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Plasmid Profiles of Antibiotic Resistant Bacteria Associated with Biofilms from Ground Water Sources in Ado-Ekiti, Ekiti State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AFT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The presence of microbial biofilms in our drinking water sources poses a significant health risks to human because biofilm serves as an environmental reservoir of pathogenic microorganisms; the bacteria in biofilms are usually antibiotic resistant and therefore their multiple resistance genes may be harbored on the resistant plasmid.
Aim: The study investigated the plasmid profiles of antibiotic resistant bacteria associated with biofilms from ground water sources in Ado-Ekiti, Ekiti State Nigeria.
Methodology: One hundred samples of water were collected randomly from wells and boreholes, isolation and identification of bacteria from the biofilms of the water samples were carried out by using standard microbiological procedures, antibiotic sensitivity of the isolates was carried out and

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multiple antibiotic resistant indexes of the bacterial isolates were calculated. The plasmid profiles of the bacterial isolates was also determined.

Results: Results showed that a total of 209 bacteria were isolated from the biofilms of the two ground water sources; these include *Streptococcus faecalis*, *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi* and *Shigella dysenteriae*. *S. faecalis* from borehole and well water samples had the highest occurrence of (37.5%) and (49.5%) respectively. *Shigella dysenteriae* had the lowest occurrence of (1.8%) from borehole water while *Staphylococcus aureus* from well occurred least (2.1%). Both Gram positive and the Gram negative bacterial isolates showed considerable resistance to the different antibiotics. The percentage occurrence of the multiple antibiotic resistant (MAR) bacterial isolates was 106 (52.5%) with the highest percentage (63.4%) from the biofilms of borehole water samples. 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 study area. Of the 10 MAR isolates selected and examined for plasmid analysis, it was discovered that only six isolates harbored plasmids with molecular size range of 300-950 bp. Plasmid curing and antibiotic sensitivity test after curing showed that curing of plasmids was effective in four isolates (*P. vulgaris* B11, *E. coli* W21, *Enterobacter aerogenes* W25 and *Strept. faecalis* B25) and partially effective in two isolates (*Salmonella typhi* B4 and *E. coli* B37).

Conclusion: Well and borehole water must be treated at the point of use, water storage vessels must be washed regularly and there should be public enlightenment on indiscriminate use of antibiotics in order to eradicate the incidence of antibiotic resistance

Keywords: Antibiotic resistance; bacteria; biofilms; ground water sources; plasmid profile and plasmid curing.

1. INTRODUCTION

"A biofilm is an assemblage of microbial cells that is irreversibly associated with a surface and enclosed in a matrix of primarily polysaccharide material known as extracellular polymeric substance (EPS)" [1]. The presence of microbial biofilms in our drinking water sources poses a significant health risks to human because biofilm serves as an environmental reservoir of pathogenic organisms [2]. Therefore, it is essential to ensure that drinking water is safe by preventing the formation of biofilms since water is vital to life. The presence of biofilms in our drinking water can lead to occasional outbreak of waterborne and water-related diseases.

According to WHO [3], "the two main categories of relevant microorganisms that are usually involved in biofilms are: microorganisms with pathogenic properties which have been shown to be associated with water-related illness and outbreaks, and bacteria which are primarily used as indicator organisms in water analysis, indicating the presence of pathogenic organisms of faecal origin". "Water-related and 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" [4].

"Others are opportunistic pathogens which cause disease in sensitive human subgroups such as the elderly, children, immuno-compromised individuals, patients with preexisting disease or other predisposing conditions which facilitate infection by these organisms" [5]. "These organisms may attach to surfaces as primary colonizers and actively establish biofilms alone or in combination with other microorganisms" [6]. "Biofilms in drinking water such as well and bore hole can be responsible for a wide range of water quality problems such as increased bacterial levels, taste and odour changes" [7]. "Biofilms may develop within the different drinking water sources as a result of contamination, regrowth of microorganisms or from microorganisms that survived disinfection and this may lead to occurrence of waterborne diseases" [4].

Previous studies have demonstrated the presence of biofilms in water pipe network distribution systems as well as in sachet water in Abakaliki area of Ebonyi State, Nigeria [8], Research on plasmids analysis had revealed the presence of plasmids with bandwidth of 10,000 bp in *Veillonella Spp*, *Vibrio orientalis* and *Micrococcus luteus* isolated from selected water bodies in Owo, Ondo-State [9]. Similar work by Fadahunsi, et al. [10] also revealed the presence of plasmid of band width 23,130 bp in multidrug-resistant enterobacteriaceae isolated from

hawked soymilk samples in the Polytechnic of Ibadan community Nigeria. But, there is no information about the plasmid analysis of bacteria associated with biofilms in ground water sources in Ekiti State, Nigeria. Since plasmids have always been associated with antibiotic resistance, it becomes imperative to assess the plasmid profiles of bacteria that are associated with biofilms from ground water sources in Ado-Ekiti, Ekiti State, Nigeria.

