODRW** **Strain ID: (230B)**
TTTTGAGGTCCGTCACTTTCGCTTCTTCCCTACTAAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTTGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCG
TAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCATGGTGAGCCATTACCTCACCATCTAGCTGATAGGCCGCGAGTCCATCCAGAACCGATA
AATCTTTCCACCTACACACCATGCGGTGATAGGTCGTATCCAGTATTAGACACCGTTTCCAGTGCTTATCCCAGAGTTCAGGGCAGGTTACTCACGTGTTACTCACCCGTTCGCCACTCTTCCACCCAGC
AAGCTGGGCTTCATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCGTCC

Organisms: Uncultured bacterium clone **Accession no: GQ058163.1** **Source: Dam 5 OWODRW** **Strain ID: (235)**
TNTCTCAGGTCGTTCAGCCACCTACACGTAGGTGGGTTTATTCCCTGGCAAAAGCAGTTTACAACCCATAGGGCAGTCGTCCTGCACGCGGCATGGCTGGTTCAGGGTTGCCCCCATTGACCAATATTC
CTTACTGCTGCCTCCCGTAGGAGTCTGGTCCGTGTCTCAGTACCAGTGTGGGGGATTCTCCTCTCAGAGCCCCTAGACATCGTCGCCTTGGTGGGCCGTTACCCCACCAACTAGCTAATGTCACGCGAG
CCCATCCATATCCTATGAATATTTGACCCCTCCCCGATGCCGGGATGGGGTGTCATGCGGTGTTAATCCGAATTTCTTCGGGCTATCCCCCTGATATGGGTAGGTTGCTCACGCGTTACGCACCCGTGCG
CCACTCTCACCAGATGGTAGCAAGCTACCACTGGATCCCGTCCGACTTGCATGTATTAGGCCTNCCGCTAGCGTTCATCC

Organisms: ***Proteus mirabilis*** **Accession no: AB626123.1** **Source: Dam 5 OWODFW** **Strain ID: (201)**
TAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCT
GGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATATGGGTTCATCCGATAGTGCAAGGTCCGAAG
AGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCT
CCTGTTACCGCTCGACTTGCATGTGT

Organisms: ***Alcaligene sp*** **Accession no: HQ161777.1** **Source: Dam 5 OWODFW** **Strain ID: (198)**
TGNGNACCGTCAGCAGTATCCCGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCNTAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATT
CCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCG
GCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTCCGCC
ACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAATC

Organisms: ***Alcaligenes sp*** **Accession no: JF707602.1** **Source: Dam 5 OWODFW** **Strain ID: (198)**
GNTTNTGCGATCCGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCC
AAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCG
ATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATG
TGTAAAGCATCCCGCTAGCGTTCAAT

Organisms: ***Alcaligenes faecalis*** **Accession no: HQ161777.1** **Source: Dam 5 OWODFW** **Strain ID: (197)**
GTATCTGNGATNCGTCAGCAGTATCCCGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCA
AAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGA
TATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGT
CCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organisms: ***Alcaligenes faecalis*** **Accession no: JN162124.1** **Source: Dam 5 OWODFW** **Strain ID: (197)**
GCTTCTGNGAACGTCAGCAGTATCCCGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCNTAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCA
AAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGA
TATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGT
CCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organisms: *Aquitalea sp* **Accession no: AM989077.1** **Source: Dam 5 OWODFW** **Strain ID: (352)**
GCTTCTCGGTCTGGTCATCCCCCAGCGATATTAGCGCTAGGGATTTCCTCCCTGACAAAAGTCCTTTACAACCCGAAGGCCTTCTTCAGACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCA
AAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACTGATCGATGCCTTGGTGAGCCTTTACCTCACCAACTAGCTAATCAGA
CGTCGGCCGCTCGAATAGCGCAAGGTCCTAAGATCCCCTGCTTTCCTTCTCAAAGCGTATGCGGTATTAGCTATCCTTTCGGATAGTTATCCCCCACTACTCGGCACGTTCCGACGCATTACTCACCCGT
TCGCCACTCGTCAGCGGAGCAAGCTCCCTGTTACCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAAT

Organisms: *Pseudomonas otitidis* **Accession no: GU428467.1** **Source: Dam 5 OWODFW** **Strain ID: (350)**
TNCTGNTGGTACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGNAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCTTTACCTCACCAACTAGCTAATCCGACC
TAGGCTCATCTGATAGCGTGAGGTCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTC
CGCCGCTGAATCATGGAGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: *Klebsiella sp* **Accession no: JN036433.1** **Source: Dam 5 OWODFW** **Strain ID: (352)**
GCTNTCGNGGGTACGTCAATCGATGAGGTTATTAACCTNACCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCC
ATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACNTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCG
TCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAA

Organisms: *Morganella morganii* **Accession no: HM122053.1** **Source: Dam 5 OWODFW** **Strain ID: (199)**
GCTTTTGNGGTACGTCAATTGATGAGCGTATTAAGCTCACCACCTTCCTCCCGACTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCTCACCTACNAGCTAATCCCA
TATGGGTTCATCTGATGGCGCGAGGCCCGAAGGTCCCCCGCTTTGGTCCGAAGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCGCCATCAGGCAGATCCCCATACATTACTCACCCG
TCCGCCGCTCGTCAGCAGAGAAGCAAGCTTCTCCCTGTTACCGCCCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organisms: *Bacillus pumilus* **Accession no: AY792029.1** **Source: Dam 5 OWODFW** **Strain ID: (200A)**
CGGCTTTGGTNGGTNCGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGG
AAGATTCCCTACAGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCG
CCGCGGGTCCATCTGTAAGTGACAGCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGT
TACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: *Bacillus sp* **Accession no: JF683264.1** **Source: Dam 5 OWODFW** **Strain ID: (200)**
TTNGGTGGTACNGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTAGTCCCAGTGTGGCC
GATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCGCGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTC
AAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGC
ATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: *Bacillus sp* **(202B)** **Accession no: JF683264.1** **Source: Dam 5 OWODFW** **Strain ID: (339)**
GGTNGGTNCGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATT
CCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCGCGG
GTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTC
ACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: *Staphylococcus sp* **Accession no: HQ730688.1** **Source: Dam 5 OWODFW** **Strain ID: (202)**
GATGGTACGTCAGATGTGCACAGTTACTTACACATTTGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCC
CTACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGTATCGTTGCCTTGGTAAGCCGTTACCTTACCAACTAGCTAATACGGCGCGGGT
CCATCTATAAGTGATAGCAAAACCATCTTTTACTTTAGAACCATGCGGTTCCAAATGTTATCCGGCATTAGCTCCGGTTTCCCGAAGTTATTCCAGTCTTATAGGTAGGTTACCCACGTGTTACTCACCC
GTCCGCCGCTAACTTCAAAGGAGCAAGCTCCTTATCCGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organisms: *Alcaligenes sp* **Accession no: HQ730686.1** **Source: Dam 5 OWODM1** **Strain ID: (272B)**
GCCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGNCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGT
GTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGGATCTCCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCC
CCTTTCCCCCGTAGGNNNATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTCCGCCACTCGCCACCAAGAGAGCAAGCTCTCTCGTGCTG
CCGTTCGACTTGCATGTGT

