Original Research Article

Prevalence and Antibiotic Resistance Patterns of Biofilm-Producing *Escherichia coli* strains from Water Sources around major Slum in Port Harcourt City.

Abstract

Slum is characterized by uncontrollable development around urban areas, new settlements creation and decreased human welfare level. It is associated with increasing environmental problems; hence this study was aimed to assess the prevalence and resistance patterns of biofilm-producing *E. coli* strains in water sources around major slums in Port Harcourt city. A of three hundred and sixty water samples comprising surface water (120), Borehole (120) and well water (120) samples were collected from five different slums namely:Bundu, Egede, Enugu, Ibadan and Etche watersides over a period of twelve months. The water samples were processed and screened for the presence of *E. coli* using standard microbiological techniques. The isolates were subjected to biofilm production assays and antibiotic susceptibility testing. Results revealed that out of the 360 water samples, 291 (80.8%) were positive for *Escherichia coli* across the three water sources; 120 (100%) for surface water, 98(81%) for well water and 73(60%) for borehole water. Further analysis revealed that out of the 291 *Escherichia coli* isolates from the three water sources, 180(62%) were positive for biofilm production, 90(31%) showed moderate biofilm production while 21(7%) were non biofilm producing strains. The antibiotic susceptibility testing showed that the biofilm-producing *E. coli* exhibited high levels of resistance to commonly used antibiotics, including Augmentine, Ceftazidime,Cefuroxime, andCloxacillin The percentage resistance of *Escherichia coli* isolated from water sources inBundu, Egede, Enugu, Etche and Ibadan waterfronts, were 100% resistant to Ceftazidime (CAZ) and Cefuroxime (CRX) respectively. For Cloxacillin (CXM), *Escherichia* showed the following resistance Enugu and Ibadan (82.5%), Etche and Egede (79.2%) and Bundu (66%). Whereas *Escherichia coli* isolated from Bundu, Enugu, and Ibadan were 100% while those isolated from Egede, and Etche were 75% resistant to Augmentin. On the other hand, for *Escherichia coli* from Etche and Egede were both 8.3 and 15% resistant to Gentamycin. In present study, it was observed that *Escherichia coli* were100%susceptible toNitrofurantoin, Ofloxacin and Ceftriaxone across the five slums. The high prevalence of biofilm-producing *E. coli* and their resistance to multiple antibiotics suggest the need for urgent intervention strategies to prevent the spread of antibiotic-resistant bacteria in these areas.

Keywords: Biofilm-producing,*Escherichia coli,* Water sources, Slums, Antibiotic resistance pattern, Public Health, Port Harcourt.

Introduction

Port Harcourt, the capital of Rivers State is one of the fastest growing urban centers in Nigeria. This growth is induced by population drift from rural areas to urban

centersin search of employment and greener pastures in the city. The increasing growth resulted to the development of informal settlements along the water-fronts popularly known as “waterside” to house these immigrants from the rural areas who are mostly low income earners. These slums lack basic amenities such as toilets, waste collection points, access roads, and good water supply (Wokekoro and Inyang, 2007; Ogbonna *et al*., 2021).

The slums in Port Harcourt city lack access to potable water supply, they solely depend on well and borehole water as their main sources of water supply. Within these communities there are various improperly managed sanitation systems, including domestic waste, solid waste dumpsites,abattoir activities and other anthropogenic activities. Hence the possibility of the groundwater from these wells and boreholes are being contaminated by potential pathogens as well as other microorganisms. Bacterial contamination of drinking water is a global public health threat, placing persons at risk for a host of diarrheal and other diseases as well as chemical intoxication (Ogbonna *et al*., 2021).

Biofilms are complex communities of microorganisms that adhere to surfaces and are encased in a protective matrix of extracellular polymeric substances (EPS). In the case of *E. coli*, biofilms can be formed on various surfaces such as medical devices, food processing equipment, and natural environments (Okafor *et al.,* 2005). However, in aquatic environments, microorganisms have the ability to adhere to solid surfaces and form biofilms due to different unfavorable conditions that required adaptations. 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 (Suma *et al.*, 2014).

The formation of biofilms by *E. coli* involves several stages. Initially, the bacteria attach to a surface using specialized adhesion molecules. They then multiply and produce Extracellular polymeric substances (EPS) which areorganic polymers of microbial origin involved in bacterial cells interactions with their environment, which helps to anchor the biofilm and provide protection against external factors such as antibiotics and the host immune system. The biofilm structure provides a favorable environment for the bacteria to thrive and communicate with each other through a process known as quorum sensing (Suma *et al.*, 2014).