2. MATERIALS AND METHODS

2.1 Collection of Water Samples

One hundred samples of water were collected randomly from well and borehole within Ado-Ekiti metropolis in sterilized bottles. The samples were transported to the laboratory in ice packed cooler, the samples were later removed from the cooler and kept in a safe place at room temperature.

2.2 Isolation of Bacteria from the Biofilms of Drinking Water

"Isolation of bacteria from the biofilm samples obtained from the ground water sources was carried out using pour plate method as described by Sam" [11]. The water samples were allowed to stand and stored for a period of three weeks, this was done to ensure that biofilms had actually formed in the drinking water samples. Biofilm samples were collected by swabbing the inner side of the surface in contact with the drinking water supplies starting from day one and later at interval of seven days (weekly) until the total bacteria counts were significant. The bacteria were isolated by using nutrient agar, MacConkey and blood agar at the third week of storage when the total bacteria counts became significant.

2.3 Identification of Bacterial Isolates

The identification of the bacterial isolates was carried out by cultural, morphological examinations and different biochemical tests using standard microbiological techniques as described by Fawole and Oso [12].

2.4 Antibiotic Sensitivity Test

The antibiotic sensitivity testing was carried out using disc diffusion techniques as described by CLSI [13]. The test was carried out by using gram positive and gram negative disks containing different antibiotics which were placed aseptically on the inoculated Muller Hinton agar plates. The plates were incubated at 37 ^{0}C for 24 hours and observed for the presence of growth and zones of inhibition.

2.5 Multiple Antibiotics Resistant index of Bacterial Isolates

The multiple antibiotic resistance (MAR) index of the bacteria isolates was determined according to the method used by Oluyege et al. [14]. It was calculated by dividing the number of antibiotics to which each isolate was resistant to by the total number of antibiotics using the relation $I = \frac{N}{T}$ where I is MAR index, N the number of antibiotics to which each isolate was resistant, and T the total number of antibiotics used.

2.6 Determination of Plasmid Profiles of Bacterial Isolates

Plasmid extraction was carried out based on the methods of Molina-Aja et al. [15] with little modification. Ten (10) representative multiple antibiotics resistant isolates (MAR) which included four isolates from biofilms of well water (*Streptococcus faecalis* W27, *Enterobacter aerogenes* W23, *E. coli* W21 and *E. aerogenes* W25) and six isolates from biofilms of borehole water (*Pseudomonas aeruginosa* B7, *Salmonella typhi* B4, *Proteus vulgaris* B11, *Staph. aureus* B3, *E. coli* B37 and *Strept. faecalis* B25) were selected for plasmid analysis. Five milliliter of overnight broth culture of each isolate was centrifuged in an Eppendorf tube at 10,000 rpm for 2 minutes. The supernatant was decanted and 100ml of TET buffer was added. Two hundred ml (200) of sodium dodecyl sulphate (SDS) / NAOH solution was added, mixed gently and left for 5 minutes at room temperature. One hundred and fifty (150) mL of KOAC was added, left on ice for five minutes and later centrifuged. Four hundred (400) mL of the supernatant was transferred carefully into a clean micro centrifuge tube, this supernatant contains the plasmid DNA. 400 mL ice cold ethanol was added, gently mixed and left in a deep freezer (-18 to – 20 °C) for 10-30 minutes. This was centrifuged for ten minutes at high speed, the supernatant was decanted and the pellet was dissolved in fifteen mL of TBE buffer and stored in a freezer.

Agarose solution was prepared and allowed to cool down to about 50^{0}C, Ethidium bromide (EtBr) was added to a final concentration of

approximately 0.2 – 0.5 µg/m. The agarose was poured into a gel tray with the comb in place, a molecular weight ladder was carefully loaded into the first lane with loaded dye added to each sample in the well. The agarose gel was placed in the electrophoresis unit which was filled with TBE buffer. The gel was run at 120 V and visualized in UV- trans- illuminator (FOTO UVI-1430).