Organisms: *Klebsiella pneumoniae* **Accession no: JF919909.1** **Source: Dam 5 OWODM1** **Strain ID: (274)**
GCTNTCGNGGGTACGTCAATCGATGAGGTTATTAACCTNANCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCNNANGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCC
ATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACNTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCG
TCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: *Pantoea agglomerans* **Accession no: AY335552.1** **Source: Dam 5 OWODM1** **Strain ID: (214)**
TTNGGGTACGTCAATTGCTGNGGTTATTAACCACAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATAT
TCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTG
GGCACATCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGC
CACTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organisms: *Pseudomonas sp* **Accession no: HQ832880.1** **Source: Dam 5 OWODM1** **Strain ID: (260)**
GGCTTTCGGTGGTACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTC
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGGGCCATTACCCCACCAACTAGCTAATCC
GACCTAGGCTCATCTGATAGCGTGAGGNCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACC
CGTCCGCCGCTGAATCATGGAGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: *Proteus mirabilis* **Accession no: GU420970.1** **Source: Dam 5 OWODM1** **Strain ID: (273)**
GGGTACGTCATTGATAAGGGTATTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTNAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCC
ACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATATGGGTTC
ATCCGATAGTGCAAGGTCCGAAGAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCCGCCGCT
CGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: *Morganella morganii* **Accession no: AM931264.1** **Source: Dam 5 OWODM1** **Strain ID: (215A)**
GGCTTTTGNGGTACGTCATTGATGAGCGTATTAAGCTCACCACCTTCCTCCCGACTGAAAGTACTTTACAACCCNAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCTCACCTACNAGCTAATCCCA
TATGGGTTCATCTGATGGCGCGAGGCCCGAAGGTCCCCCGCTTTGGTCCGAAGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCGCCATCAGGCAGATCCCCATACATTACTCACCCG
TCCGCCGCTCGTCAGCAGAGAAGCAAGCTTCTCCCTGTTACCGCCCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organisms: *Bacillus altitudinis* **Accession no: HQ432811.1** **Source: Dam 5 OWODM1** **Strain ID: (271A)**
TGGTNCGTCAGGTGCANGCAGTTACTCTTGCACTTGCTTCTTCCCTAACAACAGAGACTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCC
CTACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGT
CCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCAC
CCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organisms: ***Bacillus stratosphericus*** **Accession no: FM992660.1** **Source: Dam 5 OWODM1** **Strain ID: (171A)**
TNTGGTTGGTNCGTCAGGTGCAAGCAGTTACTCTTGCACTTGCTTCTTCCCTAACAACAGCAGACTTTACGATCCGAAAACCTTCATCACTCACGCGGCGGTTGCTCCGTCAGACTTTCGTCCATTGCGG
AAGATTCCCTACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCG
CCGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTG
TTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGT

Organisms: ***Bacillus altitudinis*** **Accession no: HQ432811.1** **Source: Dam 5 OWODM1** **Strain ID: (271B)**

TGGTNCGGTCAGGTGCANGCAGTTACTCTTGCACTTGCTTCTTCCCTAACAACAGAGACTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATT
CCCTACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCANGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCNTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCG
GGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGNTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACT
CACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCG

Organisms: ***Bacillus sp*** **Accession no: HE613375.1** **Source: Dam OWODM1** **Strain ID: (273)**
AAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGNTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGT
AGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTGACAG
CCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATC
CGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATG

Organisms: ***Bacillus cereus*** **Accession no: FN667584.1** **Source: Dam 5 OWODM1** **Strain ID: (215B)**
TTTGGTTGGTNNGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACG
CGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCNGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCC

Organisms: ***Bacillus sp*** **Accession no: HM063898.1** **Source: Dam 5 OWODM1** **Strain ID: (245A1)**
GNTACGGTCAGGAGCCGGCAGTTACTCCGGCACTTGCTTCTTCCCTGACAACAGCAGATTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATT
CCCTACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGG
GCCCATCTGTAAGTGATAGCCGAAACCATCTTTCAGCATAGCCTCATGAGAGGTTGTGCATTATCCGGTATTAGCTCCGGTTTCCCGAAGTTATCCCAGTCTTACAGGCAGGTTGCCCACGTGTTACTC
ACCCGTCCGCCGCTGACTTCAGGGAGCAAGCTCCCATCCGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Lysinibacillus sp*** **Accession no: HE586366.1** **Source: Dam 5 OWODM1** **Strain ID: (213A)**
GCCAGTTACTACTGTACTTGTTCTTCCCTTACAACAGAGTTTTACGAACCGAAATCCTTCTTCACTCACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTA
GGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGCCCATCCTATAGCGACAGC
CGAAACCGTCTTTTAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCACGTGTTACTCACCCGTCCGCCGCTAACGTC
GAAGGAGCAAGCTCCTTCT

Organisms: ***Leucobacter komagatae*** **Accession no: EU370411.1** **Source: Dam 5 OWODM1** **Strain ID: (274)**
GNTTTTGAGGTNGGTCCTTGCGCTTCTTCCCTACTAAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACNTNGNCGTTGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCC
CGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCATGGTGAGCCATTACCTCACCATCTAGCTGATAGGCCGCGAGTCCATCCAGAACCG
ATAAATCTTTCCACCTACACACCATGCGGTGATAGGTCATATCCAGTATTAGACACCGTTTCCAGTGCTTATCCCAGAGTTCAGGGCAGGTTACTCACGTGTTACTCACCCGTTCGCCACTCTTCCACCC
AGCAAGCTGGGCTTCATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCGTC

