Antibiotic resistant bacteria and their resistance genes in biofilm samples isolated from model water distribution systems of hospitality homes in Benin City, Nigeria

Batteri resistenti agli antibiotici e loro geni resistenti in campioni di biofilm isolati dai sistemi di distribuzione dell'acqua modello di case di ospitalità a Benin City, Nigeria

Olisaka Frances Ngozi[1,2,*], Ekhaise Fred Osaro[2]

[1] *Department of Basic Sciences, Benson Idahosa University, P.M.B 1100, Benin, Edo state, Nigeria*
[2] *Department of Microbiology, University of Benin, P.M.B 1154, Benin, Edo state, Nigeria*

*Corresponding author:
Olisaka Frances Ngozi, Department of Basic Sciences, Benson Idahosa University, P.M.B 1100, Benin, Edo state, Nigeria; Ph. + +23 08091022609, e-mail: francesolisaka@gmail.com or folisaka@biu.edu.ng

Water distributed in residential facilities such as Hotels, Inns and Guest houses are generally intended for several purposes like drinking and bathing. It is not sterile, regardless the water treatment been applied. Microbial presence in pipe-borne water results in the colonization of the distribution systems infrastructure and biofilm formation. Biofilms are well structured multicellular communities which are buried in a self-produced extra-polymeric substance that functions as an obstacle to antibiotic diffusion. Bacteria in biofilms have some advantage over their free floating counterparts which include protection from disinfectants and ability to resist most antibiotics especially in water piped systems. The aim of this study was to investigate the antibiotic resistance and their resistance genes in biofilm isolated from faucets giving water to end users in hospitality homes. Thirty six biofilm samples were collected from 6 hospitality homes. A total of 108 biofilms bacteria were isolated using spread plate method on R2A medium. Antibiotic susceptibility test was determined using disk diffusion method. Isolates were characterized using 16S rRNA gene sequencing and three resistance genes; *Tet*A, *Tet*M and *Erm*B were detected by Polymerase chain reaction. *Alcaligenes faecalis, Bacillus cereus, Enterobacter sp, Lysinibacillus fusiformis, Methylobacterium fujisawaense, Pseudomonas aeruginosa, Providencia vermicola*, and *Serratia liquefaciens*, were isolated. *Alcaligenes* spp was the most frequently isolated in all Hospitality homes. *Tet*A, resistance gene was more prevalent. It was detected in 49% of biofilm isolates, *Tet*M, 45%, and *Erm*B, 46% of all biofilm samples. Bacteria isolated from Biofilm were highly resistant to Chloramphenicol (100%) while lowest resistance was to Imipenem (1%). In view of the above, there is therefore an urgent need for the Hotel Management to work out modalities in eliminating biofilm bacteria associated with hospitality homes, which could pose a great public health risk due to the presence of multidrug resistant bacteria harboring antibiotic resistant genes.

Key words: Biofilms, resistance genes, antibiotic resistance, tetracycline resistance, *Methylobacterium fujisawaense*

Introduction

One of the basic right of every individual is to have uninterrupted access to safe drinking water [WHO, 2003]. The world health Organisation, considers that potable water should be suitable for human consumption and for all other domestic purposes including personal hygiene [WHO, 2003]. In Nigeria, like every other developing country, obtaining potable drinking water is almost impossible. This problem is made more convoluted by the fact that most of the available water sources are not potable without some form of treatment [Oluyege et al., 2009]. Therefore, this water may be a major source of pathogenic and opportunistic microorganisms [Yi et al., 2011]. The presence and wide spread of antibiotics resistant bacteria in water in drinking water systems have been a public health issue [Xi et al., 2009]. Antibiotic resistant genes have been reported to be known contaminants of bulk water [Pruden et al., 2006]. In aquatic environments, microorganisms have the ability to adhere to solid surfaces and form biofilms [Castonguay et al., 2006]. Biofilms are bacterial communities embedded in a polysaccharide matrix, which gives them the opportunity to resist destruction by antibiotics, environmental stress, biocides and detergents [Simpson, 2008]. Bacteria resistant to antibiotics are common in areas where antibiotics are used, but antibiotic-resistant bacteria are also know to occur in aquatic environments [Klare et al.,1995]. A well know mechanism in which biofilms help to confer resistance is through the delayed penetration of the antibiotics which is due to the self-produced extra cellular polymeric substance [Costerton et al., 1999]. Bacterial growth in the distribution systems may result from the detachment of biofilm bacteria, which increases the risk of infection in humans when the water is consumed [Choi and Morgenroth 2003]. Generally, most water distribution systems are characterized by the presence of biofilms, regardless of purity and the type of pipe materials used for distribution or the presence of a disinfectant [Lehtola et al., 2006]. Bacteria in drinking water systems can therefore grow in bulk water of distribution