2.7 Plasmid Curing of the Resistant Isolates

The curing of the resistant isolates was carried out according to the method used by Ebele et al. [16] by exposing the overnight grown culture of each representative resistant isolate to an elevated temperature of 37 ºC and 10% sodium deodecyl sulphate (SDS), this was incubated at 37°C for 48 hours. A freshly made 4.5 ml nutrient broth was supplemented with 0.5 ml of the broth cultures and incubated at 37°C for an additional 24 h in order to remove their antibiotics resistance ability.

2.8 Antibiotics Sensitivity of the Cured Isolates

The antibiotics sensitivity of the cured isolates was carried out by inoculating broth cultures of each cured isolate on sterilized Muller Hinton agar plates by using spread plate technique. Both gram positive and gram negative antibiotics disks were placed separately on the inoculated plates and incubated at 37ºC for 24 hours. The plates were then observed for the presence of zones of inhibition.

2.9 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 at 95% confidence level.

3. RESULTS AND DISCUSSION

A total of 209 bacteria were isolated from the biofilms of the two ground water sources; these include *Streptococcus faecalis*, *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi* and *Shigella dysenteriae* (Figs. 1 and 2). “Their presence may be as a result of inadequate water treatment practices by the individual households, lack of good personal hygiene practiced by individuals and contamination of the drinking water with faecal materials possibly by dugging of wells or boreholes very close to septic tanks which may permit the growth of bacteria in them or seepage of faecal materials from the septic tank into it. The presence of these bacterial isolates in the biofilms of the drinking water indicate that the water is unsuitable for drinking and implies the likelihood of waterborne diseases until the water is treated” [17]. This result corroborates the findings of Etido [18], where the author observed that the water samples from borehole, well and pipe-borne available to students in Nasarawa State University Keffi were found to harbor bacteria such as *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, and *Citrobacter sp.* This result is also in accordance with PanelSahar et al. [19] where the authors observed the presence of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneu moniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacter species from the biofilm resistome in drinking water distribution systems.

A total of 97 bacteria were isolated from the biofilms of well water; *S. faecalis* had the highest occurrence (49.5%) while *Staphylococcus aureus* occurred least (2.1%). A total of 112 bacteria were isolated from the biofilms of borehole water with *S. faecalis* having the highest occurrence (37.5%) and *Shigella dysenteriae* having the lowest occurrence (1.8%) (Fig. 3). “This may due to the fact that *Strept. faecalis* and *E. coli* are the major indicator organisms and they have the ability to inhabit any part of the environment most especially water. Borehole’s water had the highest value of the mean total bacterial count (Table 1). This result showed that the total bacterial count from the biofilms of borehole water was high which implies high rate of contamination which may be due to irregular cleaning of the stored tanks and running taps and lack of treatment of the water from the borehole” [17]. This result is in line with Sunday et al. [20] where the authors obtained a high level of bacterial counts in borehole water samples from Abakaliki area of Abia State, Nigeria.