Organisms: ***Alcaligenes faecalis*** **Accession no: JN162124.1** **Source: Dam 5 OWODM2** **Strain ID: (253)**
GGNGGCTTCTGAGATCCGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCNAAGGCCTTCATCATACACGCGGGGATGGCTGGATCAGGGTTTCCCCCAT
TGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTA
ATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTC
ACCCGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organisms: ***Pseudomonas putida*** **Accession no: EU196391.1** **Source: Dam 5 OWODM2** **Strain ID: (251B)**
GCTTTTGTCGGTACGTCAAACAGCAAGGTATTCGCTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCNAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACC
TAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCGTC
CGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: ***Providencia rettgeri*** **Accession no: JN644625.1** **Source: Dam 5 OWODM2** **Strain ID: (253B1)**
GCTNTCGGNGGTACGTCAATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTAGGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCTCACCTACTAGCTAATCCC
ATATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTGAGAGATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCG
TCCGCCGCTCGTCAGCGAGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: ***Providencia rettgeri*** **Accession no: JN644625.1** **Source: Dam 5 OWODM2** **Strain ID: 209**
GTCTCTGTGGTACGTCATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTTNGGGNCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCTCACCTACTAGCTAATCCCAT
ATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTGAGAGATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTC
CGCCGCTCGTCAGCGNGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organisms: ***Alcaligenes faecalis*** **Accession no: JN162124.1** **Source: Dam 5 OWODM2** **Strain ID: (253A)**
GCTNTCTGNGATCCGTCAGCAGTATCCCGGTATTAGGGGATACCTTNTTCTTCTCTGCCAAAAGTACTTTACAACCCNNAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGT
CCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATC
CGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACC
CGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organisms: ***Escherichia coli*** **Accession no: CP003034.1** **Source: Dam 5 OWODM2** **Strain ID: (210B)**
GNTTTTNGGGTACGTCAATGAGCAAAGGTATTAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCA
TCTGGGCACATCCGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGT
CCGCCACTCGTCAGCNAAANAGCAAGCTGNTTCCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organisms: ***Escherichia coli*** **Accession no: CP003034.1** **Source: Dam 5 OWODM2** **Strain ID: (210A)**
TNTGGGGTACGTCAATGAGCAAAGGTATTAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTG
GGCACATCCGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCG
CCACTCGTCAGCNAAANAGCAAGCTGNTTCCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTG

Organisms: ***Bacillus sp*** **Accession no: AB678448.1** **Source: Dam 5 OWODM2** **Strain ID: (187)**
GGNTNTGGGTTGGTACGGTCAAGGTGCCAGCTTATTCAACTAGTCACTTGCTTCTTCCCTAACAACAGCAGATTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTC
CATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAG
CTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTA
CCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTC

Organisms: ***Bacillus sp*** **Accession no: DQ268808.1** **Source: Dam 5 OWODM2** **Strain ID: (245A2)**
GGGGCTTTGGTTGGTNCGGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTG
CGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAAT
GCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCAC
GTGTTACTCACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCT

Organisms: ***Bacillus sp.*** **Accession no: DQ268808.1** **Source: Dam 5 OWODM2** **Strain ID: (252A)**
TTTGGTNGGTNCGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACG
CGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCT

Organisms: ***Bacillus cereus*** **Accession no: FN667584.1** **Source: Dam 5 OWODM2** **Strain ID: (245B)**
TNTGGTTGGTNCGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACG
CGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCNGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTA
CTCACCCGTCCCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCC

Organisms: ***Bacillus stratosphericus*** **Accession no: FM992660.1** **Source: Dam 5 OWODM2** **Strain ID: (192B)**
TTNGGTTGGTNCGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGCC

Organisms: ***Bacillus pumilus*** **Accession no: HQ432808.1** **Source: Dam 5 OWODM2** **Strain ID: (253B2)**
TTTGGTNGGTACGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Bacillus cereus*** **Accession no: FN667584.1** **Source: Dam 5 OWODM2** **Strain ID: (186)**

GNTTCTGGTAGGNACGATCAGGTGCCAGTCTTATTCAACTAGCACTTGCTTCTTCCCTAACAACAGTAGTTTTACGCCTTTTTAANGCCTTCATCACTCACGCGGCGGTTGCTCCGTCAGACTTTCGTCC
ATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGC
TAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTAC
CCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organisms: ***Bacillus sp*** **OWODM2** **Accession no: JF426151.1** **Source: Dam 5 OWODM2** **Strain ID: (371)**
CGGTNTGGTGGACGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCTGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGA
AGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGA
CGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGT
TACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organisms: ***Bacillus pumilus*** **Accession no: AY792029.1** **Source: Dam 5 OWODM2** **Strain ID: (184A)**
GCTTTGGTNGGTACGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGA
AGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGC
CGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGT
TACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Lysinibacillus sp.*** **Accession no: JN695724.1** **Source: Dam 5 OWODM2** **Strain ID: (298)**
CTNNATAGGTACGCTCAAGGGTACAGCCAGTTACTACTGTACTTGCTTCTTCCCTTACAACAGCAGATTTTACGAACCGAAATCCTTCTTCACTCACGCGGCGATTGCTCCATCAGGCTTTCGCCCATTG
TGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAAT
GCGCCGCGGGCCCATCCTATAGCGACAGCCGAAACCGTCTTTCAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCAC
GTGTTACTCACCCGTCCGCCGCTAACGTCGAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Acinetobacter baumannii*** **Accession no: JN162444.1** **Source: Dam 5 OWODM3**
Strain ID: (193)
TTTCTGGAGTACGTCACTATCCCTAGGTATTAACTAGAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTGTCCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACTT
AGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATCCCTTTCGAGATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGTCC
GCCGCTAGGTCCGGTAGCAAGCTACCTTCCCCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATC

Organisms: ***Morganella morganii*** **Accession no: AB089245.1** **Source: Dam 5 OWODM3** **Strain ID: (169)**
TTCTGAGANCCGTCAGCAGTATCCCGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGTNAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAA
AATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGAT
ATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTC
CGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTTAAAGCATCCCGCTAGCGTTCAATC

Organisms: ***Morganella morganii*** **Accession no: AB089245.1** **Source: Dam 5 OWODM3** **Strain ID: (218B)**TTTTGNGGTACGTCATTGANAAGGTTATTAACCTTGACACCTTCCTCCCGACTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTAAGCCGTTACCTTACCTACTAGCTAATCCC
ATATGGGTTCATCTGATGGCGCGAGGCCCGGAGGTCCCCCGCTTTGGTCCGAAGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCGCCATCAGGCAGATCCCCATACATTACTCACCC
GTCCGCCGCTCGTCAGCAGAGAAGCAAGCTTCTCCCTGTTACCGCCCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: ***Myroides odoratus*** **Accession no: AB517709.1** **Source: Dam 5 OWODM3** **Strain ID: (269B)**
CGGNACGTCAATAATTACACGTAATCACATTTCTTCCCGTACAAAAGCAGTTTACAATCCATAGGACCGTCATCCTGCACGCGGCATGGCTGGTTCAGAGTTGCCTCCATTGACCAATATTCCTCACTG
CTGCCTCCCGTAGGAGTCTGGTCCGTGTCTCAGTACCAGTGTGGGGGATCTCCCTCTCAGGACCCCTAATCATCGTAGCCTTGGTATGCCGTTACCACACCAACTAGCTAATGATACGCATGCCCATCT
TTTACCGATAAATCTTTATTAATCAAACGATGCCATTCAATTAAACCATGGAGCATTAATCCGAATTTCTCCGGGCTATTCCCCTGTAAAAGGTAGGTTGCATACGCGTTACTCACCCATCCGCCGGTCT
CTAGAAAGCAAGCTCTCTATACCCCTCGACTTGCATGTGTTAGGCCTGCCGCTAGCGTTCATC