Biofilms increase the opportunity for gene transfer among bacteria (Okafor *et al.,* 2005). Bacteria that are resistant to antibiotics may transfer the genes for resistance to neighbouring susceptible bacteria (Joanne *et al.*, 2011). Also, gene transfer could convert a previous virulent commensal organism into a highly virulent pathogen (Lewis 2012). *E.coli* is an affiliate of the faecal coliform bacteria and originates in the human intestines (Environmental Protection Agency, 2001). *E. coli* bacteria serves as an excellent sign for faecal pollution in water as they can live longer than other bacteria or disease-causing organisms (Olorode *et al.,* 2015). *E. coli* is a rod-shaped

bacterium commonly found in the gastrointestinal tract and faces of warm-blooded animals. It is a member of the faecal coliform group of bacteria. This bacterium is a preferred indicator, and its presence provides direct evidence of faecal contamination from warm-blooded animals. For the safety of drinking water, the *E.coli* counts should be 0 (Environmental Protection Agency, 2001). Analysis of water for *E.coli* is important for assessing bacteriological pollution in the water. Occurrence of *E.coli* in water at high concentration means that the water is not safe for drinking and there is high possibility for waterborne infections such as diarrhoea, cholera, and many more.

E. coli biofilms are associated with various impacts and challenges. They can cause persistent infections that are difficult to treat, as the bacteria within the biofilm exhibit increased resistance to antibiotics (Sunday *et al.,* 2014). Biofilms are also implicated in the contamination of water systems, leading to outbreaks of diarrheal diseases. Additionally, biofilms formed on medical devices can compromise their functionality and increase the risk of device-related infections. It is therefore necessary to evaluate the quality of groundwater from the major slums around Port Harcourt in order to ascertain their public health impacts to the residents (Onyedibia*et al.,* 2021).

Materials and Methods

2.1: Description of Study Area

Port Harcourt is located in the Niger Delta region, Southern Nigeria. The city is situated between latitudes $3^0$37' and $3^0$56' N. and longitude $11^0$10' and $11^0$45' E, approximately 50km from the Atlantic coast(Ogbonna *et al*., 2007). Precipitation averages 3,030mm annual and a temperature average of 23℃. Figure 1 shows the map of Port Harcourt indicating the sampled locations.

2.2: Collection of samples

A total of three hundred and sixty water samples of surface, well and borehole water were obtained from five different slums within Port Harcourt namely,Bundu, Egede, Enugu, Ibadan and Etche watersides for a period of twelve months. On each sampling occasion, water samples of approximately 600ml were 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 600 ml was poured aseptically into sterile bottles, for the surface water sample, sterile bottles were submerge to about 15cm deep and fetch the water (Kpormon and Douglas 2018), and then transported to the Laboratory for analyses within 2 hours of collection in a thermos box containing an ice pack. The map of the study area is presented in Figure 1 while Plate 1 shows an overview of a slum settlement.

