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# IMIPENEM RESISTANCE AMONG GRAM NEGATIVE BACILLI FROM GREAT KWA RIVER, NIGERIA

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## ABSTRACT

Emergence of antibiotics resistance bacteria is a persistent global problem affecting public health. The occurrence and widespread resistance to carbapenems among environmental bacterial isolates can constitute a major threat to chemotherapy. This investigation was carried out to assess the antibiotics susceptibility profile of imipenem resistant bacterial isolates from Great Kwa river located in Calabar, Nigeria. From different sites of the Great Kwa River, a total of 89 bacterial isolates were screened for imipenem resistance and susceptibility to 13 antibiotics. Out of the 89 isolates, 20(27.37%) were imipenem resistant, 22(23.16%) showed intermediate resistance while 47(49.47%) were susceptible to imipenem. The resistant and intermediate resistant isolates were of the following genera: *Serratia spp* (29%), *Plesiomonas spp* (17%), *Aeromonas spp* (14%), *Acintebacter spp* (2%), *Providencia spp* (2%), *Citrobacter spp* (5%), *Klebsiella spp* (5%), *Escherichia spp* (5%), *Proteus spp* (11%), *Salomnella spp* (5%). Among the resistant and intermediate resistant isolates, the percentages that were resistant to the 13 antibiotics were: Orfloxacin (57%), Pefloxacin (21%), Gentamycin (24%), Augmentin (48%), Ampicillin (24%), Ciprofloxacin (14%), Streptomycin (12%), Chloramphenicol (29%), Septrin (33%), Sulfamethoxazole-trimethoprim (29%), Aztreonam (64%), Ceftazidime (76%) and Cefuroxime (38%). Imipenem resistant bacteria are emerging globally at a rapid rate. The resistance can be transferred to human commensals and pathogens and can constitute a threat to an effective therapy using imipenem.

**KEYWORDS:** Emergence of antibiotics resistance effective therapy using imipenem.

## INTRODUCTION

The presence of various chemical pollutants and anthropogenic acitivities in the Great Kwa River has been linked to high incidence of antibiotics resistance (Abu and Egenonu, 2008). Antibiotics in aquatic habitats is a global problem both from the ecological point of view and the public health (Deng *et al*., 2016). Though antibiotics have been shown to be present in natural water bodies with little or no anthropogenic activities, the widespread use of antibiotics for therapy and livestock rearing has led to the abundance and persistence in the environment (Smith *et al*., 2002). The resultant selective pressure in these environment has led to the emergence of bacteria resistant to various antibiotics. The genetic mutations within these bacteria which lead to evolution of traits that enable tolerance to antibiotics (Stewart *et al*., 2015). The genes encoding resistance can easily be transferred both via vertical and horizontal means. This phenomenon is further worsened considering the possibility of exchange of resistance genes between environmental and human bacteria (Threedeach *et al* 2012).

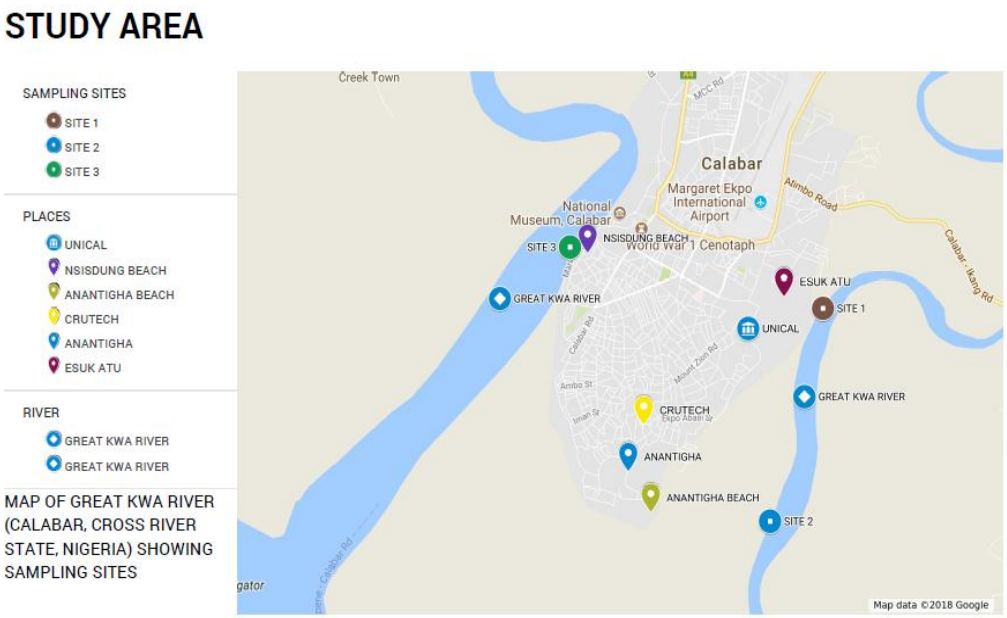
Among different antibiotics that bacterial resistance have been reported, the carbapenems are not excluded. Though some of the antibiotics observed to be ineffective for therapy are no longer used for treatment, the carbapenems (which include imipenems, meropenems, doripenem, ertapenem) are currently adopted for management of severe bacterial infections especially those associated with extended spectrum producing pathogens. The widespread use of the carbapenems can be attributed to the broad spectrum and potency against different bacterial infections. The penem ring structure of the carbapenems enhances the stability against beta lactamases compared to other beta lactam antibiotics which can be easily degraded. Carbapenems act on the bacterial cell wall and inhibit peptidoglycan synthesis by binding to the serine residue on the transpeptidases or penicillin binding proteins after penetrating the outer membrane through porins (Tam *et al*., 2005; Goyal and Rajput, 2014).