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systems, as biofilms attached to the walls of pipes [Srinivasan et al., 2008]. However, the development of biofilms inside water distribution pipes makes it possible for mixed microbial populations to occur and is considered the main source of planktonic bacteria in water supply systems [Momba et al., 2000]. Piped drinking water typically involves the treatment of water from its source prior to distribution. Bacteria have adopted different means to inactivate the antibiotics used against them [Davison, 1999]. Most of the studies concerning antibiotic resistance in the aquatic environment have focused on bulk water and do not reflect the situation in biofilms, which is the preferred pattern of life of many bacteria. These aquatic systems have been identified to be reservoirs [Zu et al., 2016]. Due to the presence of high concentrations of Antibiotic resistant bacteria and Antibiotic genes in surface waters these bacteria and their genes may be found in drinking water treatment systems, making it major public health concern [Zu et al., 2016]. A Hotel is an establishment which provides paid lodging and feeding on a short term basis [Charara et al., 2011]. The goal of every successful hotel business is to maintain a high level of customer satisfaction. Hotels are major water consumers because people tend to use more water in the hotels than in the homes [Charara et al., 2011]. Hotel size may not really matter in relation to water needs, rather its tourists' water usage patterns and factors such as seasons and climate are more important. The implication of this is that water management is the responsibility of hotels of all sizes [Kasim et al., 2014]. Hospitality homes where people lodge for several days may be a high risk environment due to the complex nature of the water distribution systems, and the possible sensitivities of most of its occupants. As a result, a greater attention is being focused on hygienic risks associated with the use of the water distribution systems, where healthy and immuno-compromised persons, as well as elderly and young children reside either for few days or for a longer period of time. Adverse health consequences associated with the use of these commercial outfits can be prevented through correct information on potential risks and effective management. The hazards posed by bacteria present in biofilm associated with water distribution systems of Hotels and guest home segment of the Hospitality industry in Benin City is of major concern. The study was aimed at investigating the antibiotic resistance and resistant gene determinants of biofilm bacteria collected from point of use faucets from the water distribution systems of some selected hotels, based on high patronage by customers and tourists in Benin City, Edo State, Nigeria.

Materials and methods

Study site and sampling

Nigeria is located at Longitude 8.6753°E and Latitude 9.0820°N. The study site Benin City in Oredo Local Government Area, is the capital of Edo State in southern Nigeria, approximately 40 kilometers North of the Benin River. It is situated 320 kilometers (200 mi) by road east of Lagos. Benin City has an estimated population of 1,147,188 people of diverse ethnic group. Samples were collected between January 2015 and November 2015 from six Hospitality Homes. Samples were collected in triplicates from three sampling points, which are Room Tap, Kitchen Tap and Shower. Before biofilm collection, the inner and the outer mouths of the faucets were cleaned with spirit and the water was allowed to run for 2 - 3 min. A total of 90 biofilms samples were collected by vigorously rubbing the inner surface of the faucets with sterile swabs soaked in sterile distilled water. Swab samples were kept wet in 3 milliliters of bacteriological (0.85%) saline at each sampling point upon collection. Samples were transported in ice box to the laboratory for microbiological analyses. The sample was mixed continuously for 1 min using a vortex mixer, serial tenfold dilutions of each sample were prepared in test tubes, using sterile saline as the diluents, to a final volume of 1 ml. Triplicate spread plates were prepared of three appropriate dilutions on R2A agar (Difco Laboratories Detroit, MI).Upon incubation, plates showing good growth were sub - cultured onto nutrient medium and further sub - cultured pure cultures of the bacteria isolates were transferred onto nutrient agar slants and incubated at 37°C for 18 - 24h. The slants were kept at 4°C in the refrigerator for further experimentations. The cultural, morphological and biochemical characterization were carried out according to the methods of Cowan and Steel [Cowan and Steel, 1965].

Antibiotic susceptibility test

Antibiotic susceptibility test was carried out using the Kirby-Bauer disc diffusion methods [Bauer et al., 1966]. The Bacterial inoculum that was used was prepared by inoculating freshly grown bacteria in 5 ml of sterile nutrient broth. The turbidity of the broth was adjusted to a 0.5 Mc Faland standard. The antimicrobial susceptibility testing was performed using Mueller-Hinton medium. The following antibiotic discs were used [CLSI, 2011], which include; Amoxicilin (Amx) 25 μg, Ofloxacin (Ofl) 5 μg, Chloramphenicol (Chl) 30 μg, Gentamicin (Gen) 10 μg, Cotrimoxazole (Cot) 25 μg, Tetracycline (Tet) 30 μg, Erythromycin (Ery) 15 μg, Imipenem (Imp) 5 μg, Netilmicin (Net) 5 μg and Cefuroxime (Crx). The discs were transferred unto Muller Hilton agar plates with a sterile forceps, then pressed down gently and incubated at 37°C for 24h. Resistance was recorded when there were no clear zones of inhibition around the respective disc and sensitivity was recorded when there was presence of inhibition. Multiple antibiotic resistance phenotypes were generated for isolates that showed resistance to three or more antibiotics.

Identification of bacteria using 16S rRNA gene sequencing

Extraction of Genomic DNA and Detection of Resistant genes

DNA was isolated from bacterial species grown on R2A agar using the QIAamp DNA Mini Kit (250) cat no 51306. (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The final volume of the

extracted DNA was 100 µl. The concentration of the purified DNA was quantified spectrophotometrically [Nanodrop 2000 Spectrophotometer, Thermo scientific, USA] until further use.

