Original Research Article

Antibiotic Resistance Pattern of Bacteria Isolated from Biofilms in Water from Groundwater Sources in Ado-Ekiti, Nigeria

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ABSTRACT

This study investigated the pattern of occurrence of antibiotic resistant bacteria in biofilms in water from groundwater sources in Ado-Ekiti, Nigeria. Water samples were collected from boreholes and wells within Ado-Ekiti metropolis over a period of 4 months (n = 100), and biofilm samples were taken at interval of seven days within the period of storage and subjected to microbiological analysis until the total bacterial counts were significant. Enumeration of bacteria in biofilms and antibiotic sensitivity of the bacterial isolates were carried out using standard microbiological methods and multiple antibiotic resistant indexes of the bacterial isolates were calculated. Results showed that a total of 202 bacterial isolates were obtained from the biofilms of the water samples and this include *Streptococcus faecalis, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Salmonella typhi* and *Shigella dysenteriae*. Of all the bacterial isolates, *Streptococcus faecalis* had the highest frequency of occurrence (90 %). The bacterial isolates from the biofilms in water from borehole had the highest bacterial count (1.11×10^4 cfu/ml) and were more resistant to antibiotics, whereas those from well had the least bacterial count (0.78×10^4 cfu/ml) and were less resistant to antibiotics. A total of 106 (52.5%) bacterial isolates displayed multiple antibiotic resistance (MAR) with indexes greater than 0.2. The findings from this study suggest high prevalence of MAR indexes indicating high source of contamination in areas where antibiotics are used in Ado-Ekiti. Water from the groundwater sources should be treated at point of use and should not be stored for too long before use to prevent the development of biofilms that may be of great significance to human health.

Keywords: Antibiotic resistance, biofilms, groundwater, bacteria, human health

1. INTRODUCTION

Water is vital to life and it is essential to ensure that the drinking water is safe by preventing the formation of biofilms ref. Despite the purification systems set up by various water suppliers and individuals, there still exist occasional outbreaks of water borne diseases ref. Waterborne diseases are caused by the presence of microorganisms most especially bacteria such as *Streptococcus feacalis*, *Escherichia coli*, *Salmonella spp* and *Shigella dysenteriae* in the water [1]. In aquatic environments, micro-organisms have the ability to adhere to solid surfaces, and form biofilms, a biofilm is a population of cells growing on a surface in contact with water and enclosed within a self-produced matrix of extracellular polymeric substance (EPS) [2]. The matrix contains polysaccharides, proteins, glycoproteins, glycolipid and DNA, the extracellular matrix allows the microbes to stick more stably to the surface and protects them from antimicrobial agents meant to destroy them [3].

Biofilms increase the opportunity for gene transfer among bacteria ref. Bacteria that are resistant to antibiotics may transfer the genes for resistance to neighboring susceptible bacteria [4]. Also, gene transfer could convert a previous virulent commensal organism into a highly virulent pathogen [5].

Bacteria within a biofilm are more resistant to antibiotics, compared to planktonic bacteria [6]. Bacterial cells in biofilms exhibit 10 to 1,000 times less susceptibility to specific antimicrobial agents compared to their planktonic counterparts ref. Antibiotic resistance is primarily the consequence of genetic transfer of resistant genes, therefore, bacteria in biofilms are usually multiple antibiotics resistant [7]. High prevalence of multidrug resistance indicates a serious need for antibiotics surveillance program [8]. Multiple antibiotic resistant (MAR) indexing has been used to differentiate bacteria from different sources using antibiotics that are commonly used for human therapy ref.

Biofilms can be responsible for increased bacterial levels, reduction of dissolved oxygen, taste and odour changes in water [9]. Among the major drinking water sources in Ado-Ekiti, the capital of Ekiti - State are borehole and well water, biofilms may develop within these drinking water as a result of contamination or regrowth of microorganisms and this may lead to the occurrence of waterborne diseases, biofilms oftentimes serve as environmental reservoirs for pathogenic microorganisms and this is of great public health significance ref. There is therefore, the need to assess these drinking waters in Ado-Ekiti for the presence of biofilms and examine the bacterial population associated with such biofilms, especially those implicated in waterborne diseases. This study investigated the pattern of occurrence of antibiotic resistant bacteria in biofilms in water from groundwater sources in Ado-Ekiti, Nigeria.