Resistance to imipenem and other carbapenems have been reported globally among environmental and clinical bacterial isolates (Buehrle *et al*., 2016; Hrenovic *et al*., 2017). This can constitute a major threat to therapy and public health if proper monitoring and regulation of the usage of the antibiotic is not implemented within and outside the hospital settings. Hence, this study was carried out to assess antibiotics susceptibility and the occurrence of imipenem resistant bacteria within the aquatic environment.

## MATERIALS AND METHODS

### Study area

The study was conducted within Calabar (4.9757° N, 8.3417° E) which is a city in Cross River State, located in the south southern region of Nigeria. The city has an area of 406 square kilometres (157 sq mi) and a population of 371,022 as at the 2006 census. The city stretches southwards and borders with the Calabar River, while to the east, it is bordered by the Great Kwa River which drains the east side of the city of Calabar (Ugbaja and Bassey, 2007).




### Collection of samples

Surface water samples were aseptically and randomly collected from three locations (Obufa Esuk Atu beach, Nsidung beach and Anantigha beach) into sterile bottles and transported to laboratory within 18 h for analysis. From each of the location, 10 surface water samples were randomly collected from different points (approximately 50m apart) and analyzed within 18 h of collection.

### Enumeration, Isolation and identification of isolates

As described by Oladipo *et al.* (2009), a 5 step ten-fold serial dilution was performed on the Great Kwa River samples and 1ml of the last two dilutions were plated using the surface plating technique on sterile MacConkey agar and incubated at 35°C. The colonies were

enumerated and the following procedures (oxidase, sugar fermentation, citrate, urease, catalase, motility indole Ornithine (MIO), and methyl Red (MR) tests) for bacterial identification according to Bergey's manual of determinative bacteriology were carried out to identify the isolates. The macroscopic, cultural, physiological and biochemical characteristics results were compared with the Bergey's manual in order to identify the isolates.

**Screening for imipenem resistant isolates and susceptibility to other antibiotics**

According to Clinical laboratory standard Institute (CLSI, 2009) guidelines for antibiotics susceptibility testing, a freshly prepared pure broth culture of the isolates which corresponds with 0.5 Macfarland was swabbed on a Muller Hinton agar plate. A 10 µg imipenem disk was placed on the agar surface and incubated for 24h at 35°C. Imipenem resistance was determined by measuring the diameter of the zones of inhibition and comparing with the standard ranges in CLSI manual (2009). The imipenem resistant isolates were subjected to susceptibility testing to the following antibiotics: sulfamthoxazole-trimethoprim (25µg), septrin (30µg), chloramphenicol (30µg), ampicillin (30µg), augmentin (30µg), gentamycin (10µg), pefloxacin (10µg), orfloxacin (10µg), ciprofloxacin (5µg), ceftazidime (30µg), aztreonam (30µg), cefuroxime (30µg), streptomycin (10µg).

**Statistical analysis**

Descriptive statistics using IBM SPSS 21 were used in analyzing the data obtained from this study.

## RESULTS

The number of Gram-negative bacterial isolates from the three sites of Great Kwa River is shown in table 1. A total of 45 colonies from Esuk Atu beach, 32 colonies and 12 colonies from Nsidun and Anantigha beaches respectively were obtained. The highest colony count was observed from samples from Esuk Atu beach.