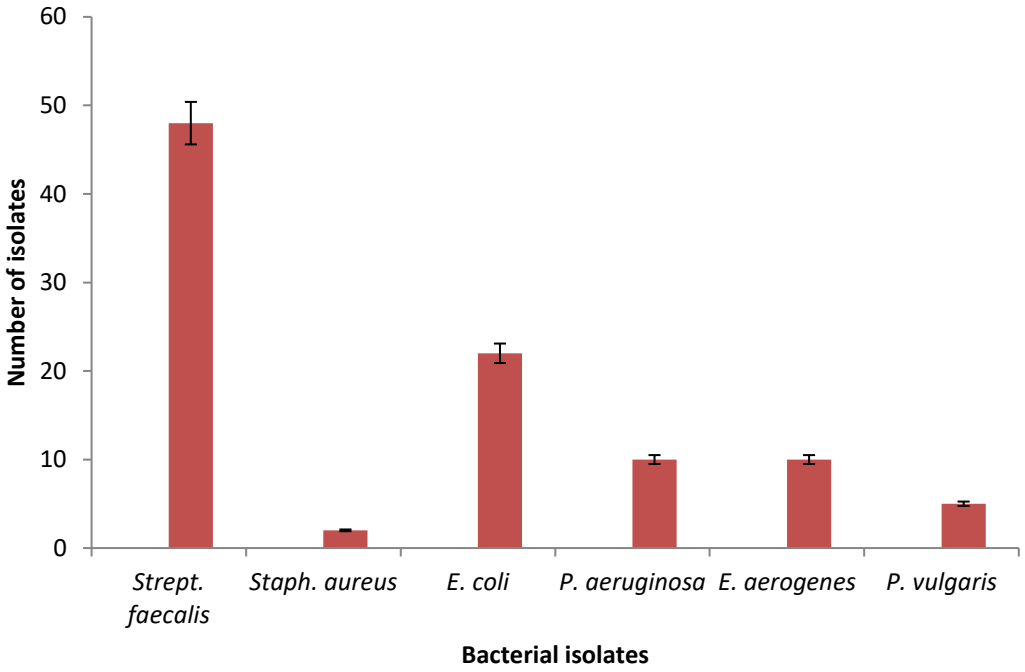

Fig. 1. Bacteria isolated from the biofilms of well water

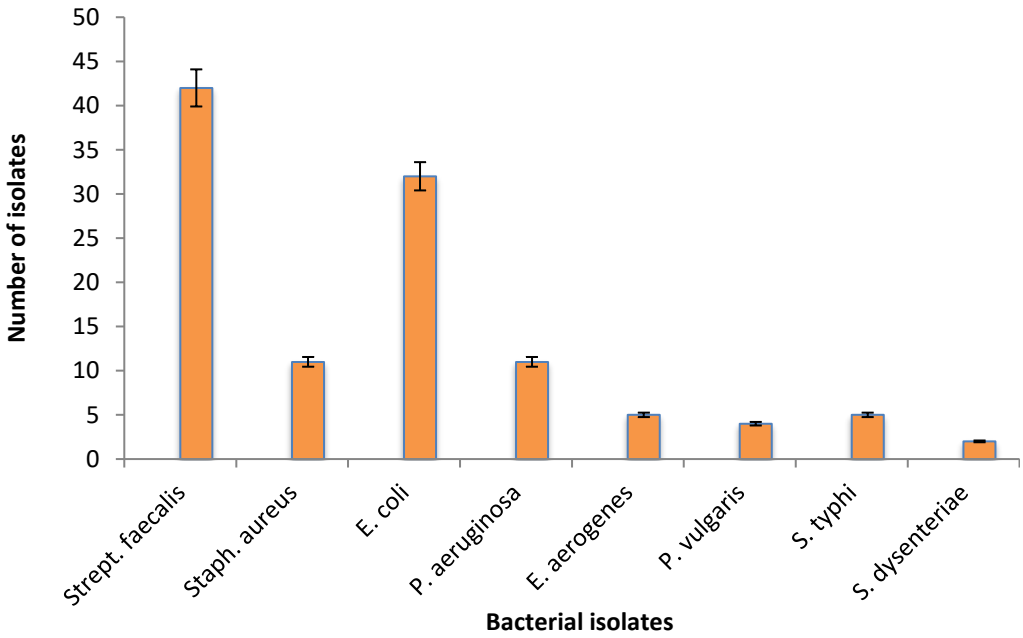

Fig. 2. Bacteria isolated from biofilms of borehole water samples

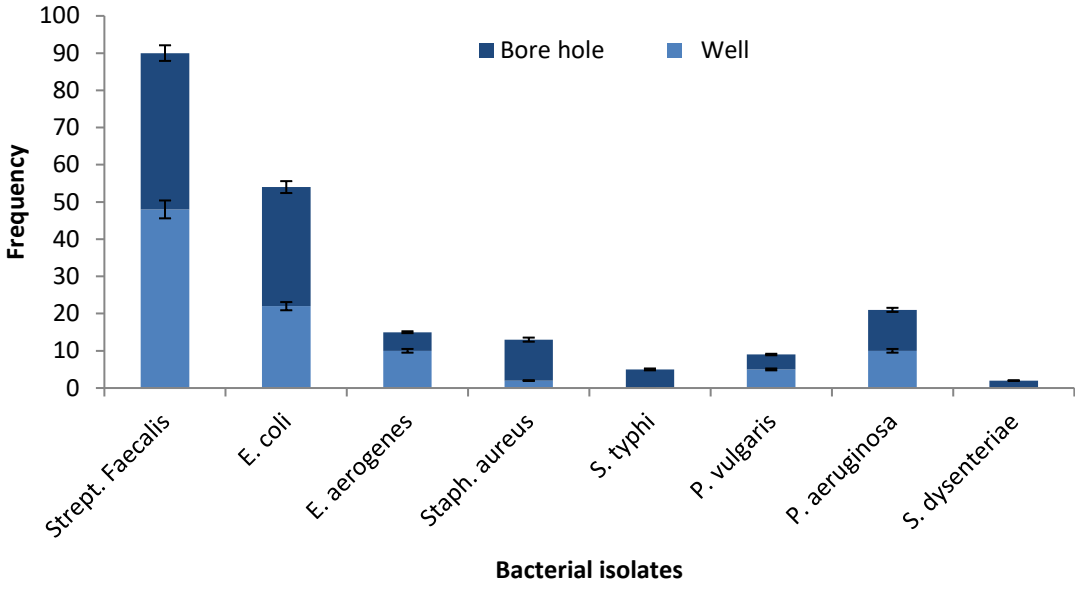

Fig. 3. Frequency of occurrence of bacterial isolates from biofilms of the water samples [17]

