Investigation of Virulence Factors and Effect of Chlorine and Sunlight on Carbapenems-Resistant *Enterobacteriaceae* from Water Samples

Busayo M. Olowe[1*], Olufunke Adelegan[2], Abisoye O. Ojo[3]
[1,]*Department of Microbiology, Faculty of Basic Medicals and Applied Sciences, Lead City University, Ibadan, Oyo State, Nigeria*
[2]*Department of Medical Microbiology and Parasitology, Afe Babalola University, Ado-Ekiti, Nigeria*
[3] *National Centre for Genetic Resources And Biotechnology (NACGRAB), Moor Plantation, Apata, Ibadan, Oyo State*

Abstract **-Carbapenems are the last line resort antibiotics in the fight against the diseases caused by Carbapenemase-Producing *Enterobacteriaceace*(CPE). The study aimed to investigate the presence of virulent factors in CPE *(Escherichia coli* and *Klebsiella pneumoniae)* and effect of sunlight and chlorine on them. Thirty water samples from streams and wells were collected and their microbial quality assessment was carried out following standard procedures. Antibiotic sensitivity testing of isolates, detection of virulence factors, OXA genes and plasmid and effect of chlorine and sunlight were carried out using standard techniques. Thirty-five isolates (*E. coli* (fifteen, 42.9%) and *K. pneumonia* (twenty, 57.1%) were recovered from the water samples and 28 (80%) demonstrated multiple antibiotics resistance (MAR). The MAR organisms were further subjected to carbapenems (meropenem and doripenem) and only 2 (33.3%) of *E. coli and* 4 (66.7%) of *K. pneumoniae* were resistant to these antibiotics. The detection of virulent factors showed that out of five carbapenem-resistant organisms (CRO) investigated, 2 (40%) possessed OXA gene of 320 bp and 4 (80%) contained plasmid of >1300bp. Also, number of colony forming unit of CRO reduced with exposure rate to sunlight and chlorine. The study showed that OXA gene and plasmid were detected in isolates and revealed health risk posed to clinical therapy. However, the study revealed that sunlight and chlorine at adequate exposure and concentration are very effective in treating water contaminated with CRO, thereby reducing the incidence of health risk in the community.**

Keywords- **Carbapenems-resistant *Enterobacteriacea*, Chlorine, Sunlight, Virulence Factors, Water Samples**

I. INTRODUCTION

The degradation of water quality from a public health or ecological point of view is a major concern in the nation[1, 2]. Readily available water sources such as surface and underground water are prune to contamination like sewage discharges or faecal loading by domestic or wild animals' defecation, malfunctioning of septic trenches, storm water drainage, municipal wastes and industrial effluents[3]. Globally, surface and underground water receive pollutants from faecal origin [4-6]. Consequently, several microorganisms (pathogenic microorganisms) are being released into the water bodies [7]. Several enteric microorganisms such as *Enterobacteriacea* among others are excreted in the faeces of infected individual or animals which may directly or indirectly contaminate drinking water[8]. *Enterobacteriaceae* naturally exit the body from the gut in feces, and are therefore ubiquitous in water and soil biomes, hence responsible for several infections such as urinary tract infections (UTI), bactereamia, intra-abdominal infections, and pneumonia[9, 10]. As a result, antibiotics are being administered to curb these infections but unfortunately the microorganisms that are responsible for the infections are becoming resistant to these commonly used antibiotics even the so called potent, last line resort antibiotic (carbapenems).

Many known genera within *Enterobacteriaceae* family exist. Among the clinically significant opportunistic pathogens include species of *Klebsiella* and *Escherichia*[11, 12]. Carbapenemase-producing *Enterobacteriaceae* were almost non-existent up to the 1990s, but are today encountered routinely in hospitals and other healthcare facilities in many countries they are almost always resistant to all β-lactams including carbapenems and many other classes [13]. This is as a result of possible mechanisms such as reduced permeability, increased efflux, β-lactamase production which the organisms adopt in resistance to these carbapenems[14-17]. Carbapenems are the most potent β-lactam agents against Gram-negative bacilli due to their substitution of the sulfur group from the penicillin-core with a carbon atom at carbon1, and a double bond between carbon2 and carbon3 instead of a single bond [18]. These structural modifications increase the affinity of carbapenems for penicillin-binding proteins rendering them less vulnerable to the wide spectrum of β-lactamases that hydrolyze other less potent β-lactam agents. Most β-lactam antibiotics work by inhibiting cell wall biosynthesis in the bacterial organism by interrupting peptidoglycan production in the bacterial cell wall[19]and are the most widely used group of antibiotics. Meropenem, imipenem, doripenem, and ertapenem are the carbapenems used in clinical settings. All members of carbapenems display a bactericidal effect on susceptible Gram-positive and Gram-negative bacteria by binding to and inactivating penicillin binding proteins (PBPs) [18, 20]. The β-lactam rings of carbapenems covalently bind to the active site of PBPs to prevent further interaction between the PBPs and the

