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Antibiotic Resistance Profile of *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Nworie River, Owerri, Imo State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

As a result of infectious organisms' development of antibiotic resistance, using antibiotics to treat infectious diseases has become more challenging. This study was undertaken to study the antibiogram of *Escherichia coli* and *Klebsiella pneumonia* isolated from Nworie river in Imo state. Water samples were collected and the two test organisms were selectively isolated for antimicrobial analysis. Routine antibiograms were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar. Nine (9) antibiotics were used for the analysis; ceftazidime (30 ug), imipenem (10 ug), meropenem (10 ug), cefotaxime (30 ug), tetracycline (30 ug), ciprofloxacin (5 ug), gentamicin (10 ug), colistin (10 ug) and polymyxin B (30 ug) (Oxoid, Basingstoke). 67 isolates of *Escherichia coli* and 75 isolates of *Klebsiella pneumoniae* were obtained from the water samples.

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Escherichia coli had a very high resistance to polymyxin B (100%), colistin (100%), cefotaxime (100%), ceftazidime (100%), ciprofloxacin (73.13%), tetracycline (82.09) and imipenem (73.13%), it exhibited a moderate resistance to meropenem (67.16%) and a low resistance to gentamicin (38.81%). *Klebsiella pneumoniae* had a very high resistance to polymyxin B (100%), colistin (100%), cefotaxime (98.67%), ceftazidime (98.67%), ciprofloxacin (86.67%), tetracycline (84), meropenem (78.67%) and imipenem (64%), it exhibited a moderate resistance to gentamicin (41.33%). Very high rates of resistance were found amongst isolates in this study and this is a cause for worry especially as the river from which these isolates were obtained is used for drinking swimming, washing etc. It is recommended therefore that more research is carried out to find out novel antibiotics that can be used in the treatment of diseases caused by these Enteriobacteriaceae; including combined chemotherapy.

Keywords: Antibiotic resistance; Escherichia coli; Klebsiella pneumonia; river; Enteriobacteriaceae.

1. INTRODUCTION

Although antibiotics are necessary for the treatment of illnesses in humans, animals, and even plants, their constant abuse and improper usage in the management of diseases has significantly exacerbated the issue of antibiotic resistance. Hospitals deal with a variety of issues every day brought on by newly discovered opportunistic infections that exhibit little to no tolerance to even novel antibiotics [1].

These antibiotic-resistant bacteria are found everywhere including in aquatic environments [2] such as oceans [3], rivers [4], lakes [5] and even sewer water [6]. One of the most crucial bacterial habitats is water, if not the most crucial one [7] and it is a key mechanism for the spread of bacteria and other microbes between different environmental compartments. This water too is very essential to every living macro organism; human, animals and plants. As a result, the existence of these antibiotic-resistant microbes in these bodies of water poses a major risk to the entire world and should not be taken for granted. As a matter of fact, the need to understand antibiotic resistance patterns of human bacterial pathogens present in the environment cannot be overemphasized [3]. This will help maintain the sanctity of clinical medicine and chemotherapy.

In nature, Enterobacteriaceae are widely spread. They mostly reside inside the intestines of humans and other animals, and when they excrete feces, their waste materials find their way into bodies of water. Enterobacteriaceae strains are the major foodborne and waterborne pathogens [8]. *Escherichia coli* is abundant in water systems [9] and there is high tendency of antimicrobial resistance in these locations. According to studies, surface water has been found to contain vancomycin-resistant enterococci (VRE), which are typically found in human feces, farm animal waste, and animal products [10,11]. The enterobacteriacea are a prevalent cause of nosocomial infections and they include strains that are extremely multi-drug resistant or pan-drug resistant, which result in a variety of illnesses with few effective treatment choices [12]. Due to its resistance to routinely used antimicrobial treatments, *Klebsiella pneumoniae*, a gram-negative bacterium, is one of the most frequent causes of infections in healthcare settings and is also one of the most challenging to treat [3].

Global public health is under threat from multidrug-resistant gram-negative bacteria [13]. Most bacteria now resist a wide range of antibiotics over time. This could be due to one or more mechanisms, such as the aggregation of genes that individually code for drug resistance inside a single cell or an increase in the expression of genes that code for multidrug efflux pumps that expel a variety of medications. This study was undertaken to study the antibiogram of *Escherichia coli* and *Klebsiella pneumonia* isolated from Nworie river in Imo state.

2. MATERIALS AND METHODS

2.1 Study Area

The study area (Nworie River) lies within latitude $5^0 29'$ to $5^0 49'$N and longitude $07^0 01'$ to $7^0 25'$E standing on an elevation of 77 m upstream, 55 m midstream and 45 m downstream above the sea level; Hand held Global Positioning System (GPS). The river is about 9.2 km in length with its source at Ubomiri in Egbeada which joins Otamiri river at Nekede. The river flows through the back of Federal Medical Centre (FMC), Alvan Ikoku College of Education, Holy Ghost College and Umezuruike Hospital. These institutions discharge their untreated wastes into the river (Nwosu et al., 2021). The river also serves as a source of drinking water for inhabitants when the public water supply fails.