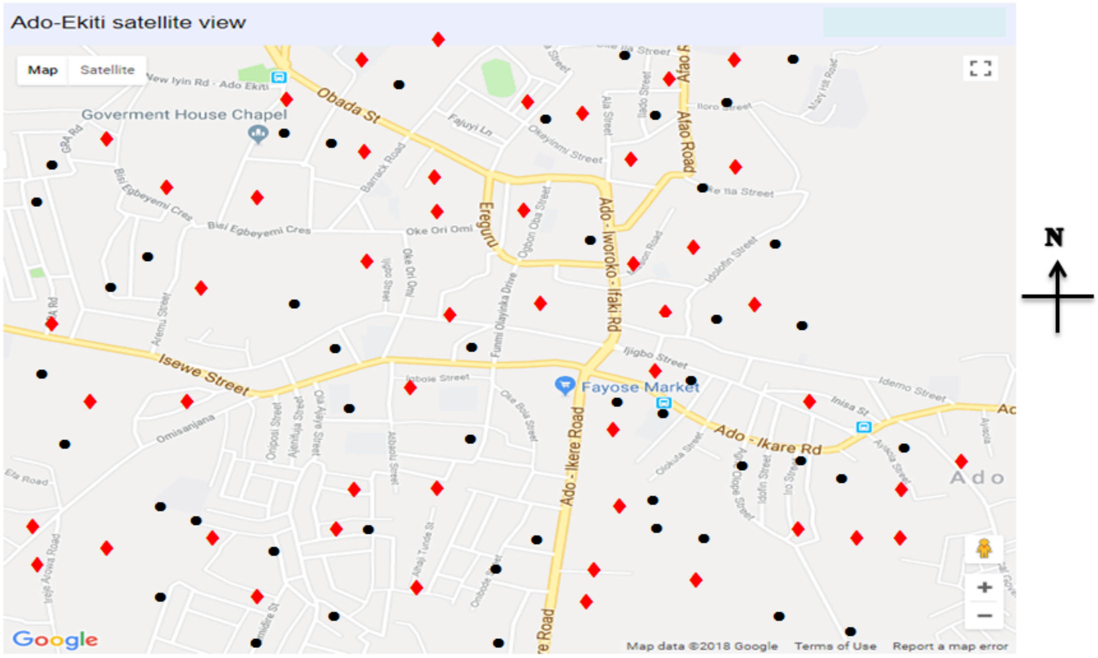
2. MATERIAL AND METHODS

2.1 The Study Area

The study area is Ado- Ekiti (Fig. 1), the capital of Ekiti State in southwest Nigeria. Its geographical coordinates are latitude 7 .62^0 north and longitude 5.22^0 east. The total area covered by Ado- ekiti is 293 km^2 (113 square meters); it also has a population of 424,340 as at 2012.

2.2 Collection of Water Sample

Water samples from borehole and well were collected randomly within Ado-Ekiti metropolis (n = 100), where n = number of samples collected. On each sampling occasion, water samples of approximately 100 ml was collected aseptically with sterile bottles via the running tap connected to the water holding tank for borehole water samples. Sterile water fetcher was used to obtain water samples from the well from which approximately 100 ml was poured aseptically into sterile bottles. All samples were labelled appropriately, transported to the laboratory and stored at room temperature.



Key:

♦ Borehole

• Well water

Fig. 1: Satellite view of the study area (Ado-Ekiti)

2.3 Isolation and identification of bacteria from the biofilms of the drinking water

The water samples were stored for a period of six weeks; this was done to ensure that biofilms had actually formed in the water samples. Biofilm samples were collected at interval of seven days (weekly) until the total bacteria counts were significant. The isolation of bacteria from the biofilm samples was carried out using pour plate method as described by Sam [10]. The inoculated plates were incubated at 37 ^{0}C for 24 hours and observed bacterial colonies were counted and expressed as colonies forming unit per milliliter. The bacterial isolates were identified by using cultural, morphological and biochemical examinations as described by Fawole and Oso, [11].