Table 1. The mean total bacterial count of bacterial isolates

Drinking water sources (n= 50)	Total bacterial count (Cfu/ml)
Well	7.80×10^3
Bore hole	11.10×10^3

Key: n = number of samples

"The Gram positive and the Gram negative bacterial isolates showed considerable resistance to the antibiotics. Some of the isolates were resistant while some were susceptible to the antibiotics, for instance, the gram positive bacteria (*Strept. faecalis* and *Staph. aureus)* from borehole showed high resistance to zinnacef (Z), amoxicillin (AM) and ampiclox (APX) and low resistance to the remaining antibiotics (Fig. 4). Also, Gram negative bacteria isolated from the biofilms of the two drinking water sources revealed that nearly all the isolates were resistant to pefloxacin (PEF), septrin (SXT), chloramphenicol (CH) and augumentin (AU) and high resistance was also observed with the remaining antibiotics (Fig. 5). Results also showed that the bacterial isolates from the biofilms of borehole were more resistant than the isolates from well water biofilms to the various antibiotics, resistance could contribute to the spread and persistence of antibiotic resistant bacteria" [17]. This result suggests that bacteria from biofilms are resistant to antibiotics than their planktonic counterpart and this is in agreement with Gilbert et al. [21] who observed that bacterial cells in biofilms exhibited 10 to 1000 times less susceptibility to specific antimicrobial agents than their planktonic counterparts. "The resistance observed in the bacterial isolates may be due to the ability of the bacteria from biofilms of the drinking water to synthesize enzymes that can neutralization the antibiotics" [2]. "Some of the bacteria may even possess adaptive mechanisms such as the possession of efflux pump which can remove the antibiotics and possession of antibiotic resistant gene" [22]. This result is in agreement with the work of Okafor et al. [8] who revealed that "bacteria isolated from the biofilms of borehole water were completely resistant to multiple antibiotics including ciprofloxacin, tetracycline, norfloxacin, ofloxacin, cefuroxime and gentamycin". Hawa et al. [23] observed that "*Pseudomonas aeruginosa* and *E. coli* isolated from the biofilms of borehole, well and tap water from greater Accra region in Ghana were resistant to cefuroxime, trimethoprim and amoxillin-clavulanate". Stephen *et al*. [24] also observed "multiple antibiotics resistance in *E. coli*, Enterobacter spp, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from Ghanaian drinking water sources"