16S rRNA gene amplification

Extracted DNA templates were subjected to PCR using set (Forward and Reverse) of primers targeting 16S rRNA genes of bacteria isolates. PCR was performed with 27F-AGAGTTTGATCMTGGCTCAG and 1492R-AAGGAGGTGWTCCARCCGCA, [Fredriksson et al., 2013]. Thermocycling conditions initial denaturation, denatured at 94°C for 5 min, denaturation at 94°C for 30 sec, Ann. temperature at 56°C for 30 sec, extension at 72°C for 45 sec, final extension at 72°C for 7 min, hold temperature at 10°C at 36 circles. The amplicon from the reaction above was loaded on a 1.5% agarose gel. The PCR product was used for another PCR reaction (same as above) that is now sequencing reaction. The product from the purification process was loaded on a 3130xl genetic analyzer (Applied Biosystems) to give the sequences. MEGA6 was used to view and analyze the obtained data. BLASTn [Altschul et al., 2007] was used to identify the best matches from Genbank based on percent sequence identity. Sequences have been deposited in GenBank and are under the Study accession # KY235303-KY235307 and KY066450 to KY066453, KY066455 and KY066457.

Antibiotics resistance genes

Genes coding for erythromycin resistance (*Ery*B)and tetracycline resistance (*Tet*M and *Tet*A were amplified with *Tet*M F (5′GTRAYGAACTTTACCGAATC3′) and *Tet*(M) R (5′ATCGYAGAAGCGGRTCAC3′), *Tet*(A) F (5′TTGGCATTCTGCATTCACTC3′) and *Tet*(A)R (5′GTATAGCTTGCCGGAAGTCG3′)and *Erm*B; F (5′GAAAAGGTACTCAACCAAATA3′)*Erm*B; R (5′ AGTAACGGTACTTAAATTGTTTAC3′). They were also amplified using the PCR reaction procedure as described for 16S rRNA, with the exception of different primer annealing temperature. Thermocycling conditions in a GeneAmp PCR system (Geneamp, Singapore) 94°C for 4 min. For initial denaturation then 31 cycles, each at 94°C for 45 seconds, 55°C for 1 min for *Tet*M and *Erm*B (60°C for 1 min for *Tet*A), 68°C for 1 min and final extension at 68°C for 8 minutes after which PCR products were separated in 1.5% agarose gel which was stained with ethidium bromide. PCR products on gel were visualized with a Gel viewer (Enduro™GDS Labnet).

Results

A total of 108 multidrug resistant bacteria were obtained from biofilm samples from three source; Room Tap, Kitchen Tap and Shower. Seventy six (70.4%) and thirty two (29.6%) Gram negative and Gram positive bacteria were isolated respectively from the hospitality homes in the study. The bacteria isolated and their frequency of occurrence are: *Alcaligenes faecalis* (26.9%), *Bacillus cereus* (20.4%), *Serratia liquefaciens* (13.9%), *Lysinibacillus fusiformis* (9.2%), *Providencia vermicola* (5.6%), *Pseudomonas aeruginosa* (7.4%), *Methylobacterium fujisawaense* (11%) and *Enterobacter* sp (5.6%). *Alcaligenes faecalis* and *Bacillus cereus* were found to be the most frequently isolated bacteria in the sessile community from the hospitality homes (Figure 1). Table 1, shows the bacteria identified by 16S rRNA gene sequencing. The study accession number and closest type strains in Genbank data base, accompanied with percentage similarity of isolates, their fragment length and family. The bacterial biofilm isolated were highly resistant to Chloramphenicol (100%) and Ofloxacin (92%), and recorded to have low resistance to Imipenem (2%). While Tetracycline and Erythromycin recorded 67% and 56% respectively. *Pseudomonas aeruginosa* (1%) was reported as the only bacterial biofilm resistant to Imipenem (Table 2). Sixty nine isolates from the sample sources which where resistant to Tetracycline and sixty one isolates resistant to Erythromycin phenotypically, harbored at least one resistant gene used for this study. Table 3 shows the percentage of antibiotics resistance genes in all isolated bacteria. The *Tet*A resistant gene, which encodes resistance by efflux pump mechanism, was the most prevalent. It was found in 49% of isolates, 45% of the isolates possessed *Tet*M resistant genes encoding for ribosomal protection protein, while 46% of the isolates possessed Erythromycin resistant genes (*Erm*B), encoding efflux mediated mechanism. Table 4 shows the different primer pairs used for this study for the amplification of 16S rRNA genes and antibiotics resistant genes. Table 5 shows a summary of the Phenotypic and Genotypic characteristics of bacterial biofilm isolated from three points (source) from six hospitality homes.

Discussion

Seventy-six (70.4%) and thirty-two (29.6%) Gram negative and Gram positive bacteria were isolated respectively from the hospitality homes in this study. The gram negative organisms were recorded to be the most isolated in this study. This is in agreement with the reports of Adesoji et al., 2015, which recorded the Gram-negative bacteria as the most predominant bacteria compared to the Gram-positive Bacteria among multi-drug resistant bacteria from selected water distribution systems in Southwestern Nigeria. A total number of eight bacteria genus were isolated: *Alcaligenes, Bacillus, Serratia l, Lysinibacillus, Providencia, Pseudomonas, Methylobacterium and Enterobacter*. These were similar to the bacteria genus reported by Adesoji [Adesoji et al., 2015]. These authors isolated a group of opportunistic and pathogenic bacteria from drinking water distribution dams from southwestern Nigeria. The presence of these organisms in these systems indicates faecal pollution of the water, making the water unfit for human consumption. September et al. [September et al., 2004] reported an increase in the number of *Pseudomonas* spp and no *Shigella*