2.4 Antibiotic sensitivity testing of the bacterial isolates

The antibiotic sensitivity testing was carried out using disc diffusion techniques as described by Ajibade *et al*. [12]. Antibiotic discs used were pefloxacin10 µg, gentamycin 10 µg, ampiclox 30 µg, zinnacef 20 µg, amoxacillin 30 µg, rocephin 25 µg, ciprofloxacin 10 µg, streptomycin 30 µg, streptrin 30 µg, erythromycin 10 µg, chloramphenicol 30 µg, sparfloxacin 10 µg, augumentin 10 µg, pefloxacin 30 µg and tarivid 10 µg. Values obtained were interpreted according to the Clinical and Laboratory standards Institute (CLSI) into resistant, intermediate and sensitive.

2.5 Multiple antibiotics resistant index of bacterial isolates

The multiple antibiotics resistance of the bacteria isolates was determined according to the method used by Oluyege *et al*. [13]. It was calculated using the relation $I = \frac{N}{A}$ where I is MAR index, N the number of antibiotics to which each isolate was resistant, and A the total number of antibiotics used.

2.6 Statistical analysis of data

Data obtained from this study were analyzed by descriptive statistical method and two-way analysis of variance (ANOVA) using SPSS version 22 and turkey HSD (honest significance difference) test at 95 % confidence level.

3. RESULTS AND DISCUSSION

A total of 202 bacteria belonging to eight genera were isolated from the biofilms of the drinking water; these include *S. faecalis*, *E. coli*, *E. aerogenes*, *S. aureus, P. aeruginosa*, *P. vulgaris*, *S. typhi* and *S. dysenteriae*. The borehole biofilm samples had the highest number of bacterial isolates (112) (Figure 2). The large storage tanks and the running pipes may be sources of contamination for borehole water if not washed or disinfected regularly. The presence of bacteria in the biofilms of the borehole water implies the likelihood of occurrence of waterborne diseases and the water is unsuitable for drinking unless subjected to water treatment processes. This result agrees with Okereke *et al*. [14] where the authors isolated bacteria belonging to the genera *Staphylococcus, Escherichia, Pseudomonas, Enterobacter, Bacillus, Klebsiella, Shigella* and *Streptococcus* from borehole water in Aba south metropolis in Nigeria.

Well water had a total of (97) bacterial isolates (figure 3), the presence of bacteria in the biofilms of the well water implies the likelihood of waterborne diseases and the water is unsuitable for drinking, some well water may be located very close to septic tanks which may promote the growth of bacteria in the well or seepage of faecal materials from the septic tank into it, it may even be as a result of introduction of faecal materials or contaminant by the fetching containers from the outside into the well. This result agrees with Pius and Joy [15], where the authors isolated *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Klebsiella pneumonia, Enterobacter aerogenes, Acinectobacter baumannii and Pseudomonas* species from well water in Imota, Lagos, Nigeria.

The result from Table 1 showed that biofilm samples from borehole water had the highest mean total bacterial count of 1.11 $\times 10^4$ cfu/mL whereas the biofilms from well water had the least mean total bacterial count of 0.78 $\times 10^4$ cfu/ml. This result showed that the total bacterial counts of bacteria isolated from the biofilms of borehole water were very high compared with the one from well water, irregular cleaning of the water storage vessels (storex tanks), running taps and lack of treatment of the water from the borehole may likely be responsible for the high total bacterial count. This result is in line with Sunday *et al*. [16] where the authors obtained a high level of bacterial counts in borehole water samples from Abakaliki area of Abia State, Nigeria.

In Figure 4, *S. faecalis* had the highest occurrence almost in all the drinking water sources, this is followed by *E. coli*, *P. aeruginosa* and *E. aerogenes* respectively, while *S. dysenteriae* had the least occurrence. This observation may likely be due to the fact that *S. faecalis* and *E. coli* are major indicator organisms and they have the ability to inhabit any part of the environment most especially water. The findings from this study agree with Chemmattu *et al*. [17], where the authors isolated high percentage of *Strept. faecalis* from drinking water in India.

The Gram positive and the Gram negative bacterial isolates showed considerable resistance to the antibiotics. Some of the isolates were resistance while some were susceptible to the antibiotics, for instance, *S. faecalis* and *S. aureus* from borehole showed high resistance to zinnacef (Z), amoxicillin (AM) and ampiclox (AM) and low resistance to the remaining antibiotics (Figure 5). Resistance could contribute to the spread and persistence of antibiotic resistant bacteria. This result implies that bacteria from biofilms are resistant to antibiotics than their planktonic counterpart, this result corroborates with Gilbert *et al*. [2] who observed that bacterial cells in biofilms exhibit 10 to 1000 times less susceptibility to specific antimicrobial agents than their planktonic (freely suspended) counterparts.