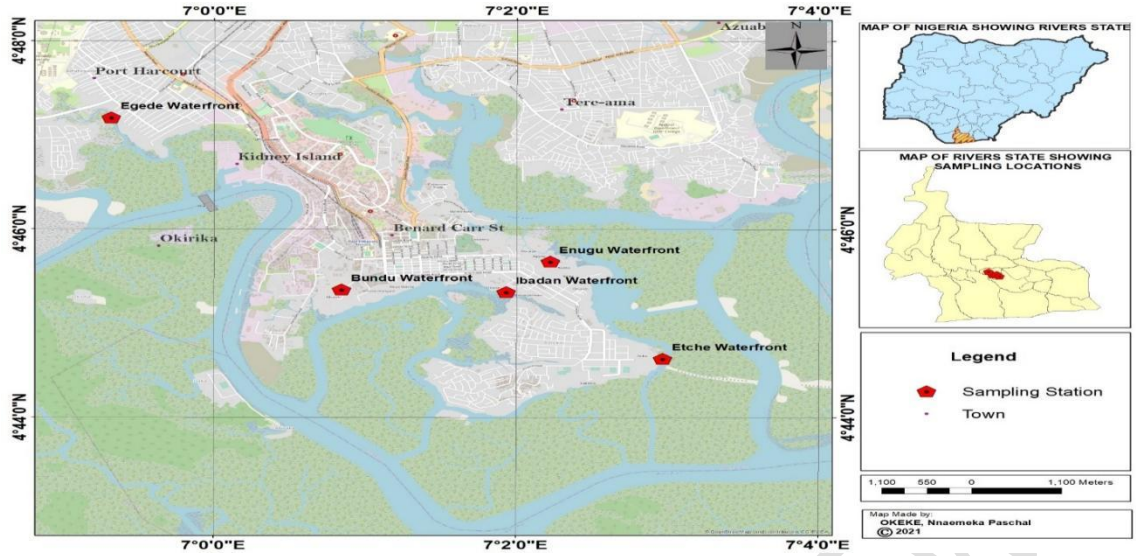


Figure.1: Map of Rivers State showing the various Study Locations

Plate 1: An Overview of Slum Settlement

Source: Researcher's Field Survey 2021

Bacteriological Analyses of the Water

Normal saline was used as diluent at a concentration of 0.85% sodium chloride (NaCl). Eosin methylene blue agar (EMBA) was used for the determination of faecal coliform (*Escherichia coli*). Faecalcoliformswereisolated and enumeratedas described by Prescott *et al* (2005). Colonies that appeared on the EMB agar plates after 24 hours of incubation at 44.5°C were observed and the prevalence calculated accordingly.

Identification of Bacterial Isolates

Pure bacterial isolates were identified by the method described by Cheesbrough (2005). Pure bacterial isolates were subjected to Biochemical tests which include oxidase test, catalase test, indole test, oxidase, coagulase, methyl red test, Voges Proskauer test, starch hydrolysis test, citrate test, sugar fermentation test and triple sugar iron agar test. The identities of the bacterial isolates were confirmed by referring to the Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994).

Maintenance of pure culture

Discrete bacterial colonies that grew on the respective media were sub cultured using streak plate method onto freshly prepared nutrient agar and incubated at 44.5°C for 24 hours in order to obtain pure culture. The pure bacterial cultures were then maintained according to the method adopted by Amadi *et al.* (2014) using ten percent (v/v) glycerol suspension at -4°C.

Determination of Biofilm Producing Bacteria

Biofilm production was determined using the Congo Red Agar method. The media was prepared using the Brain Heart Infusion (BHI) agar supplemented with 5% sucrose and Congo red. Medium was composed of: BHI (37 g/L), Sucrose (50 g/L) Agar (10 g/L), Congo red stain (0.8 g/L). Congo red was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes separately from other media constituents and was added when the agar had cooled to 45°C. Plates were inoculated and incubated for 24 to 48 hours at 37°C (Mathur *et al*., 2006). Positive result was indicated by black colonies and non-biofilm producers remained pink (Douglas *et al.,* 2022).

Antibiotic Sensitivity Test

The culture medium used was Mueller Hinton agar which was prepared according to manufacturer's instruction. Bacteria suspension was prepared according to 0.5 McFarland standard. A swap stick was used to spread the suspension evenly on the surface of the medium. Using a forceps, antibiotics sensitivity disc was placed on the surface of the medium sealed with the suspension and incubated at 37^{o}C for 24hrs. The plates were observed for zone of inhibition. Clear zone of inhibition around a particular antibiotic indicated that the organism was susceptible to the antibiotic. The zones were interpreted according to the Clinical Laboratory Standard Institute (2011).

Multiple Antibiotics Resistant Index of Bacterial Isolates

The multiple antibiotics resistance of the bacterial isolates was determined according to the method used by Oluyege*et al.* (2013). It was calculated using the relation.

$I = \frac{\mathrm{N}}{\mathrm{A}}$ ……equation 1

Where I = MAR index,

N = The number of antibiotics to which each isolate was resistant,

A = The total number of antibiotics used

Statistical Analyses

Analyses of variance of the data obtained were carried out using one ANOVA.P-value of less than 0.05 was considered to be statistically significant (<0.05).