and *Salmonella* confirmed from biofilm samples of drinking water distribution systems in South Africa. In this study, *Pseudomonas aeruginosa* was identified in the biofilm samples of distributions systems of hospitality homes. Also in a study by Albrechtsen [Albrechtsen et al., 2005] *Pseudomonas* spp. was isolated from biofilm samples and also from the bulk water of the same non-chlorinated model drinking water distribution system in Denmark. It was also recorded that undermining the water source, *Pseudomonas* was considered as the most abundant bacterium in water network supply. *Pseudomonas aeruginosa* isolated from this study was resistant to seven antibiotics (Amoxicillin, Gentamicin, Chloramphenicol, Cefuroxime, Nitrofurantoin, Augmentin and Ofloxacin). In this study *Methylobacterium fujisawaense* was isolated from the biofilm of the three sampled sites. *Methylobacterium fujisawaense* is not established as pathogenic; however, rarely it may cause human infection/disease, mostly in immuno compromised patients and has also been known to be highly sensitive to Imipenem [Rice et al., 2000]. In this study, its resistant pattern is to Chloramphenicol, Cefuroxime, Augmentin and Ofloxacin. Its sensitivity to Imipenem is in agreement with Rice [Rice et al., 2000]. Methylobacterium is recorded to predominate in bathroom environments. *Alcaligenes faecalis* was isolated from the biofilm from the three sampled sites. They are opportunistic organisms and responsible for nosocomial septicemia, cystic fibrosis and otitis media. They are known to be highly resistant to antibiotics. In this study, *Alcaligenes faecalis* was recorded to be resistant to seven antibiotics; Cotrimazole, Tetracycline, Amoxicillin, Gentamicin, Chloramphenicol Augmentin and Ofloxacin. Due to its capsule forming nature, it has been implicated as strong biofilm producers. *Bacillus cereus* was isolated from the biofilm development from the three sampled sites. This finding coincides with that of Ryan and Ray [Ryan and Ray, 2004] who demonstrated that Bacillus cereus causes foodborne illness. *Bacillus cereus* isolated from the six hospitality homes studied had a 100% resistance to four antibiotics: Cefuroxime, Amoxicillin, Chloramphenicol and Ofloxacin. *Bacillus cereus* could be controlled within the distribution system if high chlorine levels are maintained [Ryan and Ray, 2004]. The Proteobacteria constituted majority of the bacterial biofilm characterized and identified. Gamma (γ) Proteobacteria was recorded as the most dominant bacteria in the biofilm while Firmicutes represented the remaining using the 16S rRNA gene. This was also reported by Kalmbach, [Kalmbach et al., 1997] who recorded the presence of the Proteobacteria subclasses in almost two third of the total bacteria found in drinking water. This study also revealed the presence of Multi drug resistant opportunistic microorganisms possessing Tetracycline and Erythromycin resistant genes in the different sources of the six hospitality homes sampled. Molecular analysis was done to study the presence of three different genes that encode resistance to tetracycline, namely, *Tet*A, *Tet*M, and *Erm*B gene that encodes resistance to erythromycin, and two tetracycline resistance genes represent each of the three known mechanisms namely, efflux pumps and ribosomal protection. The erythromycin gene *Erm*B was used because it codes rRNA methyltransferase that confers resistance to macrolides. This was also reported by Roberts [Roberts et al., 1999]. In this study, three resistance genes were carried by bacterial biofilm found in Hospitality home 3; (*Serratia liquefaciens* and *Providencia vermicola*), Hospitality home 4; (*Alcaligenes faecalis, Lysinibacillus fusiformis* and *Providencia vermicola*) and Hospitality home 6 (*Alcaligenes faecalis*) *Tet*A has been found to be the most dominant antibiotic resistance gene, encoding efflux mechanism. This was also recorded by Adesoji [Adesoji et al., 2015] in bacteria isolated from distribution systems in Southwestern Nigeria. The presence of erythromycin resistant gene, *Erm*B alone was not found in any of the bacterial biofilm samples. The possession of antibiotic resistance genes by the isolates associated with biofilms has also been reported by Xi [Xi et al., 2009] that bacteria may develop resistance though mutation or acquisition of resistance genes from other bacteria found in the consortium. This study revealed the presence of gram negative and gram-positive bacteria in the sampled sites of hospitality homes studied. The rising increase of Antibiotic resistant bacteria and antibiotic resistant genes, could be due to the over use and misuse of antibiotics in humans and animals.

Conclusion

Multidrug resistance and possession of resistant genes in biofilm bacteria is of great public health concern, especially in Hospitality homes since more water is been used there than in the homes. More research should be focused on pathogenic biofilm bacteria which are viable but not culturable (VBNC) having the ability of transferring Antibiotic Resistance Genes to other culturable bacteria in this sessile way of life. It is the responsibility of every Hotel Management, to provide safe and potable water for their guests and occupants. A good knowledge of the growth and growth needs of microorganisms' ability to grow in water distribution systems will go a long way to help managers and operators of these systems to control biofilm microorganisms .Also the availability of carefully designed and properly maintained distribution pipes and faucets, offers a great level of protection to the consumer.