peptidoglycan precursor. This covalent link renders the PBP inactive and consequently peptidoglycan synthesis ceases, and the bacteria dies as the cell wall is destabilized[21].

There are reports on presence of carbapenems-resistant organisms in water bodies[22-25] and the effect this has on therapy [25]. This development constitutes a serious global concern to the health sector. Therefore, this research investigated the virulence factors responsible for Carbapenems resistance in *Klebsiella pneumoniae* and *Escherichia coli* isolated from water sources and also proffered solution to the presence of *Klebsiella pneumoniae* and *Escherichia coli* in water sources by investigating the effect of chlorine and sunlight on these microorganisms present in water bodies.

II. MATERIALS AND METHODS

a. Sampling Site

Water samples were randomly collected from 30 different sites (10 streams and 20 wells) located around student environment in Ado/Iworoko area, Ado-Ekiti in July 2018. The description of the water bodies and activities around the water sample collection sites were documented.

b. Sample Collection

A total of 30 water samples from well water (n=10) and stream water (n=20) were collected using sterile bottles and were kept in an ice-pack box and taken to the laboratory for microbiological analyses. The parameters of activities around the collection sites were recorded.

c. Isolation of Escherichia coli and Klebsiella pneumoniae

The isolation of *Escherichia coli* and *Klebsiella pneumoniae* was done by using standard Pour-plate method. One ml of dilution 10^3 and 10^{-4} of each water sample was inoculated aseptically into molten Eosin Methylene Blue agar plate and MacConkay agar plate and swirled for even distribution of the inoculums and the plates were allowed to set, incubated at 37°C for 24hours. Thereafter, the plates were examined for growth and each distinct colony was picked and sub cultured to get a pure culture

d. Identification and Characterization of Isolates

Presumptive *Escherichia coli* showing greenish metallic sheen on EMB agar and Creamy mucoid *Klebsiella pneumoniae* on MacConkay agar were Gram-differentiated and biochemically identified according toCowan and Steel [26]. The biochemical tests carried out included Citrate utilization, Catalase test, Indole test, Voges Proskauer (VP) test and Sugar fermentation.

e. Antimicrobial Susceptibility Tests

Antibiotic susceptibility test was carried out on all the identified *Escherichia coli* and *Klebsiella pneumoniae* isolates using standard disc diffusion techniques as recommended by Clinical Laboratory Standards Institute [27]. Eight (8) antibiotics of different classes were chosen and tested based on their importance in treating human or animal infections. The antibiotics and concentration were as follow: *Aminoglycosides*: gentamycin (10µg), *Betalactam***:** augmentin (30µg), *Cephems*: Ceftazidime (5µg), Cefuroxime (30µg), Nitrofurantoin, *Fluroquinolones*: ciprofloxacin (10µg), ofloxacin (10µg) and *Penicillin***:** ampicillin (30µg). Set of identified isolates which showed multiple resistance to these antibiotics were further tested on carbapenems; meropenem and doripenem (Oxoid Ltd, Canada). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for carbapenemase disks inhibiting Enterobacteriaceae was used.

f. Investigation of Virulence Factors in Carbapenem-Resistant Organisms using PCR Techniques

A. DNA Extraction

DNA was extracted using the following procedures: pure colony of each organism grown on medium was transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. After which, cultures were centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mMTris-HCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 min. An equal volume of chloroform : isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 h. DNA was collected by centrifugation at 13000g for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer.