The study area is covered by grasses and broad leaves as well as few trees such as palm trees, mango trees. Farming is the major occupation of the inhabitants of the area while sand mining and excavation are the major activities within the study area. The gully erosion found at the area could be attributed to intensive human activities of the watershed resulting in the discharge of many untreated waste thus leading to various forms of degradation.

2.2 Sample Collection

Water samples were collected from Nworie River using sterile sample bottles. The water samples were collected from five sampling points from the river; back of Umezurike Hospital, back of Federal Medical Centre Owerri (FMC), back of Alvan Ikoku College of Education, back of Holy Ghost College and Amakohia, Owerri. Prior to collection, the sterile sampling bottles were correctly labeled with the names of the locations where the samples will be collected. Two sample bottles were used to collect water sample from one location. The bottles were firstly rinsed with the stream water before final sampling; this ensured an even mixing of the river water. After sample collection, the water samples were transported on ice to the laboratory for analysis. Sample collection was done twice and like the first time, two bottles were used to collect sample from one location.

2.3 Sample Types, Processing and Bacterial Isolates

Water samples were collected from seven (7) sites along the river course, from the upstream to downstream points. The samples were collected in sterile 1L sample containers, immediately placed on ice and transported to the laboratory for analysis. Eosin Methylene Blue Agar (Hi media) was used to selectively isolate *Escherichia coli* while MacConkey Agar (Hi media) was used for the selective isolation of *Klebsiella pneumoniae*. The water samples were serially diluted up to 10^{-3} dilution and 0.1ml of the diluted samples were spread on the well labelled agar plates in duplicates. The plates were incubated at a temperature of 37 ^{0}C for 24 – 48 hours to allow for the growth of the organisms of interest.

Confirmatory tests were carried out to ensure that the distinct colonies of interest were isolated and this was done by carrying out some biochemical tests on the isolated organisms. The biochemical tests done were Indole test, Methyl Red test, Voges-proskauer test and Citrate utilization test.

2.4 Antimicrobial Resistant Testing

Routine antibiograms were determined by the Kirby-Bauer disk diffusion technique on Muellller-Hinton Agar (Hi media). The antibiotics assayed and their concentrations were ceftazidime (30 ug), imipenem (10 ug), meropenem (10 ug), cefotaxime (30 ug), tetracycline (30 ug), ciprofloxacin (5 ug), gentamicin (10 ug), colistin (10 ug) and polymyxin B (30 ug) (Oxoid, Basingstoke).

The prepared media was poured into well labelled petri dishes. Each isolate was first subcultured to get pure culture and then standardized using McFarland 0.5 Latex standard. After standardizing the isolate, a sterile swab was used to pick an inoculum and the dried surface of the Muller Hinton agar plate was inoculated by streaking the swab over the entire agar surface. It was ensured that the inoculum was evenly distributed on the surface of the agar plate, after which the plate was allowed to sit at room temperature or about 2 – 3 minutes.

A Sterile forceps was then used to impregnate the antibiotic disks on the surface of the agar and then pressed gently to ensure there was complete contact with the agar surface. For each isolate, three Muller Hinton agar plates were used for the test; three antibiotic disks were placed in one disk to prevent over lapping of the zones of inhibition. After all the disks were placed correctly, the plates were inverted and incubated at 35^{0}C for 24 hours. After 24 hours, the zones of inhibition were measured in mm using a measuring ruler. The results were interpreted according to the criteria established by the CLSI [14].

3. RESULTS

A total of 67 *Escherichia coli* isolates were obtained while 75 isolates of *Klebsiella pneumoniae* were obtained from the water samples.

In Table 1 as shown below, *E. coli* had a 100% resistance to Polymyxin B, Colistin, Cefotaxime and Ceftazidime. The table also shows high resistance of the isolate to Tetracycline (82.09%), Ciprofloxacin and Imipenem (73.13% each). Gentamicin was highly effective against the organism as the organism showed 61.19% sensitivity to the antibiotic.

Table 2 showed the resistance profile of *Klebsiella* and it can be seen that the organism exhibited very high resistance to the antibiotics except to Gentamicin where it was moderately sensitive to the antibiotic (58.67).

Fig. 1 showed multiple resistance rates among *E. coli* and *Klebsiella* isolates obtained from the river water. It compared multiple resistance to the antibiotics between the two organisms.