**Table 1: Number of colonies of Gram-negative bacterial isolates from Great Kwa River samples.**

| Locations | Number of colony forming units | |
|---|---|---|
| Nsidun Beach | 32 | |
| Esuk Atu Beach | 45 | |
| Anantigha Beach | 12 | |
| **Total number** | 89 | |

Among these isolates, 20(27.37%) were imipenem resistant, 22(23.16%) were intermediate resistant while 47(49.47%) were susceptible to imipenem (table 2). The isolates identified were of the following genera: *Acinetobacter*, *Aeromonas*, *Citrobacter*, *Escherichia*, *Klebsiella*, *Plesiomonas*, *Proteus*, *Providencia*, *Pseudomonas*, *Salmonella* and *Serratia* (Figure 1). *Serratia spp* had the highest frequency (29%) followed by *Plesiomonas spp* (17%) and *Aeromonas spp* (14%). *Acintebacter spp* (2%) and *Providencia spp* (2%) had the least frequency. Other bacterial isolates include *Citrobacter spp* (5%), *Klebsiella spp* (5%), *Escherichia spp* (5%), *Proteus spp* (11%), *Salomnella spp* (5%).

**Table 2: Imipenem susceptibility of isolates from Great Kwa River samples.**

| Imipenem susceptibility | Total number (n=89) |
|---|---|
| Resistance | 20(27.37) |
| Intermediate | 22(23.16) |
| Susceptible | 47(49.47) |

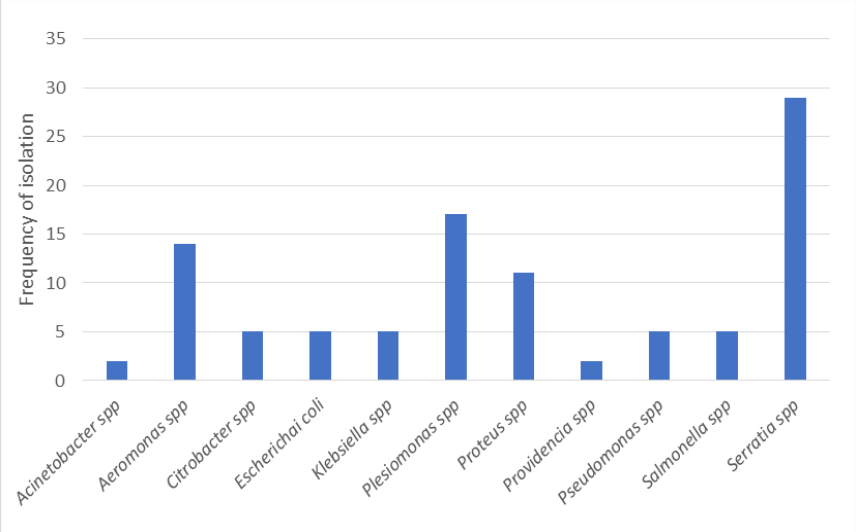



**Figure 1: Frequency of Imipenem resistant bacteria from Great Kwa river.**

**Antibiogram of imipenem resistant bacteria to other antibiotics**

Figure 1 shows the antibiotics susceptibility results of the imipenem resistant bacteria. The highest resistance of 76% was observed against ceftazidime and the least resistance of 12% was recorded against streptomycin. The percentage resistance to other antibiotics were Orfloxacin (57%), Pefloxacin (21%), Gentamycin (24%), Augmentin (48%), Ampicillin (24%), Ciprofloxacin (14%), Streptomycin (12%), Chloramphenicol (29%), Septrin (33%), Sulfamethoxazole-trimethoprim (29%), Aztreonam (64%), Ceftazidime (76%) and Cefuroxime (38%).