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Table 1: Primers used in this study for the amplifications of 16s rRNA genes and other antibiotic resistant genes

Primer pair	*Target gene/resistant genes*	*Sequence (5′-3′)*	*Anneling temperature*	*Amplicon size (bp)*	*References*
27F	16S rRNA	AGAGTTTGATCMTGGCTCAG	55	1,450	Adesoji et al., 2015
1492R	16S rRNA	GGTTACCTTGTTACGACTT	55	1,450	"
*Tet*A-F	Tetracycline	TTGGCATTCTGCATTCACTC	60	494	"
*Tet*A-R	Tetracycline	GTATAGCTTGCCGGAACTCG	60	494	"
*Tet*M-F	Tetracycline	ACACGCCAGGACATATGGAT	55	536	"
*Tet*M-R	Tetracycline	ATTTCCGCAAAGTTCAGACG	55	536	"
*Erm*B-(F)	Erythromycin	GAAAAGGTACTCAACCAAATA	55	738	Ross et al., 1990
*Erm*B-(R)	Erythromycin	AGTAACGGTACTTAAATTGTTTA	55	738	

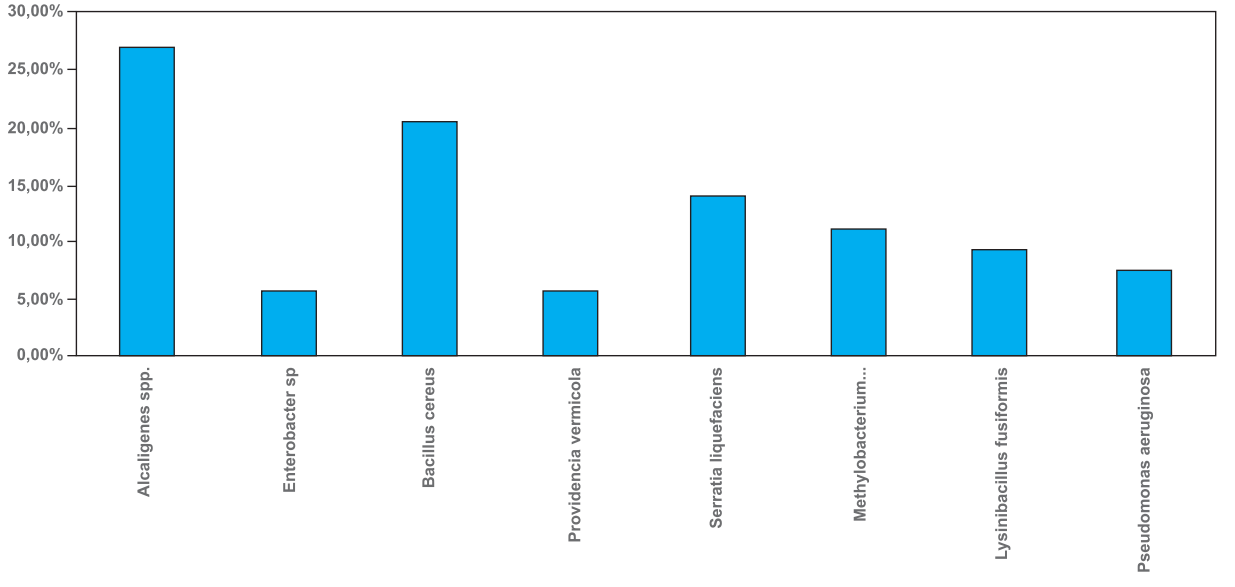

Figure 1: Percentage frequency of occurrence of bacterial biofilm in Hospitality homes studied

Table 2: Bacteria identified by 16S rRNA partial sequencing while accession number is the GenBank's number for the closest match

(Study accession number) Strains/ (GeneBank Accession No.)	*Closest type strains in GeneBank database (Accession* No.)	*Length of Fragment*	*Similarity (%)*	*Family*	*Class*	*phylum*
BT1 (KY066450)	*Providencia vermicola* JK0216^{T} (KF135454)	1,067	98	Enterobacteriaceae	γ Proteobacteria	Proteobacteria
BT2 (KY066451)	*Serratia sp* QW4 (KF737362)	1,094	85	Enterobacteriaceae	γ Proteobacteria	Proteobacteria
BT4 (KY066453)	*Serratia liquefaciens* strain OAct423 (CP006252)	1,397	99	Enterobacteriaceae	γ Proteobacteria	Proteobacteria
BT3 (KY066452)	*Bacillus cereus* strain BC-2 (KF8355391)	1,408	99	Bacillaceae	β Bacilli	Firmicutes
BT8 (KY066457)	*Alcaligenes faecalis* strain PSD10 (KP835577)	1,112	85	Alcaligenaceae	α Proteobacteria	Proteobacteria
MF (KY235304)	*Methylobacterium fujisawaense* strain MECA_7.1.3 (KT720185)	856	99	Methylobacteriaceae	γ Proteobacteria	Proteobacteria
BT (KY235303)	*Enterobacter sp* QAUEBSP01 (KT033700)	853	96	Enterobacteriaceae	γ Proteobacteria	Proteobacteria
LF (KY235305)	*Lysinibacillus fusiformis* strain PWX7 (KU942487)	842	98	Bacillaceae	Bacilli	Firmicutes

Table 3: Antibiotic resistance of bacterial biofilm isolated from distribution water systems sources of six hospitality homes