Results and Discussion

Faecal coliform counts obtained from the three water sources across the five waterfronts communities revealed that surface water samples ranged from 4.09 cfu/ml log_{10} (Egede waterfront) to 4.15 cfu/ml log_{10} (Bundu waterfront). Well water samples ranged from 3.04 cfu/ml log_{10} (Etche waterfront) to 3.46 cfu/ml log_{10} (Bundu waterfront) while counts for borehole water samples ranged from 2.90 - 3.46 cfu/ml log_{10} (Etche waterfront) to 3.39 - 3.46 cfu/ml log_{10} (Bundu waterfront) (Figure 2). ~~In the present study, it is observed that surface water recorded the highest counts followed by well and borehole water respectively~~. In this study surface water had high microbial counts followed by well water while borehole water recorded the lowest count across the five slums. The results of the T. test analysis showed that there was a significant differences between the bacterial population obtained from surface, borehole and the well water samples at P≤0.05, the high counts recorded in the surface water samples could be attributed to the of nature activities that takes place more frequently around the slums such as open defecation into the surface water and disposal of wastes which have the ability to enhance microbial growth due to their nutritional content (Khan and Rizvi, 2011; Douglas and Longjohn, 2022).

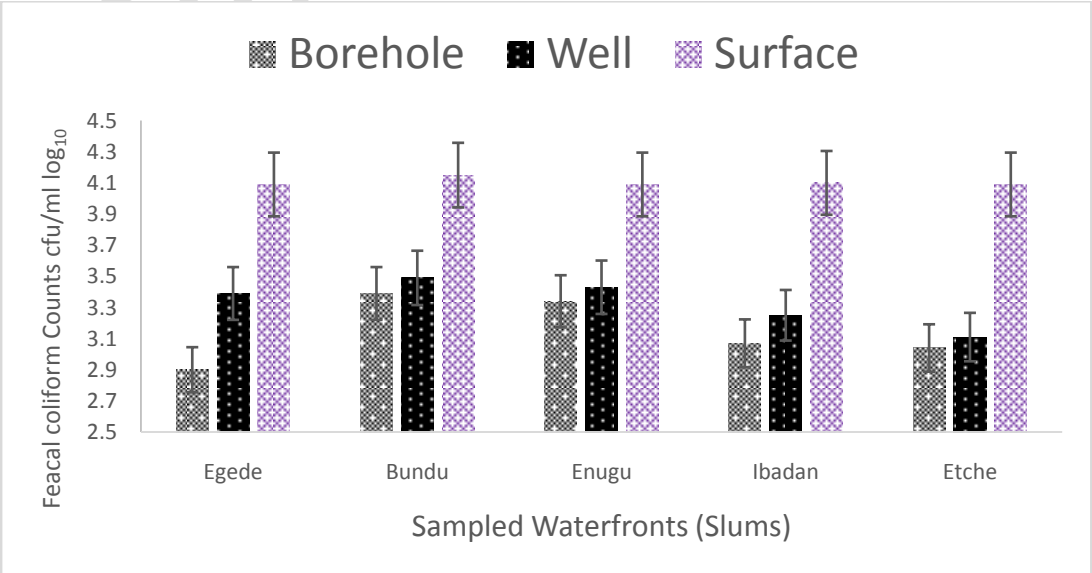


Figure 2: Mean Faecal Coliform Counts of three Water Sources from the Five Sampled Stations

Generally, faecal coliform counts of three water sources across the five slums studied were above the recommended standard by World Health Organization and Nigeria Standard for Drinking Water Quality (WHO 2010, NSDWQ 2008), which stated that faecal coliform count for a potable water should zero per milliliter (0/ml). This indicated that, the various human activities within the slums and poor environmental condition had a significant effect on the bacteriological quality of their water bodies.Prevalence of the *Escherichia coli* in the three water sources across the different slums around Port Harcourt revealed 291(80%). All surface water samples were positive for*E.coli*, 120 (100%) 98(81%) out 120 samples well water and 73(60%) out of 120 samples of the borehole water were positive of *Escherichia coli*(Figure 3). This result is in line with the report of Sunday *et al.* (2016) where the authors obtained a high level of prevalence of E.coli in surface water samples from Abakaliki area of Abia State, Nigeria.