B. Polymerase Chain Reaction for the Molecular Detection of OXA Gene

PCR preparation cocktail consisted of 2.5 µl of 5x GoTaq green reaction buffer, 0.75 µl of 25mM MgCl2, 1 µl of 0.25 mM of dNTPs mix, 0.25 µl of 10 pmol each OXA-F 5'-ACCAAAGACGTGGATGCAAT -3' and – OXA-R, 5′-TGCACCAGTTTTCCCATACA -3′ primers and 0.06 µl of Taq DNA polymerase (Promega, USA) made up to 11 µl with sterile distilled water 2 µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 55°C for 30s and 72°C for 30 seconds; and a final termination at 72°C for 10 mins. and chilled at 4°C.

The integrity of the amplified gene fragment, about 320kbp, was checked on a 1.5% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5 % agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

C. Plasmid Isolation

Plasmids were isolated using the QIAGEN Plasmid Purification mini kit. The extracted plasmids were then gel electrophoresed as previously described.

g. Effect of Chlorine and Sunlight on Carbapenems-Resistant Organisms

A. Effect of Chlorine

Inoculums of each carbapenems-resistant organisms was prepared and 0.25mg/ml of chlorine was also prepared. Four different test tubes containing sterile 9ml tryptone broth were prepared and inoculated with 1ml of dilution 10^6 of the prepared innoculum, the inoculated broth was exposed to 0.1ml of the prepared chlorine for 30 secs, 60 secs, 90 secs and 120 secs respectively, 1ml from each test tube was plated on nutrient agar plate and incubated at 37^0C for 24 hours after which the number of colonies were counted.

B. Effect of Sunlight

This was performed on an intense sunny day. six test tubes containing 9ml tryptone broth was prepared and inoculated with 1ml of dilution 10^6 of the prepared inoculum, the inoculated broth was exposed for one hour, two hours, three hours, four hours, five hours and six hours respectively, 1ml was plated from each test tube on nutrient agar plate after each time interval and incubated at 37^0C for 24 hours after which, the number of colony was counted.

III. RESULTS AND DISCUSSION

The information depicted on Table 1 and 2 revealed the detailed description of the sampling sites. Different activities which could impact the water source were observed and are shown in the tables. Some of the observation made included the activities around the sampling site, distance of activities from the water bodies, presence of dumpsite around the place, the settings of the community and type of soil in the area. All these provided the sanitary information of the water samples. A total of 36 isolates on EMB and MacConkey agar was recovered from the samples. Table 3 showed the percentage occurrence of theses isolates from the water samples collected from Ado town. A total of 27 isolates from wells was recovered of which 11 (40.7%) were identified as *Escherichia coli* and 16 (59.2%) were *Klebsiella pneumoniae* while 9 isolates were recovered from streams of which 4 (44.4%) were identified as *Escherichia coli* and 5 (55.5%) were *Klebsiella pneumoniae.*

The results on susceptibility testing of isolates from water samples collected from student environment, Ado/Iworoko area, Ado-Ekiti are presented in Figure 1. It shows that *Klebsiellapneumonia* were more resistant to the antibiotics relatively compared with *Escherichiacoli*. It was also found that ofloxacin was very potent against these two organisms as little or no organisms showed resistance against these antibiotics. Figure 2 depicts the susceptibility testing of multiple resistant isolates to carbapenems (doripenem and meropenem) and it was shown larger percentage (78.6%) of the isolates were susceptible to doripenem but all the isolates were resistant to meropenem.

Figure 3 reveals the agarose gel electrophoresis of the amplified OXAgenes; of band size approximately 320bp; from five carbapenems-resistant bacteria isolates. Organisms *E. coli* 9, *K. pneumoniae* 5, *K. pneumoniae* 10, *K. pneumoniae* 12 and *K. pneumoniae* 16 were loaded in Lane 1,, 2, 3, 4, and 5 respectively and gel image indicates presence of the OXA gene (320bp) in only *E. coli* 9 and *K. pneumoniae* 10 isolates. While Figure 4 shows the plasmid profile of these carbapenems-resistant isolates loaded into Lane 1 to Lane 5 as in Figure 3, and it was found that all the carbapenems-resistant isolates carried plasmids in them except isolates in lane 4 (*K. pneumoniae* 12).

Tables 4 and 5 reveal the effect of chlorine and sunlight respectively on *Klebsiella pneumoniae* and *Escherichia coli.* It was observed that as the rate of exposure increases, the microbial count of *Klebsiella pneumoniae* and *Escherichia coli* reduces. That is, the microbial load reduces with the rate of exposure. Table 4 reveals that chlorine disinfection was effective at exposure rate of 120 secs while Table 5 shows that exposure rate of 6 hours is very effective in disinfecting water contaminated with these microorganisms.