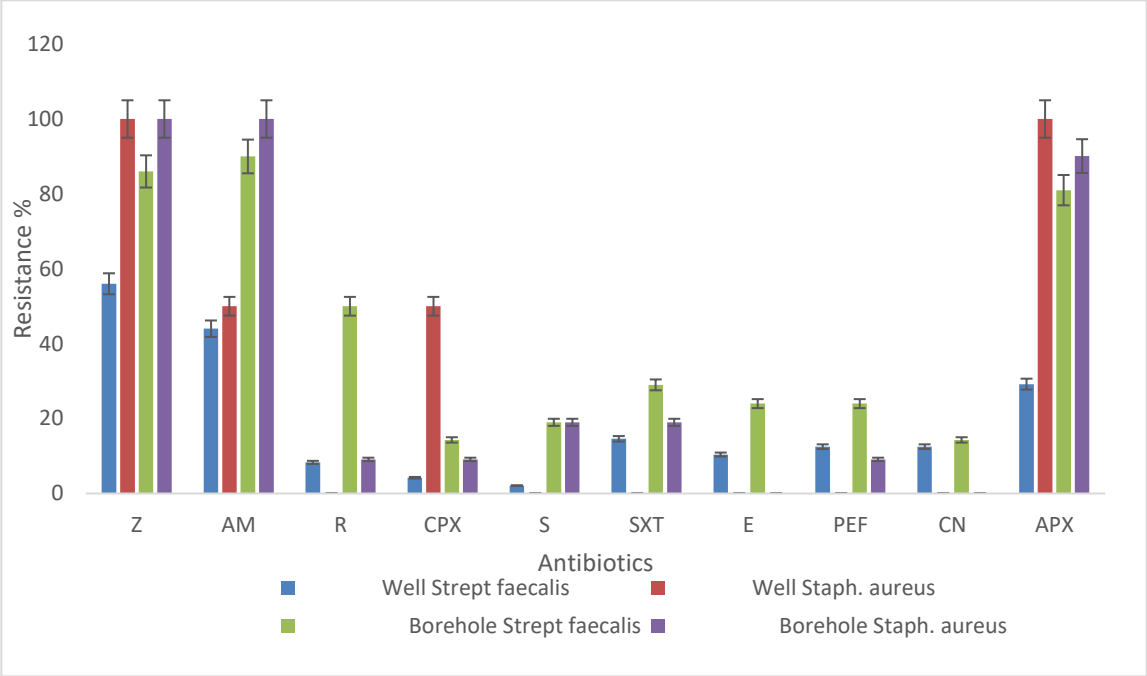

Fig. 4. Antibiotic resistance of gram positive bacterial isolates [17]

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

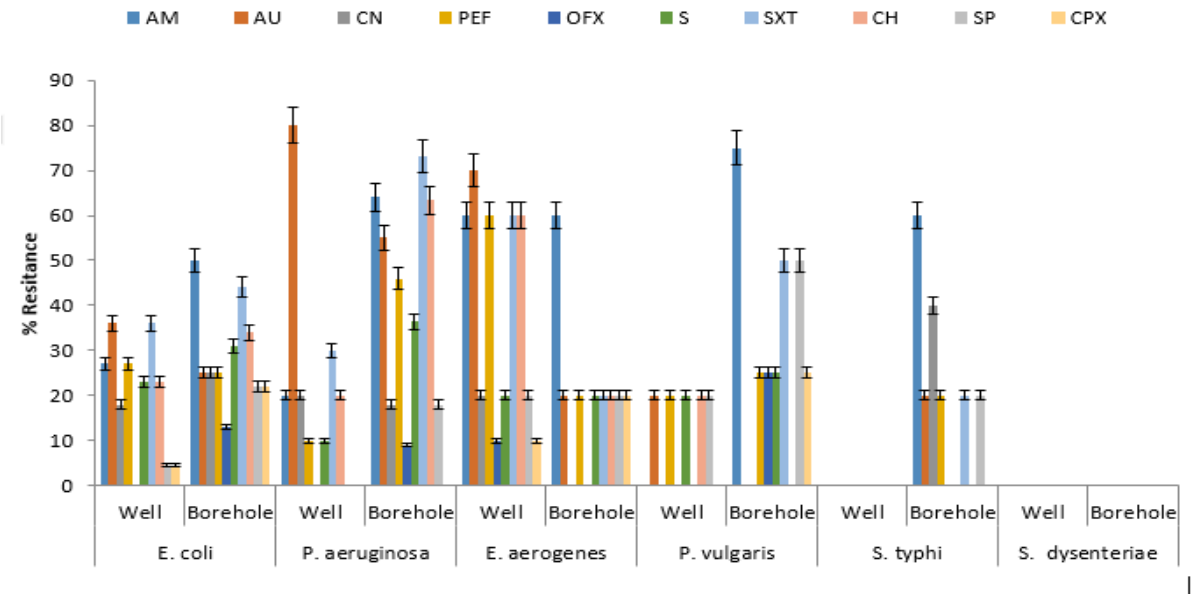

Fig. 5. Antibiotics resistance of gram negative bacterial isolates

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)

"The percentage occurrence of MAR bacterial isolates from the biofilms of borehole and well water showed that out of the 209 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 (Table 2)" [17]. This finding agrees with Okafor et al. [8] "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 209 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".

The multiple antibiotics resistant index ranged from 0.1 to 0.8, with MAR index 0.3 having the highest percentage, 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 % (Table 3). The MAR indexes of the majority of the bacterial isolates were above 0.2. This revealed a high prevalence of MAR indexes which indicated high risk sources of contamination in the study area. 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 time which select bacteria that are resistant to different kind of antibiotics. This work is in accordance with Oluyege et al. [14] where the authors isolated bacteria with high MAR indexes from drinking water.