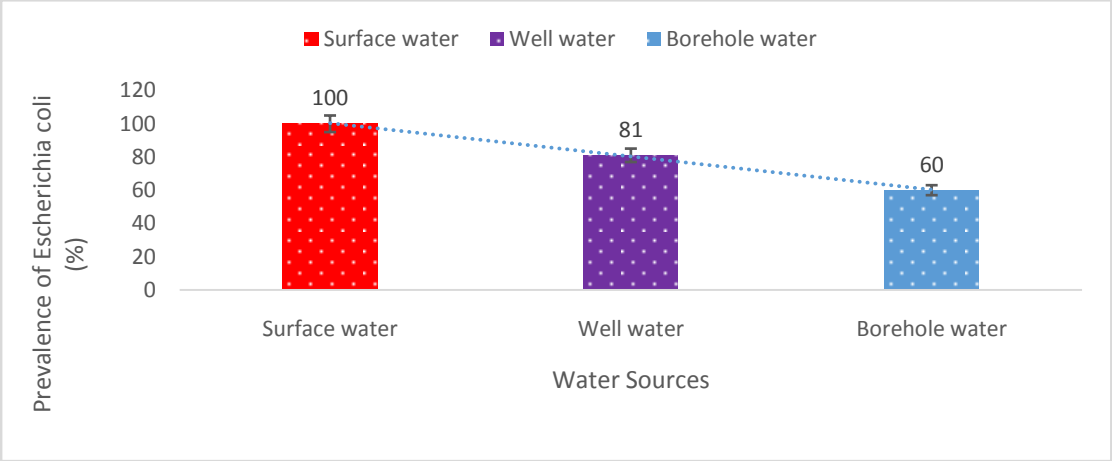


Fig. 3 Prevalence of *Escherichia coli* in the three (3) water Sources

The results of the ability of the isolates to produce biofilm are presented in Figure 4, out of the 291 *Escherichia coli* isolated from the three water sources, 180(62%) were strong biofilm producers, 90(31%) were moderate biofilm producer while 21(7%) were non biofilm producers. The presence of these biofilms producing bacteria in the groundwater samples implies the likelihood of waterborne diseases and the water is unsuitable for drinking, some well and borehole water may be located very close to septic tanks, or surface water where residents of slums defecate openly 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 contaminants by the containers used to fetch the water from the outside into the well.

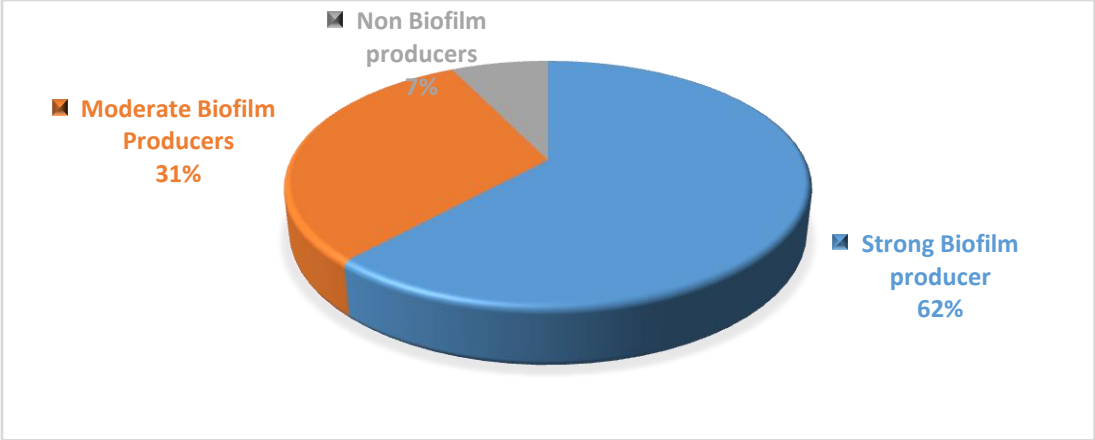


Figure 4: Percentage of *Escherichia coli* Biofilm Production from the three Water sources

The results of the antibiotic resistant pattern indicates that the*E.coli* isolated from three water sources exhibited high level of resistance to Augmentine, Ceftazidime,Cefuroxime, andCloxacillin (Figure 5-7), whereas some of the *E.coli* were barely susceptible and marginally intermediate to other antibiotics tested. The percentage resistance of *Escherichia coli* isolated from water sources inBundu, Egede, Enugu, Etche and Ibadan waterfronts, were 100% resistant to Ceftazidime (CAZ) and Cefuroxime (CRX) respectively. For Cloxacillin (CXM), *Escherichia* showed the following resistance Enugu and Ibadan (82.5%), Etche and Egede (79.2%) and Bundu (66%). Whereas *Escherichia coli* isolated from Bundu, Enugu, and Ibadan were 100% while those isolated from Egede, and Etche were 75% resistant to Augmentine. On the other hand, for *Escherichia coli* from Etche and Egede were both 8.3 and 15% resistant to Gentamycin. In present study, it was observed that *Escherichia coli* were 100%susceptible to Nitrofurantoin, Ofloxacin and Ceftriaxone. Similar treads were also observed in other waterborne pathogens isolated. High level of antibiotic resistant isolated were prevalent and the result is in comparative with the study of Chigor*et al.,* (2010) and Idia *et al.,* (2006) which shows MDR cases ranging from 61.2% to 97.1% in aquatic isolates.