Table I. Sanitary observations made at the Streams sampling sites located in Ado-Ekiti

Water Samples	Name of collection site	Activities around the place	Distance of activities from the stream	Presence of dumpsite close to the place?	Are people living around the streams?	Settings of the community	Use of the stream	Type of soil around the water
1	Odoejestream 1	Mechanic Workshop	40 ft	No	No	Rural	Washing	Sandy
2	Odoeje stream 2	Block Industry	20 ft	Yes	No	Rural	For making bricks and washing	Sandy
3	Odo-ofin	Car wash	15 ft	No	No	Rural	Washing	Sedimentary rock
4	Gofamint Church stream	Church	20ft	No	Yes	Urban	Construction	Sandy
5	TREM Stream	Farming	20ft	No	No	Rural	Construction	Loamy soil
6	Ureje stream	Mechanic, Meat selling	25 ft	Yes	Yes	Urban	Washing	Sandy soil
7	Bamgboye stream	Car wash	20 ft	Yes	Yes	Urban	Washing	Sandy soil
8	Ajebandele Stream	Dumpsite	15ft	Yes	Yes	Rural	Washing	Sandy soil
9	Nicky stream1	Car wash	20 ft	Yes	No	Rural	Washing	Sandy soil
10	Nicky stream 2	Mechanic	25ft	No	Yes	Rural	Washing	Sandy soil

The resistance of enteric microorganisms to commonly used antibiotics as a result of carbapenemase production is a global health threat as carbapenems are important antibiotics of last resort. In this study, *Escherichia coli* and *Klebsiella pneumoniae* were recovered from well and stream water samples. The occurrence of these organisms in drinking water sources affirms the sanitary observation (Table 1 and 2) made during sampling. This corroborates the findings of Aswani, et al. [28] and Kim [21] who also recovered these organisms from their work. The study also showed that resistance to commonly used antibiotics was noticed among the isolates. This is similar to the study by Ahmed, et al. [29] who reported 84.37% resistance to commonly used antibiotics. Also, resistance to meropenem and doripenem was observed among the multiple-resistant *Escherichiacoli* and *Klebsiellapneumoniae*. This resistance to doripenem and meropenem was reported by Negi, et al. [30]. The resistance observed in the organisms could probably be as a result of the three fundamental mechanisms of antimicrobial resistance which are enzymatic degradation of antibacterial drugs, alteration of bacterial proteins that are antimicrobial targets, and changes in membrane permeability to antibiotics [31].

Antibiotic resistance can be either plasmid mediated or maintained on the bacterial chromosome[32]. Beta-latam gene (OXA) and plasmids in few of the selected carbapenems-resistant isolates were detected. The presence of these genes and plasmids in the tested organisms affirmed their resistance to carbapenems. This could probably account for the resistance observed in the clinical therapy in health care sector. The carbapenems resistance observed in this study is similar to the findings byManoharan, et al. [33] who reported 17% resistance to carbapenems in *Enterobacteriaceae*. Kaur, et al. [20]also reported a considerable resistance of *E. coli* and *K. pneumoniae* to carbapenems drugs. The high resistance profile of bacterial organisms to meropenem as discovered in this study is also reported byLuyt, et al. [34]; Negi, et al. [30]; Bhatia, et al. [35]. The presence of carbapenems-resistant organisms in drinking water sources poses health risk to the users. And from this study, the investigation of chlorine and sunlight disinfection showed that these agents are effective against carbapenems-resistant organisms at adequate exposure and concentration.

IV CONCLUSION

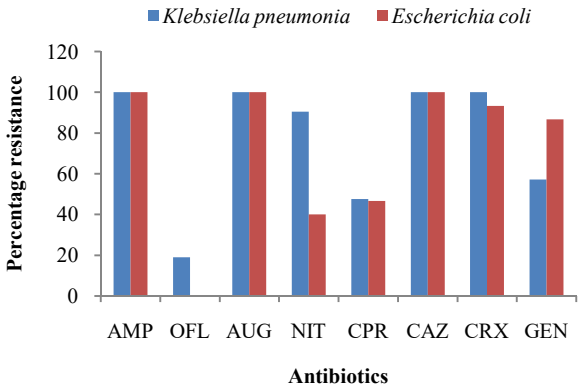
This study revealed the presence of carbapenem-resistant *E. coli* and *K. pnuemoniae*in drinking water samples in the study area. The presence of which pose high health risk to the communities who relied on this water samples for consumption or domestic use. However, findings from this study proffer the solution to this problem. It was found that sunlight and chlorine at adequate exposure and concentration are very effective in treating water contaminated with carbapenem-resistant *E. coli* and *K. pneumoniae* thereby reducing the incidence of health risk in the community.