Table 1. Antibiotic resistance profile (%) of *E. coli* isolates from Nworie River water

Antibiotic	No of isolates (n = 67)	
	Susceptible	**Resistant**
Polymyxin B	0 (0)	67 (100%)
Colistin	0 (0)	67 (100%)
Cefotaxime	0 (0)	67 (100%)
Ceftazidime	0 (0)	67 (100%)
Ciprofloxacin	18 (26.87%)	49 (73.13%)
Tetracycline	12 (17.91%)	55 (82.09)
Meropenem	22 (32.84%)	45 (67.16%)
Imipenem	18 (26.87%)	49 (73.13%)
Gentamicin	41 (61.19%)	26 (38.81%)

Table 2. Antibiotic resistance profile (%) of *Klebsiella pneumoniae* isolates from Nworie River water

Antibiotic (Conc)	No of isolates (n = 75)	
	Sensitive	**Resistant**
Polymyxin B	0 (0)	75 (100%)
Colistin	0 (0)	75 (100%)
Cefotaxime	1 (1.33%)	74 (98.67%)
Ceftazidime	1 (1.33%)	74 (98.67%)
Ciprofloxacin	10 (13.33%)	65 (86.67%)
Tetracycline	12 (16%)	63 (84%)
Meropenem	16 (21.33%)	59 (78.67%)
Imipenem	27 (36%)	48 (64%)
Gentamicin	44 (58.67%)	31 (41.33%)

Table 3. Comparative Resistance profile of *Escherichia coli* and *Klebsiella pneumoniae* isolated from Nworie river water

Antibiotics	Organisms		Total resistance
	***Klebsiella* (n = 75)**	***E. coli* (n = 67)**	**N = 142**
Polymyxin B	75 (100%)	67 (100%)	142 (100%)
Colistin	75 (100%)	67 (100%)	142 (100%)
Cefotaxime	74 (98.67%)	67 (100%)	141 (99.30%)
Ceftazidime	74 (98.67%)	67 (100%)	141 (99.30%)
Ciprofloxacin	65 (86.67%)	49 (73.13%)	114 (80.28%)
Tetracycline	63 (84%)	55 (82.09)	118 (83.10%)
Meropenem	59 (78.67%)	45 (67.16%)	104 (73.24%)
Imipenem	48 (64%)	49 (73.13%)	97 (68.31%)
Gentamicin	31 (41.33%)	26 (38.81%)	77 (40.14%)

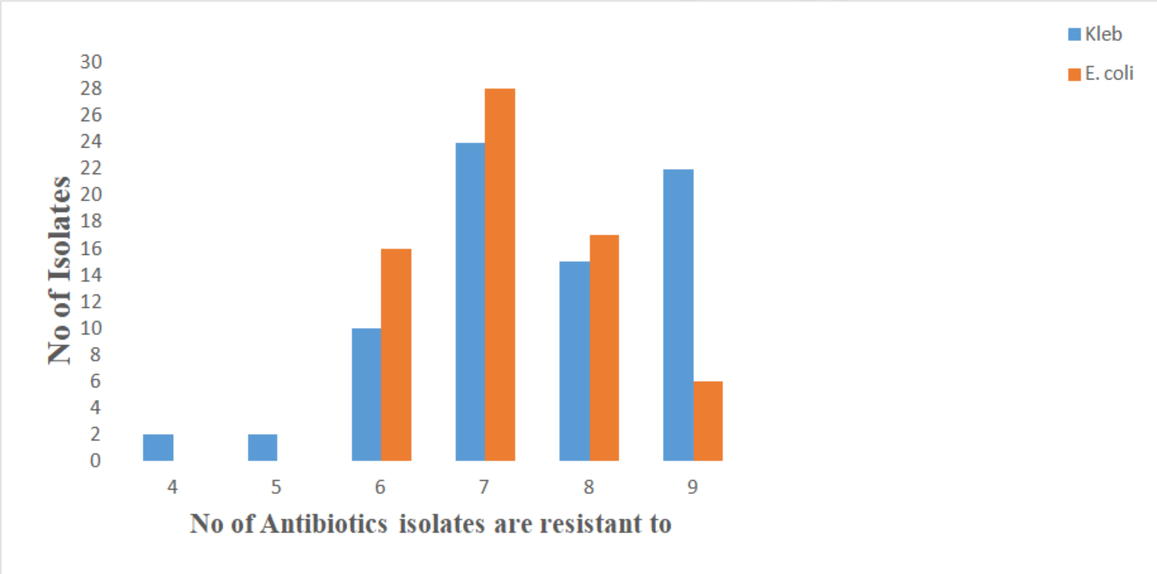

Fig. 1. Multiple resistance rates among *E.* coli and *Klebsiella* isolates from Nworie River, Imo State, Nigeria

4. DISCUSSION

From the result, all isolates expressed 100% resistance to Polymyxin B and Colistin. This was similarly observed in the research carried out by Carl et al. [15] when they observed two *E. coli* isolates that also showed 100% resistance to polymyxin B and colistin. Farhadi et al. [16] observed 62.22% resistance of *Klebsiella*. However, in the work of Naveed-ur-Rehman et al. [17], there was 100% sensitivity of *Klebsiella pneumoniae* to Polymyxin B and that led to the use of the antibiotic to cure eight (8) out of fourteen (14) patients (57.1%). Polymyxins were most widely used to fight against carbapenem resistant organisms such as *E. coli* and *K. pneumoniae* [18]. This study suggests that these organisms have actually become very resistant to these polymyxins and honestly, it is a cause for worry, especially in the light of the limited available antibiotic options and high mortality rates associated with infections caused by these organisms.