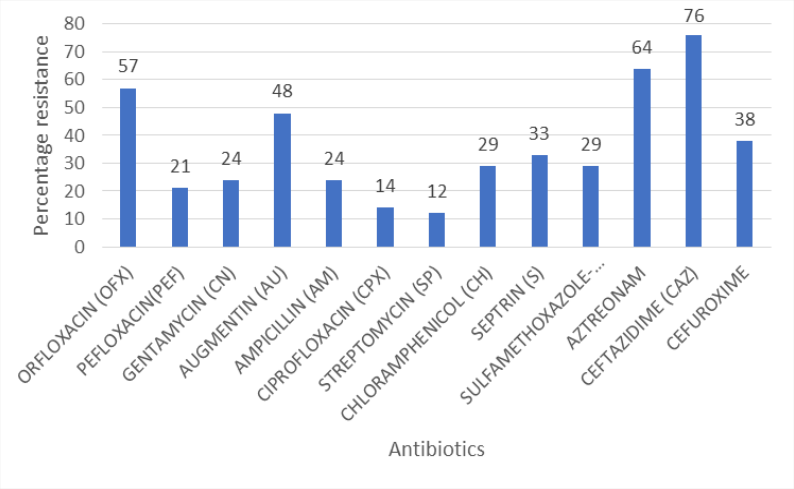



**Figure 2: Antibiogram of imipenem resistant bacterial isolates from Great Kwa River.**

## DISCUSSION

Imipenem belong to the carbapenems group of antibiotics which are used for treatment of infections caused by ESBL producing pathogens. In this study, out of a total of 89 non-duplicate isolates from Great Kwa river samples, 42(47.2%) showed resistance to imipenem judging from the diameter of the zones of inhibition and comparison with CLSI, 2005 standards. Resistance to imipenem among these gram negative isolates could be due to outer membrane impermeability, altered penicillin binding proteins, efflux pump mechanism and secretion of metallo-beta lactamases. In a study in India by Bashir *et al* (2011), 38 (13.42%) of *Pseudomonas* isolates from a total of 283 isolates were resistant to Imipenem; Hemlatha *et al.* (2005) also reported that 16% of *Pseudomonas* isolates tested, showed resistance to imipenem. Chika *et al*. (2014) reported 27% of the 99 isolates of the genera *Escherichia, Klebsiella* and *Pseudomonas* screened showed resistance to imipenem as well as showed MBL production. Similarly, a high resistance to imipenem was observed by Chika *et al.* (2016) among *Esherichia coli* and *Klebsiella species* isolates and also by Enwuru *et al*. (2011) who evaluated the occurrence of MBL producing *Esherichia coli* and *Klebsiella species* isolated from community and hospitalized subjects. A closely Similar result of 47% imipenem resistance was observed by Aghamiri *et al*. (2014). Also, Rajput *et al* (2010) stated 28% were resistant to imipenem. The differences in imipenem resistance recorded by the different researchers and in this study, can be attributed to the different rate of usage of carbapenems and antibiotics control policies in the geographical settings.

The imipenem resistant bacteria also showed resistance to other antibiotics (orfloxacin, pefloxacin, gentamycin, augmentin, ampicillin, ciprofloxacin, streptomycin,

chloramphenicol, septrin, aztreonam, ceftazidime and cefuroxime) commonly used for treatment of bacterial infections. The resistance was not just restricted to the beta lactam antibiotics but also to other classes of antibiotics including the fluoroquinolones and aminoglycosides. Kittinger *et al* (2016) also reported carbapenem resistant bacteria showing resistance to other antibiotics while Rose *et al* (2009) observed resistance mostly against the ampicillins. Similar to findings from this study, McDonnell and Treonis (2004) observed low resistance to ciprofloxacin and suggested that there may be no natural reservoir for the antibiotic. This multiple resistance phenotype can be attributed to the presence of plasmids carrying genes encoding resistance to different antibiotics (Magiorakos *et al*., 2012). The existence of genes encoding both beta-lactamases and aminoglycosidases (Kumarasamy *et al*., 2010) which confer multiple antibiotics resistance phenotype to the bacteria have been reported in previous studies. Apart from synthesis of these enzymes, other resistance mechanism such as the efflux pump mechanism can also mediate the multidrug profile of these isolates (Farinas and Martinez-Martinez, 2013).

The observation of multiple resistance to antibiotics among different genera of bacteria in this study shows widespread transfer of resistance genes through vertical and horizontal transfer mechanisms. This is further facilitated by the existence of mobile genetic elements which can be easily exchanged among diverse bacterial genera and families (Juan Nicolau and Oliver, 2010). The genetic transfer can also extend to environmental bacteria and human commensals and pathogens (Threedeach *et al* 2012; Edet *et al*., 2017). Various studies to determine the sources of the antibiotics resistant bacteria in natural water bodies have attributed it to be from livestock industry (Hagedorn *et al*., 1999), untreated waste discharge from hospital settings (Silbergeld *et al*., 2008) and anthropogenic activities like human excrement. According to McDonald *et al* (1997), the environmental bacteria may be a potential threat in the future than the clinical bacteria.

## CONCLUSION

Our findings show that imipenem resistant bacteria also exhibit multiple antibiotics resistant phenotype. The upsurge of imipenem resistant bacteria in various geographical settings can be linked to negligence and non-existence of control measures to check carbapenem usage, this calls for various local and international organizations to consistently and periodically conduct antibiotics surveillance studies to avoid useful antibiotics from being ineffective in the nearest future and to reduce indiscriminate use of antibiotics by the public and physicians.

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