Table II. Information on observation made at the well sampling sites

S/N	Site of collection	Construction: Poorly or properly?	Presence of cover?	Well depth	Specific fetcher? Yes/No	Water cloudy or transparent?	Water taste?	Septic tank close? If yes, how close?	Activitiees around the well
1	Rabex well	Properly	Yes	Not deep	No	Transparent	Tasteless	No	Student Hostel
2	Olugbenro hostel well	Properly	Yes	Very deep	Yes	Transparent	Tasteless	No	Student Hostel
3	Iyalaje well	Properly	Yes	Very deep	No	Transparent	Tasteless	No	Vegetation; shops
4	Canaan land well	Properly	No	Not deep	Yes	Transparent	Tasteless	No	Dumpsite
5	Firstday well	Properly	Yes	Not deep	No	Transparent	Tasteless	No	Student hostel
6	God is good well	Properly	Yes	Very deep	Yes	Transparent	Tasteless	No	Student hostel
7	New wine suite well	Properly	No	Very deep	No	Transparent	Tasteless	No	Student hostel
8	Ewedu well	Properly	Yes	Not deep	Yes	Transparent	Tasteless	No	Vegetation
9	Mercy domain well	Properly	Yes	Very deep	No	Transparent	Tasteless	Yes, 4ft far	Vegetation
10	Rehoboth well	Properly	Yes	Not deep	No	Transparent	Tasteless	No	Vegetation
11	Engineer China well	Properly	Yes	Very deep	No	Transparent	Tasteless	No	Student Hostel
12	Elite Castle well	Properly	Yes	Very deep	No	Transparent	Tasteless	Yes, 2ft far	Vegetation
13	MBT villa well	Properly	Yes	Very deep	Yes	Transparent	Tasteless	Yes	Student Hostel
14	Ekundayo well	Properly	No	Very deep	No	Transparent	Tasteless	Yes,7ft far	Student hostel
15	New Castle well	Properly	Yes	Very deep	No	Transparent	Tasteless	No	Vegetation
16	CBN well	Poorly	Yes	Not deep	No	Transparent	Tasteless	Yes	Student hostel
17	Flochris hall well	Properly	Yes	Deep	No	Transparent	Tasteless	Yes	Shops
18	Dave hostel well	Properly	No	Deep	No	Transparent	Tasteless	No	Dumpsite
19	Achievers lodge well	Properly	Yes	Very deep	Yes	Transparent	Tasteless	No	Dumpsite, vegetation
20	Mosunmayo hostel well	Properly	Yes	Not deep	No	Transparent	Tasteless	No	Refuse

Table III: Percentage occurrence of bacterial isolates from water samples collected from Ado/Iworoko Environ, Ado-Ekiti.

Bacterial Isolates	Wells (% occurrence)	Streams (% occurrence)
Klebsiella pneumoniae	16 (59.26)	5 (55.56)
Escherichia coli	11 (40.74)	4 (44.44)
Total	27 (100)	9 (100)



Key: AMP- Ampicillin; OFL- Ofloxacin; AUG – Augmentin; NIT- Nitrofurantoin; CPR- Ciprofloxacin; CAZ- Ceftazidime; CRX- Cefuroxim; GEN- Gentamicin