Organisms: ***Psychrobacter pulmonis*** **Accession no: HQ698579.1** **Source: Dam 5 OWODM3** **Strain ID: (191)**
GCTNTCTGAGCTAATGTCATCGTCCATGGGTATTAACCATGGAGTCTTCTTCACTGCTTAAAGTGCTTTACAACCCNTNGGNCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTGCCCCCATTGTCC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCCGGGCCGTGTCTCAGTCCCGGTGTGGCTGATCATCCTCTCAGACCAGCTACAGATCGTCGCCATGGTAGGCCTTTACCCCACCATCTAGCTAATCCG
ACTTAGGCTCATCTAATAGCGAGAGCACAAGTTGCCCCCTTTCTCCCGTAGGTCGTATGCGGTATTAATTCGAGTTTCCCCGAGCTATCCCCCACTACTAGGTAGATTCCTAAGTATTACTCACCCGTCC
GCCGCTCGTCAGCGAGAAGCAAGCTTCTCCTGTTACCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATCTG

Organisms: ***Proteus vulgaris*** **Accession no: JN630888.1** **Source: Dam 5 OWODM3** **Strain ID: (372)**
GTNTNGCGGGTACGTCATTGCTAAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATA
TGGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTC
CGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCA

Organisms: ***Proteus vulgaris*** **Accession no: JN630888.1** **Source: Dam 5 OWODM3** **Strain ID: (190)**
GGCTTTTGGGGTACGTCATTGCTAAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATA
TGGGTTCATCCGATAGCGCAAGGTCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTC
CGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: ***Proteus vulgaris*** **Accession no: JN630888.1** **Source: Dam 5 OWODM3** **Strain ID: (171B)**
GGTCTNTGGGGTAGTCATTGCTAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATAT
GGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCC
GCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAG
GCCTGCCGCCAGCGTTCAAT

Organisms: ***Serratia marcescens*** **Accession no: FJ607982.1** **Source: Dam 5 OWODM3** **Strain ID: (218A)**
GGCTTTCGGAGTACGTCATTGATGAGCGTATTAAGCTCACCACCTTCCTCCTCGCTGAAAGTGCTTTACAACCCNAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCCCACCTACTAGCTAATCCCA
TCTGGGCACATCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGT
CCGCCGCTCGTCACCCAGAGAGCAAGCTCNCTTGTGCTACCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAAT

Organisms: ***Bacillus sp.*** **Accession no: DQ268808.1** **Source: Dam 5 OWODM3** **Strain ID: (269A)**
TTTGGNTGGTACGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACG
CGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCT

Organisms: ***Bacillus sp.*** **Accession no: JF766690.1** **Source: Dam 5 OWODM3** **Strain ID: (168B)**
GGTTTGGTNGGTACGTCAGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGCTTTACGATCNGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGG
AAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATGCG
CCGCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTG
TTACTCACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Bacillus sp*** **OWODM3** **Accession no: JF683264.1** **Source: Dam 5 OWODM3** **Strain ID: (167B)**
TTTGGTNGGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCCT

Organisms: ***Lysinibacillus sp*** **Accession no: FR775037.1** **Source: Dam 5 OWODM3** **Strain ID: (219*B)**
GNTTTATAGGTNCGTCAGGTCAGCCAGTTACTACTGTACTTGTTCTTCCCTTACAACAGAGTTTTACGAACCGAAATCCTTCTTCACTCACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTGGAAGA
TTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGC
GGGCCCATCCTATAGCGACAGCCGAAACCGTCTTTCAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCACGTGTTAC
TCACCCGTCCGCCGCTAACGTCGAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Bacillus sp*** **Accession no: JF426151.1** **Source: Dam 5 OWODM3** **Strain ID: (188)**
GGTNGGTACGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGAT
TCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCG
GGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACT
CACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organisms: ***Bacillus pumilus*** **Accession no: HQ432808.1** **Source: Dam 5 OWODM3** **Strain ID: (166)**
TGGTNGGTNCGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGAT
TCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCG
GGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACT
CACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organisms: ***Bacillus sp*** **Accession no: JF683264.1** **Source: Dam 5 OWODM3** **Strain ID: (167A)**
GTNGGTACGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCATTGCGGAAGATTCC
CTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCGCGGGT
CCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCAC
CCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Bacillus sp*** **OWODM3** **Accession no: JF766690.1** **Source: Dam 5 OWODM3** **Strain ID: (168A)**
GGCTNTGGTNGGTACGTCAAGGGTACCGCCCTATTCGAACGGTACTTGCTTCTTCCCTAACAACAGCAGCTTTACGATCNTGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCA
TTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGCT
AATGCGCCGCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACC
CACGTGTTACTCACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Bacillus sp*** **Accession no: JF766690.1** **Source: Dam 5 OWODM3** **Strain ID: (194)**
GGCTTTGGTTGGTCCGTCAAGGGTACCGCCCTATTCGAACGGTACTTGCTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTG
CGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGCTAAT
GCGCCGCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCAC
GTGTTACTCACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organisms: ***Bacillus thuringiensis*** **Accession no: JN377782.1** **Source: Dam 5 OWODM3** **Strain ID: (270)**

TGGNTGGNCCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGCTTCTTCCCTAACAACAGCAGATTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGGTTGCTCCGTCAGACTTTCGTCCATTGCG
GAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGC
GACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGT
GTTACTCACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCAT

Organisms: ***Bacillus sp*** **Accession no: DQ122248.1** **Source: Dam 5 OWODM3** **Strain ID: (373)**
GGCGGCTTTGGTNGGTNCGTCAGGTACGAGCAGTTACTCTCGTACTTGTTCTTCCCTAACAACAGAGTTTTACGACCNTNAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGC
GGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATG
CACCGCGGGCCCATCTGTAAGTGATAGCCGAAACCATCTTTCAATCATCTCCCATGAAGGAGAAGATCCTATCCGGTATTAGCTTCGGTTTCCCGAAGTTATCCCAGTCTTACAGGCAGGTTGCCCACG
TGTTACTCACCCGTCCGCCGCTAACGTCATAGAAGCAAGCTTCTAATCAGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