E. coli expressed 100% resistance to cefotaxime and ceftazidime while *Klebsiella pnuemoniae* had 98.67% resistance to the two antibiotics. In a similar work carried out by Duru et al. [19], all *E. coli* isolates obtained had 100% resistance against cefotaxime and ceftazidime. A similar observation was made by Chikwendu et al. [12] where *Klebsiella pneumoniae* isolated from pig waste had 100% resistance to both cefotaxime and ceftazidime while *E. coli* isolated from chicken waste had high resistance to the two *antibiotics* (92%). High resistance for cefotaxime (78%) was also observed by Chikwendu et al. [20] in *E. coli* isolated from piggery and chicken environments.

This study observed high resistance of the isolated organisms to ciprofloxacin. *Klebsiella pneumoniae* had a resistance of 86.67% while *E. coli* had a resistance of 73.13% to the antibiotic. In 2015, a study carried out by Frederick et al. [21] showed that *E. coli* isolated from some drinking water sources in Ghana had very low resistance to ciprofloxacin (1.79%) and a very high susceptibility to the antibiotic (94.64%). However, the results in this study had a similar observation with that of Anyadoh-Nwadike et al. [22], where *E. coli* isolated from diabetic patients' wounds had a 100% resistance to ciprofloxacin while *Klebsiella* had 25% resistance and 75% intermediate susceptibility to the antibiotic. Ciprofloxacin being a broad spectrum fluoroquinolone has a hundred-fold greater antimicrobial activity than its parent compound, Nalidixic acid and as such was preferably used in the treatment because it was highly active against members of the *Enterobacteriaceae* including *E. coli* and *Klebsiella pneumoniae* [23]. The recent resistance of these organisms to ciprofloxacin may probably be attributed to frequent exposure to the antibiotic. For example, Federal Medical Center and Umezurike Hospital discharge their effluents into a parts of the Nworie River where the water samples were collected. There was also high resistance to tetracycline and meropenem for both isolates.

However, the isolates had a moderate susceptibility for imipenem; 58.67% for *Klebsiella pneumoniae* and 61.19% for *Escherichia coli.* Gentamicin was the most effective against both *Klebsiella pneumoniae* and *Escherichia coli*. *Klebsiella pneumoniae* had 41.33% resistance to gentamicin while *E. coli* had 38.81% resistance to the antibiotic. The low resistance of both organisms to gentamicin has been reported in many studies. Chikwendu et al. [20] reported low resistance of *E. coli* and *Klebsiella* isolated from the environment to gentamicin (25.5% and 38.5% respectively). Reuben et al. [23] also reported low resistance (25%) of *E. coli* and other Enterobacteriaceae to Gentamicin. This low resistance may be due to discouraged use of the drug coupled with the intravenous route of administration leading to restriction of indiscriminate use of these antibiotics [23]. However, in a study carried out by Chikwendu et al. [24], *Salmonella* isolates from the same river showed a 100% resistant rate to imipenem and highly sensitive to Gentamicin and ciprofloxacin.

Generally, it was observed that *Klebsiella pneumoniae* isolated from Nworie river had higher resistance to almost all the antibiotics than *Escherichia coli* isolated from the same river; this is shown in Fig. 1.

5. CONCLUSION

Very high rates of resistance were found amongst the isolates in this study. The water samples used for this study were gotten from Nworie river in Imo state; a river where many activities go on. The Federal Medical Centre discharges its effluents into a part of this river and this could have contributed to the reason for this high resistance found in the organisms isolated from the river.

Treatment of infectious diseases have been difficult in recent times because of the continuous rise in antibiotic resistant microorganisms. It is therefore very important that people who drink the water or swim in this river be sensitized on the high resistance observed in bacteria found in the river and negative implication on their health.

The use of combined antibiotic therapy may be recommended when infections caused by multiple antibiotic resistant bacteria occur since research has established its efficacy. There is a great need for all hands (lecturers, media, non-governmental organizations, health workers) to be on deck in sensitizing the public on the need to avoid self-medication when they are sick. The government should also provide clean and portable water for everyone so that everyone will have access to safe drinking water at little or no cost.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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