Fig. 1: Percentage resistance of bacterial isolates to commonly used antibiotics

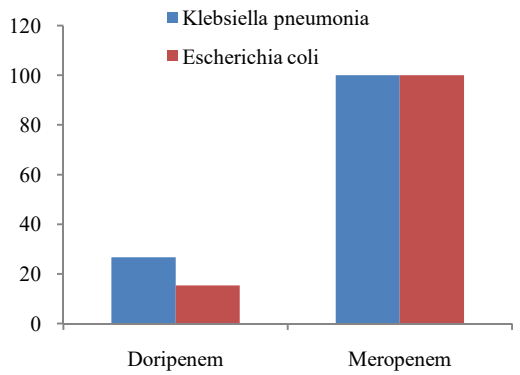
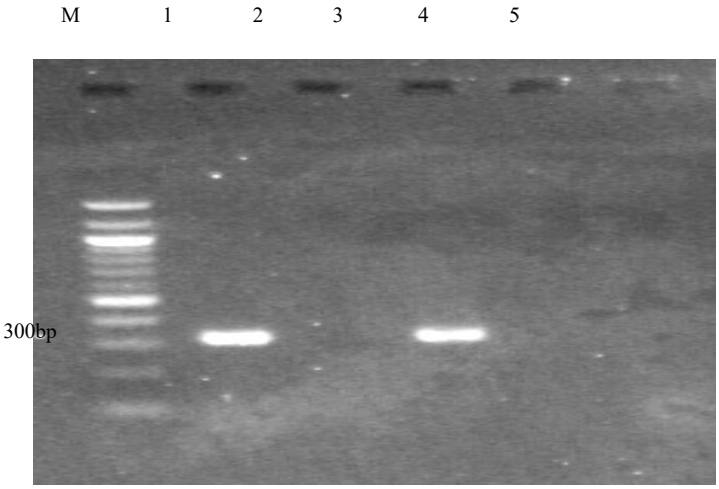
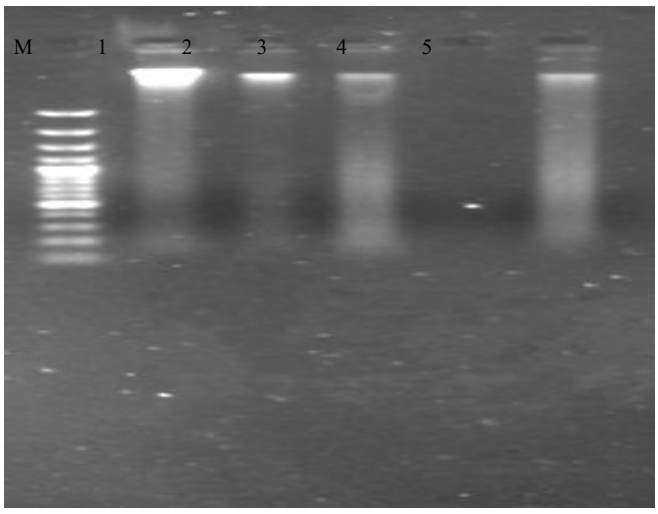


Figure 2: Percentage Resistance of Multiple Resistant Isolates to Carbapenems (doripenem and meropenem)



Lane 1- *E coli* 9; Lane 2- *K. pneumoniae* 5; Lane 3 - *K. pneumoniae* 10, Lane 4- *K. pneumoniae* 12 and Lane 5 - *K. pneumoniae* 16

Fig 3: Agarose gel electrophoresis of the amplified OXA genes from 5 carbapenems-resistant bacteria isolates (Band size approximately 320bp).



Lane 1- *E coli* 9; Lane 2- *K. pneumoniae* 5; Lane 3 - *K. pneumoniae* 10, Lane 4- *K. pneumoniae* 12 and Lane 5 - *K. pneumoniae* 16

Fig 4: Agarose gel electrophoresis of the extracted plasmid (>1300bp) from five carbapenems-resistant bacteria isolates.

Table IV: The effect of chlorine on *Klebsiella pneumoniae* and *Escherichia coli recovered from* water samples collected from Ado/Iworoko.

Exposure rate (seconds)	*Klebsiella pneumoniae* (log cfu/ml)	*Escherichia coli* (log cfu/ml)
No Chlorine (Control)	TNTC	2.56
0 secs	2.84	2.47
30 secs	2.53	2.39
60 secs	2.44	2.21
90 secs	2.17	2.06
120 secs	1.51	-

TNTC – Too numerous to count

Table 5: The effect of Sunlight on *Klebsiella pneumoniae* and*Escherichia coli recovered from* water samples collected from Ado/Iworoko.