The resistance ability of bacteria could be due to the fact that the bacteria from biofilms of drinking water may have enzymes that could cause neutralization to antibiotics [18]. Some of the bacteria may even possess adaptive mechanisms such as the possession of efflux pump which can remove or pump out the antibiotics and some of the bacteria may even have antibiotic resistant gene [19]. The Gram positive isolates (*S. faecalis* and *S. aureus*) are significantly different in their resistance to antibiotics at ($P \leq 0.05$), but the effect of the antibiotics on *S. faecalis* are significantly different from one another while there is no significant difference in the effects of antibiotics on *S. aureus*.

From the results of antibiotic resistance of all Gram negative bacteria isolated from the biofilms of the two drinking water sources (Figure 6); it was observed that nearly all the bacteria isolates were resistant to pefloxacin, septrin, chloramphenicol and augumentin and high resistance was also observed with the remaining antibiotics, this shows the ability of the bacterial isolates to be resistance to multiple antibiotics. The bacterial isolates from the biofilms of borehole were more resistance than the isolates from well water biofilms. This result corroborates the work of Okafor *et al*. [2] who revealed that the bacteria isolated from the biofilms of borehole water were completely resistant to ciprofloxacin, tetracycline, norfloxacin, ofloxacin, cefuroxime and gentamycin; this showed that they exhibited multiple antibiotics resistance.

The result of multiple antibiotic resistance profile of the isolates (Table 2) revealed that all the bacteria isolates from the borehole and well water exhibited 5 (MAR) resistant patterns that is resistant to three or more antibiotics, resistance of the bacterial isolates to 3 antibiotics 34 (16.3 %) was the highest, this is followed by resistance to 4 and 5 antibiotics 22 (10.5 %), and resistant to 5 antibiotics, resistance to 6 antibiotics was the least. The ability of the bacterial isolates to be resistant to multiple antibiotics may be because of frequent use or over usage of antibiotics.

This observation is in line with Osundiya *et al*. [8] where the authors revealed multiple antibiotics resistance in *Pseudomonas* spp. and *Klebsiella* species. Similar studies by Mbiml *et al*. [20] also revealed the resistance of each of *S. aureus*, *E. aerogenes*, *P. aeruginosa* and *Salmonella* species to seven antibiotics, while *Proteus* species were resistant to eight antibiotics, *E. coli* strains were resistant to five antibiotics, while *Enterococcus* species and coagulase negative *Staphylococci* were each resistant to 3 antibiotics.

Table 3 showed the percentage occurrence of MAR bacterial isolates from the biofilms of borehole and well water; of the 202 bacterial isolates, 106 (52.5 %) were MAR isolates with the highest percentage (63.4 %) from the biofilms of borehole water, indicating a high prevalence of MAR in this study. This finding agrees with Okafor *et al*. [2] who isolated MAR isolates which were resistant to at least seven commonly used antibiotics. The high percentages of MAR isolates found in the biofilms of the drinking water most especially borehole indicated that water is a major reservoir of antibiotic resistant bacteria. It could also be a reflection of misuse or abuse of antibiotics in the environment. A total of 16 bacterial isolates out of the 202 isolates had MAR index of 0.1, 17 isolates had MAR index of 0.2 and 106 of the isolates had MAR index greater than 0.2. This means that 106 out of the 202 bacterial isolates showed resistance to one or more antibiotics.

In table 4 the multiple antibiotics resistant index ranged from 0.1 to 0.8, with MAR index 0.3 having the highest percentage, this is followed by MAR index of 0.2 having (13.6 %) and MAR index of 0.1 having 11.3 %, while the lowest percentage MAR index of 0.6 had (4.5 %). The MAR indexes of the majority of the bacterial isolates were above 0.2. This revealed a high prevalence of MAR indexes which indicates high risk source of contamination in the areas where antibiotics are used. The high MAR index values may be due to the widespread use of antibiotics and the continuous use of a single antibiotic over a period of weeks or months which select bacteria that are resistant to different kind of antibiotics. This work is in accordance with Oluyege *et al*. [13] who isolated bacteria with high MAR indexes from drinking water.