Of the 10 MAR isolates selected and examined for plasmid analysis, four isolates from the biofilms of borehole harboured plasmid; *Strept. faecalis* B25 (No 10) harboured a plasmid of 900 bp, two isolates *Proteus vulgaris* B11 (No 4) and *Salmonella typhi* B4 (No 6) harboured a plasmid of 750 bp while the fourth isolate *E. coli* B37 (No 8) harboured a dual- plasmid of 300 bp and 900 bp. Two isolates from the biofilms of well water harboured plasmid, with *E. coli* W21 (No 7) habouring a plasmid of 950 bp while the other one *Enterobacter aerogenes* W25 (No 9) harboured a plasmid of 900 bp. No plasmid was detected in the remaining 4 isolates (Plate 1). It was discovered that only six isolates harbored plasmids with molecular size range of 300- 950 bp. These findings are consistent with the work of Kroll et al. [25] who reported that plasmids may be present in an individual cell in varying number and sizes, ranging from one to several hundreds. Two isolates (*Proteus vulgaris* B11 and *Salmonella typh*i B4) showed the same plasmid size of 750 bp, another two isolates *Enterobacter aerogenes* W25 and *Strept. faecalis* B25 also harbored the same plasmid size of 900 bp, but these isolates that had the same plasmid size did not have the same resistant phenotype (Table 4), possession of plasmid of the same molecular weight or size may suggest common origin or source. *E.coli* W21 harbored a plasmid of 950 bp and the eighth isolates *E. coli* B37 harbored a dual plasmid of 300 and 900 bp, this isolate was resistant to all the antibiotics, this may likely be the reason for the possession of double plasmid. The presence of plasmids in the six isolates showed that their resistance to antibiotics were mediated by plasmids, it implies that plasmids can be transferred to susceptible bacteria in the environment through horizontal gene transfer. This result is in accordance with the work of Mbim et al. [26] who observed that the presence of resistant genes in the plasmids of bacteria explains to a large extent the antibiotics resistance among the isolates. Similarly, Falegan et al. [27] demonstrated that multiple resistance genes are harbored on resistance plasmids (R-plasmids), some of which are conjugative. In this study, 4 of the 10 MAR representative isolates examined for plasmid DNA showed a negative result indicating the absence of plasmid in the isolates. Thus, suggesting that the resistance in these isolates may not be mediated by plasmids and may likely be chromosomal borne.

Table 2. 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	209	106 (52.5)

Table 3. 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 (209)	16	17	34	22	22	12	16

Table 4. Antibiotic resistant phenotype and plasmid analysis of the representative isolates

Isolates/ identification No	Sources	Antibiotic resistant phenotype	No of plasmid detected	Estimated size in bp
*Strept. faecalis*W27(1)	Well water	CN, APX, Z, AM, E	-	-
Staph. aureus B3 (2)	Borehole	APX, Z, AM, S, SXT	-	-
Ps. aeruginosa B7 (3)	Borehole	CH, AM, AU, SXT	-	-
Pr vulgaris B11 (4)	Borehole	PEF, S, SP, CPX, AM	1	750
Enter. aerogenes W23 (5)	Well water	PEF, S, CH, CPX, AU, SXT	-	-
Sal. typhi B4 (6)	Borehole	PEF, CH, AM, AU, CN, SXT	1	750
E. coli W21 (7)	Well water	PEF, CH, SP,CPX, AM, AU, SXT	1	950
E. coli B37 (8)	Borehole	PEF, OFX, S, CH, SP, CPX, AM AU, CN, SXT	2	300, 900
Enter. aerogenes W25(9)	Well water	PEF, OFX, AM, AU	1	900
Strept. faecalis B25 (10)	Borehole	APX, Z, AM, R	1	900

*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), APX = ampiclox (30 µg), Z = zinnacef (20 µg), R = rocephin (25 µg), E = erythromycin (10 µg)*