The high percentage of resistance to commonly used antibiotics recorded in this study may be caused by mechanisms such as the synthesis of low affinity-β-lactams binding proteins, the production of penicillinase, and transferable genetic elements which included plasmids that may contain different resistant genes and the ability of the isolates to produce biofilms which increase the opportunity for gene transfer among bacteria (Ogbonna *et al*., 2007). In addition to having structural barriers, biofilm-forming *E.coli* can undergo physiological changes such as slow growth rate

and producing persistent cells. In these occasions, antibiotics cannot inhibit, kill, or eradicate these slow-growing and persistent cells which are found inside the biofilm matrix (Okafor *et al.,* 2005). According to the report of Lewis, (2012), biofilms promote genetic exchange among bacterial cells through horizontal gene transfer, allowing the transfer of antibiotic resistance genes between different species or strains of bacteria. This genetic exchange can further enhance antibiotic resistance within the biofilm and potentially spread it to other bacteria in the surrounding environment. The high percentage susceptibility of the test isolates to Ofloxacin is in agreement with reports by other authors on multiple-drug resistance bacteria (Ogbonna and Azuonwu, 2019) not only that but also the main drivers of antimicrobial resistance include the misuse and overuse of antimicrobials, lack of access to clean water, sanitation and hygiene for both humans and animals; poor infection and disease prevention and control in health-care facilities and farms; poor access to quality, affordable medicine.

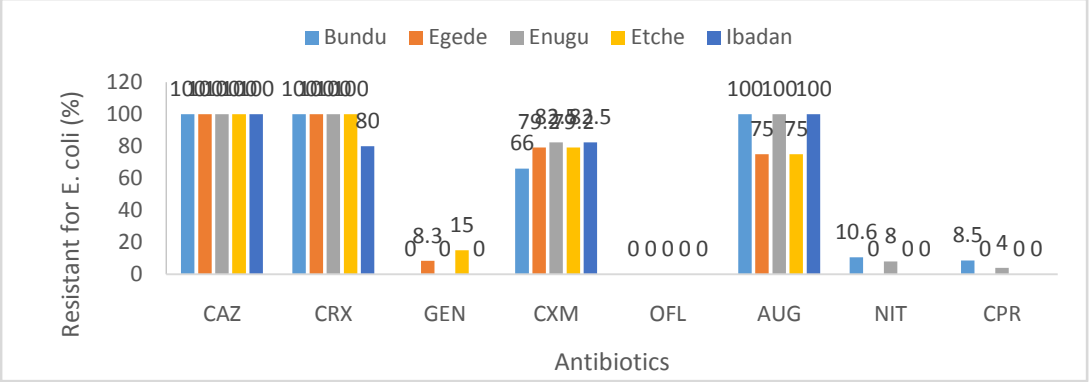
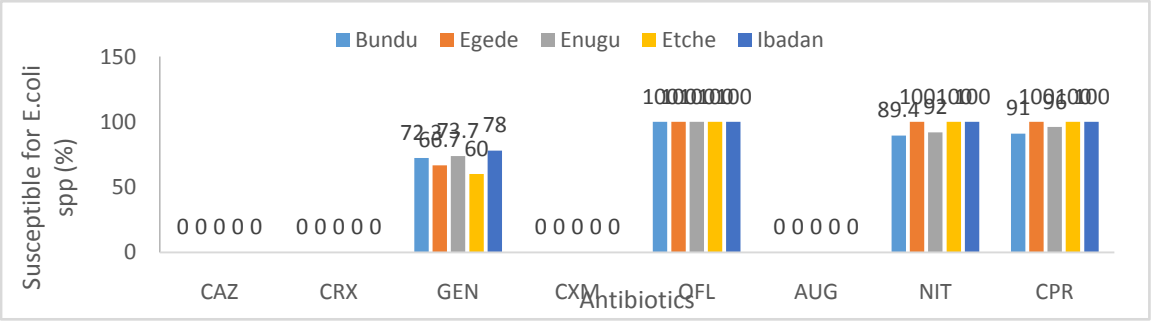


Figure 5: Resistance Pattern of Biofilm producing *Escherichia coli* Isolated from three(3) water sources around major Slums in Port Harcourt city.