Antibiotics	[a]*A.sp n=29*	*S. liquefaciens n=15*	*P. vermicola n=6*	*P. aeruginosa n=8*	*M. fujisawaense n=12*	*E. sp n=6*	*B. cereus n=22*	*L. fusiformis n=10*	*Mean resistance (%)*
Tetracycline	(26) 90%	(14) 93%	(6) 100%	(5) 62.5%	(8) 67%	(2) 33%	(2) 9%	(6) 60%	64%
Cotrimalzone	(29) 100%	(15) 100%	(6) 100%	(5) 62.5%	(6) 50%	(5) 83%	(20) 90%	(2) 20%	81%
Amoxicillin	(28) 96.5%	(15) 100%	(6) 100%	(8) 100%	(3) 25%	(6) 100%	(22) 100%	(3) 30%	84%
Gentamicin	(29) 100%	(15) 100%	(6) 100%	(8) 100%	(2) 17%	(6) 100%	(16) 72%	(10) 100%	85%
Chloramphenicol	(29) 100%	(15) 100%	(6) 100%	(8) 100%	(12) 100%	(6) 100%	(22) 100%	(10) 100%	100%
Imipenem	(0) 0%	(0) 0%	(0) 0%	(1) 10%	(0) 0%	(0) 0%	(0) 0%	(0) 0%	01%
Cefuroxime	(5) 17%	(12) 80%	(3) 50%	(8) 100%	(12) 100%	(6) 100%	(22) 100%	(4) 40%	67%
Netilmicin	(3) 10%	(2) 10%	(0) 0%	(6) 75%	(6) 50%	(4) 66%	(11) 50%	(10) 100%	39%
Erythromycin	(3) 10%	(8) 53%	(6) 100%	(7) 87.5%	(8) 67%	(6) 100%	(13) 59%	(10) 100%	56%
Nitrofurantoin	(6) 20%	(15) 100%	(3) 50%	(8) 100%	(2) 17%	(5) 83%	(19) 86%	(8) 80%	61%
Augmentin	(29) 100%	(15) 100%	(6) 100%	(8) 100%	(12) 100%	(2) 33%	(2) 9%	(10) 100%	78%
Ofloxacin	(28) 96%	(13) 86%	(6) 100%	(8) 100%	(12) 100%	(1) 17%	(21) 95%	(10) 100%	92%

[a]A.sp - *Alcaligenes sp*; S. liquefaciens - *Serratia liquefaciens*; P. vermicola - *Providencia vermicola*; P. aeruginosa - *Pseudomonas aeruginosa*; M. fujisawaense - *Methylobacterium fujisawaense*; E. sp - *Enterobacter sp*; B. cereus - Bacillus cereus; L. fusiformis - *Lysinibacillus fusiformis*

Table 4: Antimicrobial resistance genes in biofilms bacteria isolated from Hospitality Homes in Benin City, Nigeria

Resistant gene	*Number of Bacteria tested based on Phenotypic resistance*	*Proportion of bacterial carrying the resistance*
Tetracycline (*Tet*A)	69	(34) 49%
Tetracycline (*Tet*M)	69	(31) 45%
Erythromycin (ErmB)	61	(28) 46%

Table 5: Summary of Bacterial biofilm isolated from three points from all Hotels with their resistance genes