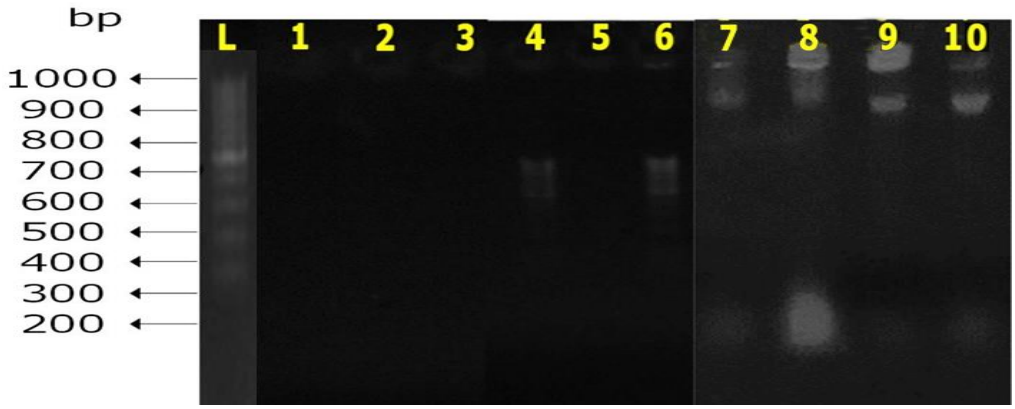

Plate 1. Plasmid profile of bacterial isolates from the biofilms of drinking water

L = Molecular weight ladder (200 to 1000bp) Lane 1 = Strept. faecalis W 27, lane 2 = Staph. aureus B3, lane 3 = Pseudomonas aeruginosa B7, lane 4 = Proteus vulgaris B11, lane 5 = Enterobacter aerogenes W23, lane 6 = Salmonella typhi B4, lane 7 = E. coli W21, lane 8 = E. coli B37, lane 9 = Enterobacter aerogenes W25, lane 10 = Strept. faecalis B25

Table 5. Antibiotic resistant phenotype of the cured bacterial isolates

Isolates		Sources	Antibiotics resistant phenotype
Strept. Faecalis W 27	(1)	Well water	CN, APX, Z, AM,
Staph. aureus B 3	(2)	Borehole	S, SXT
Pseudomonas aeruginosa B7	(3)	Borehole	CH, AM, SXT
Proteus vulgaris B11	(4)	Borehole	-
Enterobacter aerogenes W23	(5)	Well water	CH, S, AU
Salmonella typhi B4	(6)	Borehole	AU
E. coli W 21	(7)	Well water	-
E. coli B 37	(8)	Borehole	CN
Enterobacter aerogenes W25	(9)	Well water	-
Strept. faecalis B 25	(10)	Borehole	-

*S = streptomycin (30 µg), AU = augumentin (30 µg**)**, SXT = septrin (30 µg), CH= chloramphenicol (10 µg), CN = gentamycin (10 µg), APX = ampiclox (30 µg), Z = zinnacef (20 µg), AM = amoxicillin (30 µg**)**, R = rocephin(25 µg), PEF = pefloxacin (30 µg).*

Plasmid curing and antibiotic sensitivity test after curing showed that curing of plasmids was effective in four isolates (*P. vulgaris* B11, *E. coli* W21, *Enterobacter aerogenes* W25 and *Strept. faecalis* B25) and partially effective in two isolates (*Salmonella typhi* B4 and *E. coli* B37) (Table 5). The isolates were no longer resistant to the antibiotics after the plasmid curing procedure, this is an indication that the isolates have been cured of their plasmids, and therefore the resistance was plasmid mediated. However, plasmid curing was not effective in the other isolates indicating that their resistance were chromosomal mediated. This result is in line with Lavanya et al. [28] who observed that most resistant isolates from fermented milk were cured of their plasmid, that is, their resistance were plasmid-borne and few of the isolates were resistant against tested antibiotics after curing (chromosomal-borne) as compared with the initial resistant pattern before curing (pre-curing). Similar work by Ebele et al. [16] also showed that plasmid curing was effective in *E. coli* (xiii), *Staph. aureus* (iii) and *Klebsiella pneumonia (*vii) isolated from different clinical specimens at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria.

4. CONCLUSION

Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, *Streptococcus faecalis*, *Staphylococcus aureus Escherichia coli*, *Enterobacter aerogenes*, and *Proteus vulgaris* were isolated from the biofilms of well and borehole. High level of contamination of bacterial isolates was revealed indicating that most of the water supplies were unfit for human consumption if kept for long and do not meet the World Health Organization (WHO) drinking water standards. 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 209 bacterial isolates were resistant to one or more antibiotics and the percentage of multiple antibiotics resistant isolates (MAR) was 106 (52.5)%. The presence of plasmids which ranged from 300-950bp in six isolates out of the 10 representative MAR isolates and the ability of these isolates to be cured of their plasmids indicated that their resistance to antibiotics were mediated by plasmids, while the resistance of the remaining isolates were chromosomal based.

Findings from this study suggests that the well and borehole water must be treated at the point of use and water Stored tanks or water storage vessels) must be washed regularly. Well and borehole must be sited far away from septic tanks and there should be 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that No generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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