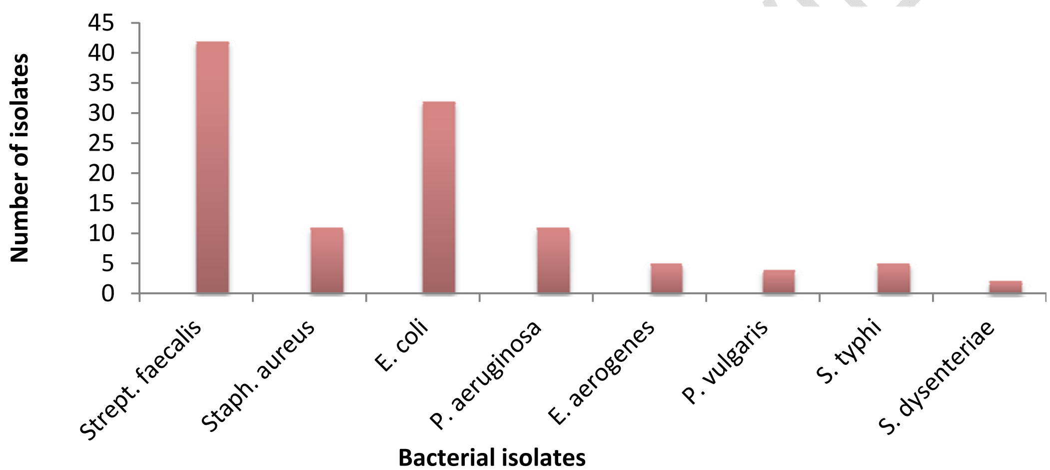


Figure 2: Bacteria isolated from biofilms of borehole water

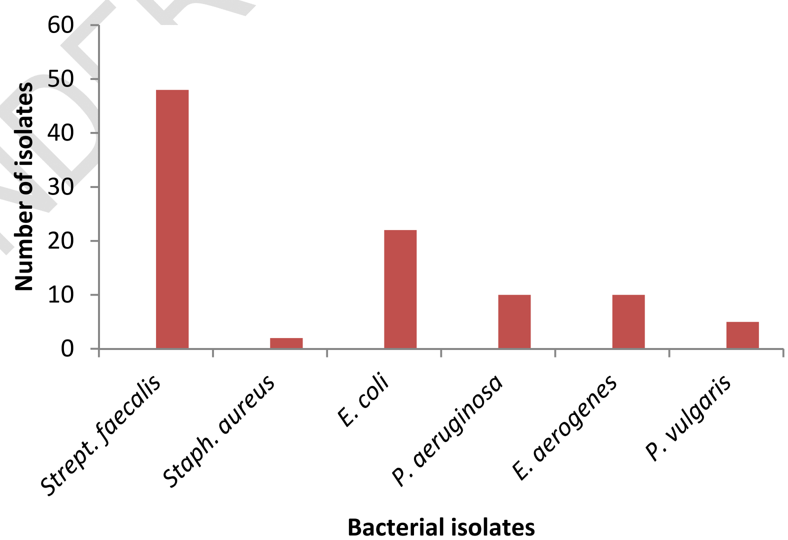


Figure 3: Bacteria isolated from the biofilms of well water

Table 1. The mean total bacterial count of bacterial isolates from biofilms of borehole and well water

Drinking water sources	Total bacterial count (cfu/ml)
Well (n = 50)	0.78×10^4
Bore hole (n = 50)	1.11×10^4