Exposure rate to Sunlight (seconds)	*Klebsiella pneumoniae* (log cfu/ml)	*Escherichia coli* (log cfu/ml)
No exposure to Sunlight (control)	2.99	TNTC
1 hour	2.97	TNTC
2 hours	2.78	TNTC
3 hours	2.63	3.04
4 hours	2.59	2.94
5 hours	1.81	2.06
6 hours	0	0

TNTC – Too numerous to count

ACKNOWLEDGEMENT

The authors wish to thank Mr. Tope who supported the practical work in the laboratory and also the Management of Science Laboratory Technology Department, Federal Polytechnic, Ado-Ekiti, Nigeria for the permission granted to carry out our research work in their research laboratory

REFERENCES

[1] L. Dhaouadi, H. Besser, F. Wassar, and A. R. Alomrane, "Assessment of natural resources in tunisian Oases: degradation of irrigation water quality and continued overexploitation of groundwater," Euro-Mediterranean Journal for Environmental Integration, vol. 6, no. 1, pp. 1-13, 2021.

[2] J. O. Ighalo and A. G. Adeniyi, "A comprehensive review of water quality monitoring and assessment in Nigeria," Chemosphere, p. 127569, 2020.

[3] X. Jia, J. J. Klemeš, S. R. W. Alwi, and P. S. Varbanov, "Regional water resources assessment using water scarcity pinch analysis,"