HOTEL	*S/N*	*Bacteria*	*Source*	*Resistant Genes*	*b Resistant phenotype*
HOTEL 1	1	*Enterobacter sp*	Room tap	*Tet*A + *Tet*M	Crx, Gen, Ofl, Aug, Nit, Tet, Ery, Chl, Amx, Cot
	2	*Alcaligens faecalis*	Kitchen	*Tet*M + *Erm*B	Crx, Gen, Ofl, Aug, Nit, Tet, Ery, Amx, Chl, Cot
HOTEL 2	1	*Alcaligens faecalis*	Shower, Kitchen, Room tap	*Tet*A	Crx, Gen, Cxm, Aug, Tet, Ery, Amx, Chl, Cot
	2	*Lysinibacillus fusiformis*	Kitchen, Room tap	*Tet*A + *Tet*M	Crx, Gen, Cxm, Aug, Tet, Nit, Ery, Amx
	3	*Providencia vermicola*	Room tap	*Tet*A + *Erm*B	Crx, Gen, Cxm, Aug, Nit, Ery, Amx
HOTEL 3	1	*Serratia liquefaciens*	Shower	*Tet*A + *Tet*M + *Erm*B	Crx, Gen, Cxm, Aug, Tet, Ery, Amx, Chl, Cot
	2	*Bacillus cereus*	Shower	*Tet*A + *Tet*M	Crx, Gen, Cxm, Aug, Tet, Amx
	3	*Alcaligens faecalis*	Shower	*Tet*A + *Tet*M	Crx, Gen, Cxm, Tet, Ery, Amx, Chl
	4	*Providencia vermicola*	Kitchen	*Tet*A + *Tet*M + *Erm*B	Crx, Gen, Cxm, Aug, Tet, Nit, Ery, Amx
	5	*Pseudomonas aeruginosa*	Shower	*Tet*A + *Erm*B	Crx, Gen, Cxm, Ofl, Aug, Tet, Nit, Ery, Amx, Chl, Cot
	6	*Lysinibacillus fusiformis*	Shower	*Tet*A + *Erm*B	Crx, Gen, Cxm, Ofl, Aug, Tet, Nit, Ery, Amx, Chl, Cot
	7	*Providencia vermicola*	Room tap	*Tet*A + *Erm*B	Crx, Gen, Cxm, Ofl, Aug, Cpr, Ery, Tet
	8	*Pseudomonas aeruginosa*	Room tap	*Tet*A + *Tet*M	Crx, Gen, Cxm, Ofl, Nit, Cpr, Amx, Chl, Cot, Imp
HOTEL 4	1	*Serratia liquefaciens*	Room tap	*Tet*A + *Tet*M	Crx, Gen, Ofl, Aug, Tet, Ery, Amx, Chl
	2	*Serratia liquefaciens*	Kitchen	*Tet*A + *Erm*B	Gen, Aug, Tet, Ery, Amx, Chl, Cot
	3	*Alcaligenes faecalis*	Kitchen	*Tet*A + *Tet*M	Crx, Cxm, Ofl, Aug, Tet, Cpr, Ery, Amx
	4	*Alcaligenes faecalis*	Shower	*Tet*A + *Tet*M + *Erm*B	Crx, Gen, Ofl, Aug, Tet, Cpr, Ery, Amx
	5	*Lysinibacillus fusiformis*	Kitchen	*Tet*A + *Tet*M + *Erm*B	Crx, Gen, Ofl, Aug, Tet, Cpr, Amx
HOTEL 5	1	*Providencia vermicola*	Shower	*Tet*A + *Tet*M + *Erm*B	Crx, Gen, Aug, Tet, Nit, Ery, Amx, Chl, Cot
	2	*Providencia vermicola*	Room tap	*Tet*M + *Erm*B	Crx, Gen, Ofl, Aug, Nit, Cpr, Ery, Amx, Chl, Cot
	3	*Methylobacterium fujsawaense*	Room tap	*Tet*M + *Erm*B	Crx, Gen, Ofl, Tet, Aug, Nit, Cpr, Ery, Amx, Chl, Cot
	4	*Bacillus cereus*	Room tap	*Tet*M	Crx, Gen, Ofl, Nit, Cpr, Ery, Amx, Chl, Cot
HOTEL 6	1	*Methylobacterium fujisawaense*	Room tap	*Tet*A + *Erm*B	Crx, Gen, Cxm, Aug, Tet, Ery, Amx, Chl, Cot
	2	*Alcaligens faecalis*	Shower	*Tet*A + *Tet*M + *Erm*B	Crx, Gen, Cxm, Aug, Tet, Nit, Cpr, Ery, Amx, Chl, Cot
	3	*Bacillus cereus*	Room tap	*Tet*A	Crx, Gen, Ofl, Aug, Nit, Cpr, Tet, Ery, Amx, Chl, Cot

References

Aderiye, B.I., Igbedioh, S.D., Adebobuyi, A.A., 1992. *Incidence of coliforms in well water and anti-outbreak of water borne diseases; environmental considerations and empirical evidence from Owo Nigeria.* ActaMediterr di Patolog. E Tropic., **11** (1), 2 - 7.

Adesoji, A.T., Ogunjobi, A.A., Olatoye, I.O., Douglas, R.D., 2015. *Prevalence of tetracycline resistance genes among multi-drug resistant bacteria from selected water distribution systems in Southwestern Nigeria.* Anna. Clin. Microbiol. Antimicrob., **14**, 35.

Albrecmsen, H., Arvin, E., Martiny, A.C., Molin, S., 2005. *Identification of bacteria biofilm and bulk water samples from a nonchlorinated model drinking water distribution system: Detection of a large nitrite-oxidizing population associated with Nitrospira spp.* Appli. Environ. Microbiol., **71** (12), 8611 - 8617.

Altschul, S.F., Madde, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 2007. *Gapped BLAST and PSI-BLAST: a new generation of protein databa research programs.* Nucl. Acids Res., 25, 3389 - 3402.

Bartram, J., Cotruvo, J., Exner, M., Fricker, C., Glasmacher, A., 2004. *Heterotrophic plate count measurement in drinking water safety management.* Report of an expert meeting Geneva, 24 - 25/04/2002. Int. J. Food Microbiol., **92**, 241 - 247.

Bauer, A.W., Kirby, W.M., Sherirs, J.C., Turck, M., 1966. *Antibiotic susceptibility testing by standardized single disk method.* Amer. J. Clin. Pathol., **45**, 433 - 496.

Berry, D., Xi, C., Raskin, L., 2006. *Microbial ecology of drinking water distribution systems.* Curr. Opin. Biotechnol., **17**, 297 - 302.

Burmeister, A.R. 2015. *Horizontal Gene Transfer.* Evol. Med. Public Health, **1**, 193 - 194.

Castonguay, M., Van der Schaff, S., Koester, W., Krooneman, J., Vanderr Meer, W., Landini, H.P., 2006. *Biofilm formation by Escherichia coli is stimulated by synergistic interactions and co adhesion mechanism with adherence proficient bacteria.* Res. Microbiol., **157**, 471 - 478.

Charara, N., Cashman, A., Bonnell, R., Gehr, R., 2011. *Water use efficiency in the hotel sector of Barbados.* JoST, **19** (2), 231 - 245.

Chen, K.T., Chen, C.J., Chiu, J.P., 2001. *A school waterborne outbreak involving both Shigella sonnei and Entamoebahistolytical.* J. Environ. Health, **64**, 9 - 13.

Choi, Y.C., Morgenroth, E., 2003. *Monitoring Biofilm Detachment under dynamic changes in shear stress using Laser-based particle size.* Water Sci. Technol., **47** (5), 69 - 76.