n = number of samples

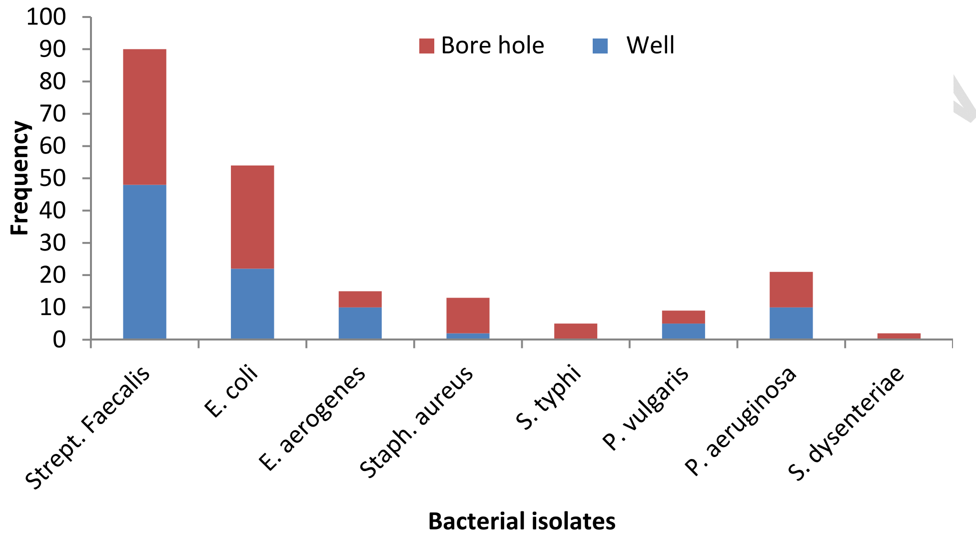
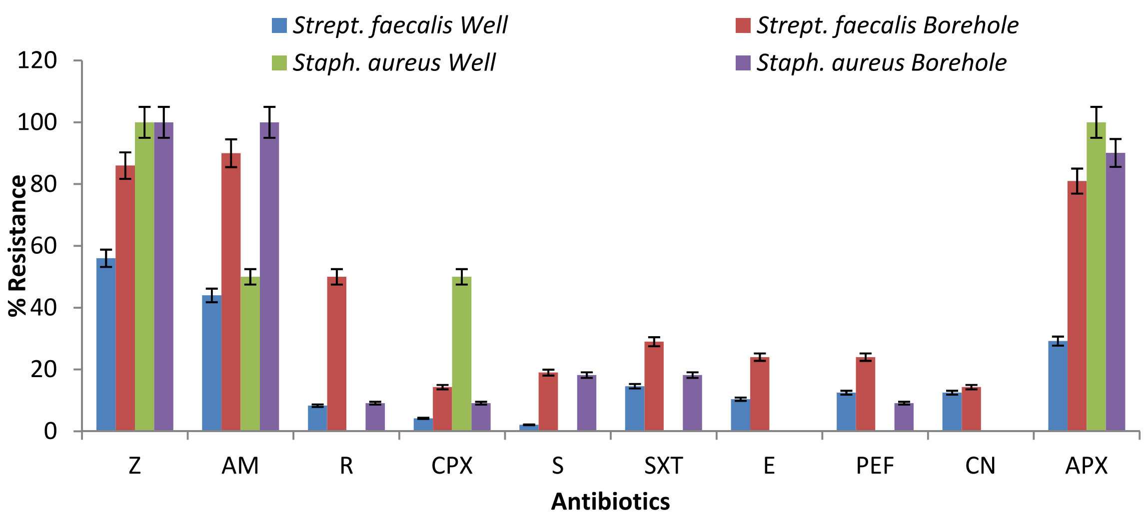
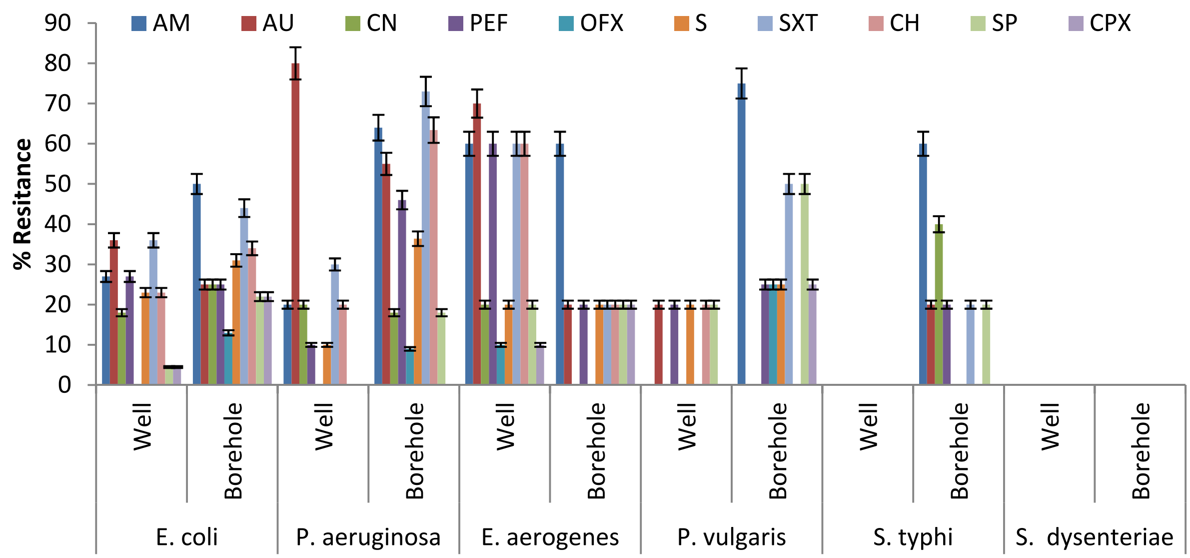