Resources, Conservation and Recycling, vol. 157, p. 104749, 2020.
[4] L. Paruch and A. M. Paruch, "Cross-tracking of faecal pollution origins, macronutrients, pharmaceuticals and personal care products in rural and urban watercourses," Water Science and Technology, vol. 83, no. 3, pp. 610-621, 2021.
[5] N. Sasakova et al., "Pollution of surface and ground water by sources related to agricultural activities," Frontiers in Sustainable Food Systems, vol. 2, p. 42, 2018.
[6] P. Verlicchi and V. Grillini, "Surface water and groundwater quality in South Africa and mozambique—Analysis of the Most critical pollutants for drinking purposes and challenges in water treatment selection," Water, vol. 12, no. 1, p. 305, 2020.
[7] N. Pichel, M. Vivar, and M. Fuentes, "The problem of drinking water access: A review of disinfection technologies with an emphasis on solar treatment methods," Chemosphere, vol. 218, pp. 1014-1030, 2019.
[8] W. H. Organization, "A global overview of national regulations and standards for drinking-water quality," 2018.
[9] J. G. Holt, "The shorter Bergey's manual of determinative bacteriology," The shorter Bergey's manual of determinative bacteriology. 8th edition., 1977.
[10] I. Bashir et al., "Assessment of bacteriological quality of borehole water in Wamakko local government, Sokoto state, Nigeria," Novel Research in Microbiology Journal, vol. 2, no. 6, pp. 175-184, 2018.
[11] K. L. Wyres and K. E. Holt, "Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria," Current opinion in microbiology, vol. 45, pp. 131-139, 2018.
[12] S. Tissera and S. M. Lee, "Isolation of extended spectrum β-lactamase (ESBL) producing bacteria from urban surface waters in Malaysia," The Malaysian journal of medical sciences: MJMS, vol. 20, no. 3, p. 14, 2013.
[13] Y. Tiersma, "β-Lactamase-producing bacteria How can I resist you?," 2013.
[14] A. E. Kizny Gordon et al., "The hospital water environment as a reservoir for carbapenem-resistant organisms causing hospital-acquired infections—a systematic review of the literature," Clinical infectious diseases, vol. 64, no. 10, pp. 1435-1444, 2017.
[15] T. R. Walsh, J. Weeks, D. M. Livermore, and M. A. Toleman, "Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study," The Lancet infectious diseases, vol. 11, no. 5, pp. 355-362, 2011.
[16] S. Tofteland, U. Naseer, J. H. Lislevand, A. Sundsfjord, and Ø. Samuelsen, "A long-term low-frequency hospital outbreak of KPC-producing Klebsiella pneumoniae involving intergenus plasmid diffusion and a persisting environmental reservoir," PLoS One, vol. 8, no. 3, p. e59015, 2013.
[17] M. Fakruddin, R. M. Mazumdar, A. Chowdhury, and K. S. B. Mannan, "A preliminary study on virulence factors & antimicrobial resistance in extra-intestinal pathogenic Escherichia coli (ExPEC) in Bangladesh," The Indian journal of medical research, vol. 137, no. 5, p. 988, 2013.
[18] G. G. Zhanel et al., "Comparative review of the carbapenems," Drugs, vol. 67, no. 7, pp. 1027-1052, 2007.
[19] Y. Rui, W. Lu, S. Li, C. Cheng, J. Sun, and Q. Yang, "Integrons and insertion sequence common region 1 (ISCR1) of carbapenem-non-susceptible Gram-negative bacilli in fecal specimens from 5000 patients in southern China," International journal of antimicrobial agents, vol. 52, no. 5, pp. 571-576, 2018.
[20] M. Kaur, S. Gupte, and T. Kaur, "Detection of carbapenem resistant gram-negative bacteria in clinical isolates from a tertiary care hospital," J Bacteriol Mycol Open Access, vol. 2, no. 1, p. 00011, 2016.
[21] H. C. Kim, "Isolation of Carbapenemase Producing Enterobacteriaceae in the Greater Toronto Area's Sewage Treatment Plants and Surface Waters, and their Comparison to Clinical CPE from Toronto," 2016.
[22] T. R. Walsh, "Emerging carbapenemases: a global perspective," International journal of antimicrobial agents, vol. 36, pp. S8-S14, 2010.
[23] N. Woodford, D. W. Wareham, B. Guerra, and C. Teale, "Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making?," Journal of Antimicrobial Chemotherapy, vol. 69, no. 2, pp. 287-291, 2014.
[24] D. E. Harmon, O. A. Miranda, A. McCarley, M. Eshaghian, N. Carlson, and C. Ruiz, "Prevalence and characterization of carbapenem-resistant bacteria in water bodies in the Los Angeles–Southern California area," Microbiologyopen,vol. 8, no. 4, p. e00692, 2019.
[25] C. Kittinger et al., "Enterobacteriaceae isolated from the river Danube: antibiotic resistances, with a focus on the presence of ESBL and carbapenemases," PloS one, vol. 11, no. 11, p. e0165820, 2016.
[26] Cowan and Steel, Manual for the Identification of Medical Bacteria. Cambridge, U. K: Cambridge University Press, 1991.
[27] Clinical Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement (CLSI document M100- S24). Wayne, PA: Clinical and Laboratory Standards Institute, 2013.
[28] S. M. Aswani, U. Chandrashekar, K. Shivashankara, and B. Pruthvi, "Clinical profile of urinary tract infections in diabetics and non-diabetics," The Australasian medical journal, vol. 7, no. 1, p. 29, 2014.
[29] O. I. Ahmed, S. A. El-Hady, T. M. Ahmed, and I. Z. Ahmed, "Detection of bla SHV and bla CTX-M genes in ESBL producing Klebsiella pneumoniae isolated from Egyptian patients with suspected nosocomial infections," Egyptian Journal of Medical Human Genetics, vol. 14, no. 3, pp. 277–283-277–283, 2013.
[30] A. Negi, M. Anand, A. Singh, A. Kumar, C. Sahu, and K. N. Prasad, "Assessment of doripenem, meropenem, and imipenem against respiratory isolates of Pseudomonas aeroginosa in a tertiary care hospital of North India," Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine, vol. 21, no. 10, p. 703, 2017.
[31] D. J Wolter and P. D Lister, "Mechanisms of β-lactam resistance among Pseudomonas aeruginosa," Current pharmaceutical design, vol. 19, no. 2, pp. 209-222, 2013.
[32] J. D. Pitout and K. B. Laupland, "Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern," The Lancet infectious diseases, vol. 8, no. 3, pp. 159-166, 2008.
[33] A. Manoharan, K. Premalatha, S. Chatterjee, D. Mathai, and S. S. Group, "Correlation of TEM, SHV and CTX-M extended-spectrum beta lactamases among Enterobacteriaceae with their in vitro antimicrobial susceptibility," Indian journal of medical microbiology, vol. 29, no. 2, p. 161, 2011.
[34] C.-E. Luyt et al., "Imipenem, meropenem, or doripenem to treat patients with Pseudomonas aeruginosa ventilator-associated pneumonia," Antimicrobial agents and chemotherapy, vol. 58, no. 3, pp. 1372-1380, 2014.
M. Bhatia, P. S. Loomba, B. Mishra, and V. Dogra, "Comparative Evaluation of In-Vitro Doripenem Susceptibility with Other Carbapenem Antibiotics among Gram Negative Bacterial Isolates Obtained from VAP Patients in a Super-Speciality Hospital: A Pilot Study," International Journal of Medical Research & Health Sciences, vol. 6, n o. 4, pp. 36-41, 2017.