Clinical and Laboratory Standards Institute Standards guidelines (CLSI), 2011. *Performance standards for antimicrobial susceptibility testing: twelfth informational supplement.* NCCLS document M100-S12. PA, USA. 44 pp.

Cowan, S.T., Steel, K.J., 1965. *Manual for the Identification of Medical Bacteria.* Cambridge University Press, Cambridge, UK.

Critchley, M.M., Cromar, N.J., McClure, N.C., Fallowfield, H.J., 2003. *The influence of the chemical composition of drinking water on cuprosolvency by biofilm bacteria.* J. Appl. Microbiol., **94**, 501 - 507.

Davison, J., 1999. *Genetic exchange between bacteria in the environment.* Plasmid, **42**, 73 - 91.

Elhariry, H., Gherbawy, Y., El-Deeb, B., Altalhi, A., 2012. *Molecular Identification and Biofilm forming ability of Culturable Aquatic Bacteria in Microbial Biofilms formed in Drinking water Distribution Networks.* Geomicrobiol. J., **29** (6), 561 - 569.

Fredriksson, N.J., Hermansson, M., Wilén, B.M. 2013. *The Choice of PCR Primers Has Great Impact on Assessments of Bacterial Community Diversity and Dynamics in a Wastewater Treatment Plant.* PLoS ONE, **8** (10), 76431.

Kasim, A., Gursoy, D., Okumus, F., Wong, A. 2014. *Importance of Water management in Hotels: A Framework for sustainability through innovation.* JoST, **87**, 34 - 44.

Kalmbach, S., Manz, W., Szewzyk, U., 1997. *Dynamics of biofilm formation in drinking water: phylogenetic affiliation and metabolic potential of single cells assessed by formazan reduction and in situ hybridization.* FEMS Microbiol. Ecol., **22**, 265 - 279.

Klare, I., Heier, H., Claus, H., Bohme, G., Martin, S., Seltmann, S., Hakenbeck, R., Atanassova, V., Witte, W. 1995. *Enterococcus faecium strains with vanA-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community.* Microb. Drug Resist., **1**, 265 - 272.

Lehtola, M., Laxander, M., Miettinen, I., Hirvonen, A., Vartiainen, T., Martikainen, T., 2006. *The effects of changing water flow velocity on the formation of biofilms and water quality in pilot distribution systems consisting of copper or polyethylene.* Water Res., **40**, 2151 - 2160.

Momba, M., Kfir, R., Venter, S., Cloete, T., 2000. *An overview of biofilm formation in distribution systems and its impact on the deterioration of water quality.* Water SA., **26**, 59 - 66.

Oluyege, J.O., Dada, C.A., Odeyemi, A.T., 2009. *Incidence of multiple antibiotic resistant gram negative bacteria isolated from surface underground water sources in south western region of Nigeria.* Water Sci. Techol., **5** (10), 1929 - 1936.

Pruden, A., Pei, R., Storteboom, H., Carlson, K.H., 2006. *Antibiotic resistance genes as emerging contaminants: studies in northern Colorado.* Environ. Sci. Technol., **40**, 7445 - 7450.

Rice, K.C., Mann, E.E., Endres, J.L., Weiss, E.C., Cassat, J.E., Smeltzer, M.S., 2007. *The Acid murein hydrolase regulator contributes to DNA release and biofilm development in Staphylococcus aureus.* Proceedings of National Academy of Science USA, **104**, 8113 - 8118.

Roberts, M.C., Sutcliffe, J., Courvalin, P., Jensen, L.B., Rood, J., Seppala, H., 1999. *Nomenclature for macrolide and macrolide, lincosamide and streptogramin B resistance determinants.* Antimicrob. Agents Chemother., **43**, 2823 - 2830.

Ryan, K.J., Ray, C.G., 2004. *Shems Medical Microbiology*. 4th ed. New York, McGraw-Hill, 992 p.

September, S.M., Els, F.A., Venter, S.N., Brozel, V.S., 2007. *Prevalence of Bacterial pathogens in biofilms of drinking water distribution systems.* Jour. Water Health, **5** (2), 219 - 227.

Simpson, D., 2008. *Biofilm processes in biologically active carbon water Purification.* Water. Res., **42** (12), 2839 - 2848.

Srinivasan, S., Harrington, G., Xagoraraki, I., Goel, R., 2008. *Factors affecting bulk to total bacteria ratio in drinking water distribution systems.* Water Res., **42**, 3393 - 3404.

Xi, C., Zhang, Y., Marrs, F.C., Ye, W., Simon, C., Foxman, B., Nriagu, J., Marrs, F.C., Ye, W., Simon, C., Foxman, B., Nriagu, J., 2009. *Prevalance of Antibiotics resistance in Drinking water Treatment and Distribution Systems. Appl.* Environ. Microbiol., **75** (17), 5714 - 5718.

Xu, L., Ouyang, W., Qian, Y., Su, C., SU, J., Chen, H., 2016. *High- throughput Profiling of antibiotic resistance genes in Drinking water treatment plants and distribution systems.* Environ. Pollut., **213**, 119 - 126.

Yi, L., Jiao, W., Chen, X., Weiping Chen, W., 2011. *An overview of reclaimed water reuse in China.* J. Environ. Sci., **23** (10), 1585 - 1593.

World Health Organization (WHO), 2003. *Emerging issues in water and infectious disease.* Geneva.