Figure 4: Frequency of occurrence of bacterial isolates from biofilms of drinking water



Z = zinnacef 20 µg, Am= amoxacillin30 µg, R= rocephin 25 µg, CPX= ciprofloxacin 10 µg,S=streptomycin 30 µg, SXT= septrin30 µg, E= erythromycin= 10 µg, PEF= pefloxacin10 µg, CN= gentamycin10 µg, APX= ampiclox 30 µg.

Figure 5: Antibiotic resistance of Gram positive bacterial isolates from the biofilms of borehole and well water



AM = amoxicillin (30 µg), AU = augumentin (30 µg), CN = gentamycin (10 µg), PEF = pefloxacin (30 µg), OFX = tarivid (10 µg), S = streptomycin (30 µg), SXT = septrin (30 µg), CH = chloramphenicol (10 µg), SP = sparfloxacin (10 µg), CPX = ciprofloxacin (10 µg)

Figure 6: Antibiotic resistance of Gram negative bacterial isolates from the biofilms of borehole and well water

Table 2. Multiple antibiotic resistant (MAR) profile of bacterial isolates

Sources		No (%) of Isolates Resistant to				
		3 antibiotics	4 antibiotics	5 antibiotics	6 antibiotics	7antibiotics and above
Well	(n = 97)	14 (14.4)	8 (8.2)	5 (5.2)	5 (5.2)	3 (3.1)
Borehole	(n= 112)	20 (17.8)	14 (12.5)	17 (15.2)	7 (6.3)	13 (11.6)
Total	(209)	34 (16,3)	22 (10.5)	22(10.5)	12 (5.7)	16 (7.7)

Table 3: Percentage occurrence of MAR bacterial isolates from biofilms of borehole and well water

Sources	**No of isolates**	**No of multiple antibiotics resistant isolates (%)**
Well	97	35 (36.1)
Borehole	112	71 (63.4)
Total	202	106 (52.5)

Table 4: Multiple antibiotic resistance index of bacterial isolates

Sources isolates	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7 and above**
Well (n = 97)	8	12	14	8	5	5	3
Borehole (n = 112)	8	5	20	14	17	7	13
Total (202)	16	17	34	22	22	12	16

4. CONCLUSION

S. faecalis, *E. coli*, *E. aerogenes*, *S. aureus, P. aeruginosa*, *P. vulgaris*, *S. typhi* and *S. dysenteriae* were isolated from the biofilms of the drinking water after the water samples had been stored for a period of time (three weeks for well and borehole water samples, because it was at this point that the total bacterial counts of biofilm samples from the drinking water sources became significant (i e., above 40). This result revealed high level of contamination of bacterial isolates indicating that most of the water supplies were unfit for human consumption if kept for long. Consumption of these drinking water supplies may result in public health hazard. A high level of antibiotic resistance was observed among the bacterial isolates as results demonstrated that 139 of 202 bacterial isolates were resistant to one or more antibiotics and the percentage of multiple antibiotics resistant (MAR) isolates was 106 (52.5 %).

The study suggests that the well and borehole water must be treated at the point of use and should not be stored for more than three weeks before the water storage tanks (storex tanks and water storage vessels) are washed. This will serve as baseline information for individuals and water supply agencies. Well and borehole must be sited far away from septic tanks. There should public enlightenment on indiscriminate use of antibiotics, over-counter or self-prescription and over usage of antibiotics in order to eradicate the incidence of antibiotic resistance.

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