KEY: CAZ= Ceftazidime (30µg) **CRX**= Cefuroxime (30µg), **GEN**= Gentamicin (10µg), **CXM** = Cloxacillin (5µg), **OFL**= Ofloxacin (5µg), **AUG**= Augmentin (30µg),**CPR**= Ceftriaxone (30µg) **NIT**= Nitrofurantoin



(30µg),

Figure 6. Susceptibility Pattern of Biofilm producing *Escherichia coli* Isolated from three (3) water sources around major Slums in Port Harcourt

KEY: CAZ= Ceftazidime (30µg) **CRX**= Cefuroxime (30µg), **GEN**= Gentamicin (10µg),**CXM** = Cloxacillin (5µg), **OFL**= Ofloxacin (5µg), **AUG**= Augmentin (30µg),**CPR**= Ceftriaxone (30µg) **NIT**= Nitrofurantoin (30µg),

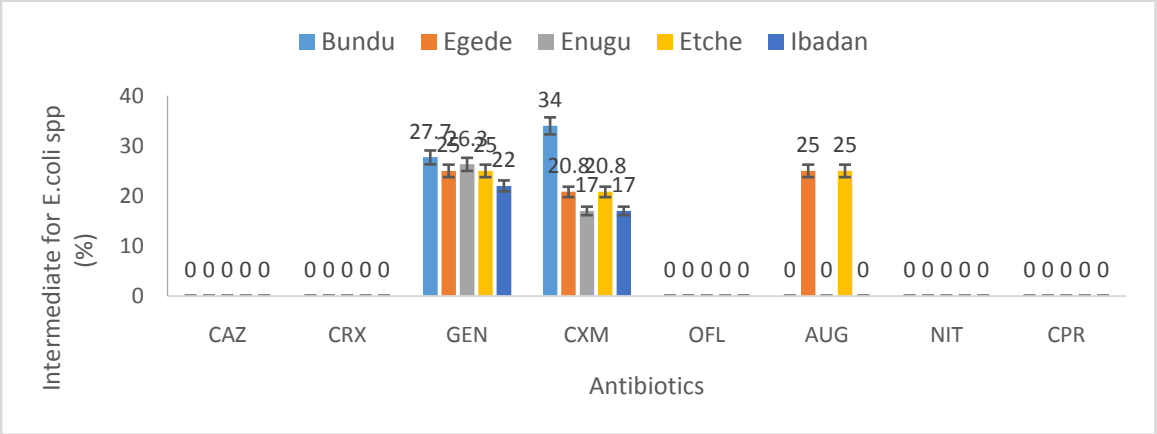


Figure 7. Susceptibility Pattern of Biofilm producing *Escherichia coli* Isolated from three (3) water sources around major Slums in Port Harcourt city.
KEY: CAZ= Ceftazidime (30µg) **CRX**= Cefuroxime (30µg), **GEN**= Gentamicin (10µg),**CXM** = Cloxacillin (5µg), **OFL**= Ofloxacin (5µg), **AUG**= Augmentin (30µg),**CPR**= Ceftriaxone (30µg) **NIT**= Nitrofurantoin (30µg)

Conclusion and Recommendations

The study revealed high prevalence of biofilm producing *Escherichia coli* in the three water sources in major slums around Port Harcourt due to poor waste management practices across the slums. The activities in the slums present numerous problems and are vulnerable to water pollution. The incidence of high bacterial contamination indicates that most of the drinking water supplies are unfit for human consumption and such water sources may predispose inhabitants/users to public health hazards. High level of antibiotic resistance was also recorded among the bacterial isolates. This study also revealed that the presence of biofilms significantly contributed to antibiotic resistance by providing bacteria with an enhanced ability to withstand and survive antibiotic treatment. This resistance can complicate the treatment of infections associated with biofilm-producing bacteria and may require alternative or combination therapies to effectively combat biofilm-associated antibiotic resistance. Therefore, it is recommended that water sources around the major slums in Port Harcourt city should be treated properly at the point of use and should not be stored for more than three weeks in order to prevent the formation of biofilms.

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