F. Nucleotide sequence of Bacteria from Dam 6 Owena-Idanre (Code: OWODW= Owena-Idanre raw water, OWIFW= Owena-Ondo treated water, OWIM1 and OWIM2= Owena-Idanre Municipal 1 and 2 taps)

Organism: ***Alcaligenes faecalis*** **Accession no: JN162124.1** **Source: Dam 6 OWIRW** **Strain ID: (177B)**
GNTTTNGCGATACGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCC
AAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCG
ATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCG
TCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Alcaligenes faecalis*** **Accession no: JN162124.1** **Source: Dam 6 OWIRW** **Strain ID: (173B)**
GCTNTCTGCGATCCGTCAGCAGTATCCCGGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGGATGGCTGGATCAGGGTTTCCCCCATTGT
CCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATC
CGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACC
CGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAA

Organism: ***Alcaligenes sp*** **Accession no: JF303893.1** **Source: Dam 6 OWIRW** **Strain ID: (174A)**
GCTATCTGCGATCCGTCAGCAGTATCTCGATATTAGGAGATACCTTNTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGT
CCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATC
CGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACC
CGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAATC

Organism: ***Aeromonas veronii*** **Accession no: HQ832864.1** **Source: Dam 6 OWIRW** **Strain ID: (258)**
TTAGCCAACAACCTTTCCTCCTCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGGTTTCCCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTC
TGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCCACCTGGGCATATCCAATCGCGCAAGGCCCGAA
GGTCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTTATCCCCCTCGACTGGGCAATTTCCCAGGCATTACTCACCCGTCCGCCGCTCGCCGGCAAAAGTAGCAAGCTA
CTTTCCCGCTGCCGCTCGACTTGCATGTGTTAGGCCT

Organism: ***Aeromonas hydrophila*** **Accession no: HQ731685.1** **Source: Dam 6 OWIRW** **Strain ID: (258)**
GGCTCTCGCGAGTACGTCACAGCTGCTGGGTATTAGCCAACAACCTTTCCTCCTCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGGTTTCCCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCC
CACCTGGGCATATCCAATCGCGCAAGGCCCGAAGGTCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTTATCCCCCTCGACTGGGCAATTTCCCAGGCATTACTCACC
CGTCCGCCGCTCGCCGGCAAAAGTAGCAAGCTACTTTCCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Acinetobacter junii*** **Accession no: AM184279.1** **Source: Dam 6 OWIRW** **Strain ID: (205B)**
TCNNGAGTACGTCCACTATCCAGTAGTATTAATACTAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCANAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTCCGCCCATTGTCCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACTT
AGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCATTCCTTTCGGAATGTTGTCCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGTCC
GCCGCTAAGCTAAGGTGCAAGCACCCTCGCTCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATC

Organism: ***Acinetobacter baumannii*** **Accession no: JF919866.1** **Source: Dam 6 OWIRW** **Strain ID: (222)**
TTGCGAGTACGTCCACTATCTCTAGGTATTAACTAAAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCNTAAGGCCTTCTTCACACACGCGGNATGGCTGGATCAGGGTTCCCCCCATTGTCCAAT
ATTCCCCACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACT
TAGGCTCATCTATTAGCGCAAGGACCGAAGATCCCCTGCTTTCTCCCCGTAGGGCGTATGCGGTATTAGCATCCTTTCGGAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGTC
CGCCGCTAGGTCCAGTAGCAAGCTACCTTCCCCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATCTG

Organism: ***Morganella morganii*** **Accession no: AB089245.1** **Source: Dam 6 OWIRW** **Strain ID: (206)**
GCTTTTGNGGTACGTCAATTGANAAGGTTATTAACCTTGACACCTTCCTCCCGACTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTAAGCCGTTACCTTACCTACTAGCTAATCCCAT
ATGGGTTCATCTGATGGCGCGAGGCCCGGAGGTCCCCCGCTTTGGTCCGAAGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCGCCATCAGGCAGATCCCCATACATTACTCACCCGT
CCGCCGCTCGTCAGCAGAGAAGCAAGCTTCTCCCTGTTACCGCCCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Myroides odoratus*** **Accession no: AB517709.1** **Source: Dam 6 OWIRW** **Strain ID: (174B)**
TCTTCGGNACGTCAATAATTACACGTAATNACATTTCTTCCCGTACAAAAGCAGTTTACAATCATAGGACCGTCATCCTGCACGCGGCATGGCTGGTTCAGAGTTGCCTCCATTGACCAATATTCCTCA
CTGCTGCCTCCCGTAGGAGTCTGGTCCGTGTCTCAGTACCAGTGTGGGGGGATCTCCCTCTCAGGACCCCTAATCATCGTAGCCTTGGTATGCCGTTACCACACCAACTAGCTAATGATACGCATGCCCA
TCTTTTACCGATAAATCTTTATTAATCAAACGATGCCATTCAATTAAACCATGGAGCATTAATCCGAATTTCTCCGGGCTATTCCCCTGTAAAAGGTAGGTTGCATACGCGTTACTCACCCATCCGCCGG
TCTCTAGAAAGCAAGCTCTCTATACCCCTCGACTTGCATGTGTTAGCCTGCCGCTAGCGTTCATC

Organism: ***Klebsiella pneumoniae*** **Accession no: CP002910.1** **Source: Dam 6 OWIRW** **Strain ID: (345)**
TCGNGGGTACGTCAATCGACNAGGTTATTAACCTTATCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTG
GGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGC
CGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Klebsiella sp.*** **Accession no: DQ989215.2** **Source: Dam 6 OWIRW** **Strain ID: (347)**
GTACGTCAATNGATAAGGTTATTAACCTTAATGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGNAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATCCCCA
CTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACA
TCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCACTCG
TCACCCGAGAGCAAGCTCTCTGTGCTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCT

Organism: ***Klebsiella pneumoniae*** **Accession no: JN545039.1** **Source: Dam 6 OWODRW** **Strain ID: (319)**
GNTNTTGCGGGTACGTCAATCGACAAGGTTATTAACCTTATCGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCNAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCC
ATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACNTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCG
TCCGCCGCTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Klebsiella oxytoca*** **Accession no: JF317350.1** **Source: Dam 6 OWIRW** **Strain ID: (175A)**
GCTCTCGCGGGTACGTCAATCGACAAGGTTATTAACCTTATCGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCNAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCC
ATCTGGGCACATCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCG
TCCGCCACTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Klebsiella sp*** **Accession no: JN036433.1** **Source: Dam 6 OWIRW** **Strain ID: (176)**
GAGGTATTAACCTCACCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCNTNAGGNCTTCTTCACACACGCGGNATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGT
AGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCANCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAG
GCCCGAAGGTCCCCCACTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGTCACCCGAGAGCAA
GCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGAGCC

Organism: Uncultured bacterium **Accession no: JN595068.1** **Source: Dam 6 OWIRW** **Strain ID: (205A)**
GCTNTTGTGGTACGTCAATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTTAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCNCACCTACTAGCTAATCCCAT
ATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTC
CGCCGCTCGTCAGCGAGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: *Providencia vermicola* **Accession no: JN092797.1** **Source: Dam 6 OWIRW** **Strain ID: (180B)**
GNTTTTGTGGTACGTCAATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCCCACCTACTAGCTAATCCCA
TATGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGT
CCGCCGCTCGTCAGCGNGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: *Proteus mirabilis* **Accession no: JN092590.1** **Source: Dam 6 OWIRW** **Strain ID: (176)**
GNTTTTNGGGTACGTCATTGATAGGGTATTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATATG
GGTTCATCCGATAGTGCAAGGTCCGAAGAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCCG
CCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: *Proteus vulgaris* OWIRW **Accession no: JN630888.1** **Source: Dam 6 OWIRW** **Strain ID: (179)**
GGCTNTCGNGGGTACGGTCAATTGCTAAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGT
GCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATC
CCATATGGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCAC
CCGTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: *Proteus mirabilis* **Accession no: GU420970.1** **Source: Dam 6 OWIRW** **Strain ID: (173A)**
GCTNTCGNGGGTACGTCAATTGATAAGGGTATTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCC
ATATGGGTTCATCCGATAGTGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCC
GTCCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAA

Organism: *Proteus vulgaris* **Accession no: JN630888.1** **Source: Dam 6 OWIRW** **Strain ID: (178B)**
GGCTNTTGGGGTACGTCATTGCTAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATAT
GGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCC
GCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: *Proteus mirabilis* **Accession no: JN092590.1** **Source: Dam 6 OWIRW** **Strain ID: (261)**
TNTCGCGGGTACGTCATTGANAAGGGTATTAACCTTATCACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTNAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCATAT
GGGTTCATCCGATAGTGCAAGGTCCGAAGAGCCCCTGCTTTGGTCCGTAGACATTATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCC
GCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGAGC

Organism: *Providencia vermicola* **Accession no: NR_042415.1** **Source: Dam 6 OWIRW** **Strain ID: (227B)**
TCTTTGTCGGTACGTCATCGTTGATGATATTAGCATCAACGCCTTCCTCCCGACTGAAAGTACTTTACAACCCTANGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAAT
ATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCCCACCTACTAGCTAATCCCATA
TGGGTTCATCCGATAGCGCAAGGACCGAAGTTCCCCTGCTTTGCTCCTAAGAGATTATGCGGTATTAGCCACCGTTTCCAGTGGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGTCC
GCCGCTCGTCAGCGAGAAGCAAGCTTCCCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: *Pseudomonas sp* **Accession no: HQ832880.1** **Source: Dam 6 OWIRW** **Strain ID: (343B)**
GTNTCTGTTGGTACGTCAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGTAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGGGCCATTACCCCACCAACTAGCTAATCCGACC
TAGGCTCATCTGATAGCGTGAGGTCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTC
CGCCGCTGAATCATGGAGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: *Pseudomonas sp* **Accession no: HQ832880.1** **Source: Dam 6 OWIRW** **Strain ID: (343A)**
TTTCTGTTGGTACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCTTNAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGGGCCATTACCCCACCAACTAGCTAATCCGA
CCTAGGCTCATCTGATAGCGTGAGGTCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCG
TCCGCCGCTGAATCATGGAGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTG

Organism: *Pseudomonas sp*. **Accession no: FJ013349.1** **Source: Dam 6 OWIRW** **Strain ID: (223)**
GGCTTTTGNGGTACGTCAAACAGCAAGGTTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCG
ACCTAGGCTCATCTGATAGCGCAAGGNCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACNAGGCAGATTCCTAGGCATTACTCACCC
GTCCGCCGCTGAATCAAGGAGCAAGCTCCCGTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: *Pseudomonas sp* **Accession no: HQ832880.1** **Source: Dam 6 OWIRW** **Strain ID: (345)**
TTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGNTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTC
TGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGGGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGTGAGGTCCGAA
GATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGAATCATGGAGCAAGCTCCACT
CATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCG

Organism: *Pseudomonas sp.* **Accession no: HQ264095.1** **Source: Dam 6 OWIRW** **Strain ID: (343B)**

TGTCGGTACGTCAAACAGCAAGGTATTCGCTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGTNAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATT
CCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGACATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGG
CTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCC
GCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: *Pseudomonas sp* **Accession no: HM352366.1** **Source: Dam 6 OWIRW** **Strain ID: (175B)**
TTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGAC
CGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGNCCGAAGGTCC
CCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCG
CTCGACTTGCATGTGTNAGGCCTGC

Organism: *Pseudomonas sp* **Accession no: HQ832880.1** **Source: Dam 6 OWIRW** **Strain ID: (342A)**
TNGGNTTTTGTGGTACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGT
CCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGGGCCATTACCCCACCAACTAGCTAATCC
GACCTAGGCTCATCTGATAGCGTGAGGNCCGAAGATCCCCCACTTTCTCCCGTAGGACGTATGCGGTATTAGCGNTCCTTTCGAAACGTTATCCCCCACTACNAGGCAGATTCCTAGGCATTACTCACC
CGTCCGCCGCTGAATCATGGAGCAAGCTCCACTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Serratia marcescens*** **Accession no: JF429936.1** **Source: Dam 6 OWIRW** **Strain ID: (348)**
GCTTTNGCGAGTACGTCATTGATGAACGTATTAAGCTCACCACCTTCCTCCTCGCTGAAAGTGCTTTACAACCCNAAGGCCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCATTACCCCACCTACTAGCTAATCCCA
TCTGGGCACATCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCAC
CCGTCCGCCGCTCGTCACCCAGGGAGCAAGCTCCCCTGTGCTACCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAAT

Organism: ***Bacillus thuringiensis*** **Accession no: JN377782.1** **Source: Dam 6 OWIRW** **Strain ID: (265)**
CGGCTTNCGGTNGGTACGTCAAGGTGCCAGCTTATTCAACTAGCACTTGCTTCTTCCCTAACAACAGCAGATTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGGTTGCTCCGTCAGACTTTCGTC
CATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAG
CTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTA
CCCACGTGTTACTCACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organism: ***Bacillus thuringiensis*** **Accession no: JN377782.1** **Source: Dam 6 OWIRW** **Strain ID: (341)**
TGGCTNCGGNTGGTACGGTCAAGGTGCCAGCTTATTCAACTAGTCACTTGCTTCTTCCCTAACAACAGCAGATTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGGTTGCTCCGTCAGACTTTCGT
CCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTA
GCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTT
ACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCAT

Organism: ***Bacillus sp*** **Accession no: JF766690.1** **Source: Dam 6 OWIRW** **Strain ID: (208)**
GGGCTNTGGGTGGTACGTCAGGTACCGCCCTATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGCTTTACCCNTNTTTAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTG
CGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCATTACCTCACCAACTAGCTAAT
GCGCCGCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTTTGAACCATGCGGTTCAAACAACCATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCAC
GTGTTACTCACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Lysinibacillus sp*** **Accession no: FR775037** **Source: Dam 6 OWIRW** **Strain ID: (172)**
TNTNAGGTACGTCAGGTCAGCCAGTTACTACTGTACTTGTTCTTCCCTTACAACAGAGTTTTACGAACCGAAATCCTTCTTCACTCACGCGGCGTTGCTCCATCAGGCTTTCGCCCATTGTGGAAGATTC
CCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGG
CCCATCCTATAGCGACAGCCGAAACCGTCTTTCAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCACGTGTTACTCA
CCCGTCCGCCGCTAACGTCNAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Proteus vulgaris*** **Accession no: JN630888.1** **Source: Dam 6 OWIFW** **Strain ID: (257B)**
GGCTNNTGGGGTACGTCATTGCTAAGAGTATTAATCTTAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCTAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCA
ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAGAGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTAGCTAATCCCAT
ATGGGTTCATCCGATAGCGCAAGGNCCGAAGAGCCCCTGCTTTGGTCCGTAGACGTCATGCGGTATTAGCCACCGTTTCCAGTAGTTATCCCCCTCTATCGGGCAGATCCCCATACATTACTCACCCGT
CCGCCGCTCGTCAGCAAGAAAGCAAGCTTTCTCCTGTTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Ralstonia sp*** **Accession no: DQ847128.1** **Source: Dam 6 OWIFW** **Strain ID: (355B)**
GGCTTTTTCGGTCATCCACCCCAGGTATTAGCCAGAGCGATTTCTTTCCGGACAAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATTGCTGGATCAGGGTTGCCCCCCATTGTCCA
AAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCGTCCTCTCAGACCAGCTACTGATCGTCGCCTTGGTGGGCCTTTACCCCACCAACTAGCTAATCAGA
CATCGGCCGCTCCTATTGCGCGAGGCCTTACGGTCCCCCGCTTTCACCCTCAGGTCGTATGCGGTATTAGCTAATCTTTCGACTAGTTATCCCCCACAACAGGGCACGTTCCGATGTATTACTCACCCGT
TCGCCACTCGCCGCCAGACCGAAGTCCGCGCTGCCGTTCGACTTGCATGTGTAAGGCATGCCGCCAGCGTTCAAT

Organism: ***Bacillus pumilus*** **Accession no: AY792029.1** **Source: Dam 6 OWIFW** **Strain ID: (357B)**
GNTTTGGTGGTACGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAA
GATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCC
GCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTT
ACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus altitudinis*** **Accession no: HQ432811.1** **Source: Dam 6 OWIFW** **Strain ID: (257A)**
GGTNNGGTCAGGTGCAAGCAGTTACTCTTGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCC
TACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTC
CATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACC
CGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTCC

Organism: ***Acinetobacter junii*** **Accession no: JN644576.1** **Source: Dam 6 OWIM1** **Strain ID: (272)**
GGCTNNTGCGAGTACGTCCACTATCCAAGAGTATTAGTCTCAGNTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACCATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGGTTCCCCCCATTG
TCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATC
CGACTTAGGCTCATCTATTAGCGCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATTCCTTTCGGAATGTTGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACC
CGTCCGCCGCTAAGATAAGGTGCAAGCACCTCATCTCCGCTCGACTTGCATGTGTTAAG
CCTGCCGCCAGCGTTCAATCT

Organism: ***Alcaligenes sp*** **OWIM1** **Accession no: JF707602.1** **Source: Dam 6 OWIM1** **Strain ID: (250B2)**
GCTTTTGAGATNCGTCAGCAGTATCCCGTATTAGGGGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGNAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCA
AAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGA
TATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGT
CCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAATC

Organism: ***Alcaligenes faecalis*** **Accession no: HM145896.1** **Source: Dam 6 OWIM1** **Strain ID: (250A2)**
GCGATCCGTCAGCAGTATCTCGTATTAGGAGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTC
CCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCGG
CCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTCCGCCA
CTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Citrobacter murliniae*** **Accession no: JN092600.1** **Source: Dam 6 OWIM1** **Strain ID: (363)**
TNTCGCGAGTACGTCAATTGCTGNGGTTATTAACCACAACNCCTTCCTCCTCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAA
TATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATC
TGGGCACATCCGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCGGGCAGTTTCCCAGACATTACTCACCCGTCC
GCCACTCGTCACCCAAGGAGCAAGCTCCTCTGTGCTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Citrobacter freundii*** **Accession no: JN644567.1** **Source: Dam 6 OWIM1** **Strain ID: (362)**
GCTNTCGCGAGTACGTCAATNGCTGCGGTTATTAACCACAACNCCTTCCTCCTCGCTGAAAGTACTTTACAACCCGNAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCC
ATCTGGGCACATCCGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCGGGCAGTTTCCCAGACATTACTCACCC
GTCCGCCACTCGTCACCCAAGGAGCAAGCTCCTCTGTGCTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Enterobacter sp*** **Accession no: GQ861528.1** **Source: Dam 6 OWIM1** **Strain ID: (360)**
TTNTCTGCGGGGTACGTCAATCGCTGAGGTTATTAACCTCAACGCCTTCCTCCCCGCTGAAAGTACTTTACAACCCTTNAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCNTTACCCCACCTACTAGCTAATCC
CATCTGGGCACATCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCC
GTCCGCCGCTCGCCGGCAAAGTAGCAAGCTACTTTCCGCTGCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATC

Organism: ***Enterococcus casseliflavus*** **Accession no: AB260996.1** **Source: Dam 6 OWIM1** **Strain ID: (250B1)**
GANCCGTCAGGGATGAACNTTTTACTCTCATCCTTGTTCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCT
ACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCC
ATCCATCAGTGACGCAAAAGCGCCTTTCAACTTTCTTCCATGCGGAAAATAGTGTTATACGGTATTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACTCACCCGTT
CGCCACTCTTTTTCTTTCGGTGGAGCAAGCTCCGGTGAAAGAAAAAGCGTTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGT

Organism: ***Klebsiella pneumoniae*** **Accession no: CP002910.1** **Source: Dam 6 OWIM1** **Strain ID: (361)**
GTCATCGANAAGGTTATTAACCTTATCGCCTTCCTCCCCGCTGAAAGTGCTTTACAACCCTTNAGGGCTTCTTCACACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGC
TGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTG
ATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCGACATTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGTCAC
CCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organism: ***Bacillus cereus*** **Accession no: AB677943.1** **Source: Dam 6 OWIM1** **Strain ID: (250)**
GNTGGTNCGTCAAGGTGCCAGCTTATTCAACTAGTCACTTGCTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACG
CGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCA

Organism: ***Alcaligenes faecalis*** **Accession no: JN162124.1** **Source: Dam 6 OWIM1** **Strain ID: (239)**
GCTATCTGCGATCCGTCAGCAGTATCCCGGTATTAGGGGATACCTTNTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGT
CCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATC
CGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACC
CGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Alcaligenes sp*** **Accession no: JF303893.1** **Source: Dam 6 OWIM1** **Strain ID: (238B)**
GGNGGCTATCTGCGATCCGTCAGCAGTATCTCGTATTAGGAGATACCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCNAAGGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATT
GTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAA
TCCGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCA
CCCGTCCGCCACTCGCCGCCAAGAGAGCAAGCTCTCTCGCGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTTCAAT

Organism: ***Brevundimonas naejangsanensis*** **Accession no: FJ544245.1** **Source: Dam 6 OWIM2** **Strain ID: (236B)**
GGCGTTTCCGGGTNCGTCATTATCGTCCCCGGGTGAAAGAATTTTACAATCCTAAGACCTTCATCATTCACGCGGCATGGCTGCGTCAGGCTTTCGCCCATTGCGCAAGATTCCCCACTGCTGCCTCCC
GTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTACTGATCGTCGCCTTGGTGAGCTTTTACCTCACCAACTAGCTAATCAGACGCGGGCCGCTCTAATGGCGAT
AAATCTTTCCCCCGAAGGGCACATTCGGTATTAGCACAAGTTTCCCTGAGTTATTCCGAACCAAAAGGCACGTTCCCACGTGTTACTCACCCGTCCGCCACTAACTCCGAAGAGTTCGTTCGACTTGCA
TGTGTTAGGCCTGCCGCCAGCGTTCGCT

Organism: Chromobacterium sp **Accession no: AB426118.1** **Source: Dam 6 OWIM2** **Strain ID: (366)**
GNTATCTTCGGTNTCTCATCCCCAGTAGGTATTAACCACTAGGATTTGCTCCCGAACAAAAGTCCTTTACAACCCTTTNGGCCTTCTTCAGACACGCGGCATGGCTGGATCAGGCTTGNGCCCATTGTC
CAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCTCTCAGACCCGCTACTGATCGTCGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCA
GACATCGGCTGCTCGTATAACGTGAGGCCTTACGGTCCCCCACTTTCCCCCTCAGGGCGTATGCGGTATTAATCCGGCTTTCGCCGAGCTATCCCCCATTACACGGTACATTCCGATGCGTTACTCACCC
GTTCGCCACTCGTCAGCGGTGCAAGCACCCTGTTACCGTTCGACTTGCATGTGTAAAGCATGCCGCCAGCGTTCAAT

Organism: ***Pseudomonas sp*** **OWIM2** **Accession no: GU726583.1** **Source: Dam 6 OWIM2** **Strain ID: (244B)**
GNTTTCTGTCGGTACGTCAAACAGCAAGGTATTCGCTTACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCNTTNGANNTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCC
AATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCG
ACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCC
GTCCGCCGCTGAATCGAAGAGCAAGCTCTTCTCATCCGCTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT

Organisms: ***Bacillus thuringiensis*** **Accession no: HQ432809.1** **Source: Dam 6 OWIM2** **Strain ID: (238A)**
GGTTNTGGTNGGTACGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGG
AAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCG
ACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTG
TTACTCACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCT

Organism: ***Bacillus sp*** **OWIM2** **Accession no: DQ268808.1** **Source: Dam 6 OWIM2** **Strain ID: (203)**
GGTNGGTCCGTCAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGAT
TCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCG
GGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACT
CACCCGTCCGCCGCTAACTTCTTGAGAGCAAGCTCTCAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCT

Organism: ***Bacillus sp*** **Accession no: JF683264.1** **Source: Dam 6 OWIM2** **Strain ID: (236A)**
GNTTTGGTGGTCGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAG
ATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATGCGCCG
CGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTA
CTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Organism: ***Bacillus pumilus*** **Accession no: AY792029.1** **Source: Dam 6 OWIM2** **Strain ID: (244)**
GGCTNTGGTNGGTACGTCAGGTGCGAGCAGTTACTCTCGCACTTGTTCTTCCCTAACAACAGCAGACTTTACGATCCNAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGC
GGAAGATTCCCTACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATG
CGCCGCGGGTCCATCTGTAAGTGACAGCCGAAACCGTCTTTCATCCTTGAACCATGCGGTTCAAGGAACTATCCGGTATTAGCTCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACG
TGTTACTCACCCGTCCGCCGCTAACATCCGGGAGCAAGCTCCCTTCTGTCCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCG

Organism: ***Bacillus cereus*** **Accession no: AB677943.1** **Source: Dam 6 OWIM2** **Strain ID: 370)**
GGCTNTGGTTGGTNCGGTCAGGTGCCAGCTTATTCAACTAGCACTTGCTTCTTCCCTAACAACAGCAGATTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATT
GCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAA
TGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCAGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCA
CGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATC

Organism: ***Lysinibacillus sp*** **Accession no: JN695724.1** **Source: Dam 6 OWIM2** **Strain ID: (244A)**
CGNTCAGGGTACAGCCAGNTACTACTGTACTTGCTTCTTCCCTTACAACAGCAGATTTTACGAACCGAAATCCTTCTTCACTCACGCGGCGATTGCTCCATCAGGCCTTTCGCCCATTGTGGAAGATTCC
CTACTGCTGCCTCNCGTAGGAGTCTGGGCCGTGTCTCANGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCNTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGG
CCCATCCTATAGCGACAGCCGAAACCGTCTTTNAGTGTTTCACCATGAGGTGAAACAGATTATTCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAACTATAAGGTAGGTTGCCCACGTGTTACTCA
CCCGTCCGCCGCTAACGNCNAAGGAGCAAGCTCCTTCTCTGTTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCGTC

Appendix 3
Publications from this study