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Full Length Research Article

PREVALENCE OF CARBAPENEM RESISTANT *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* IN URINE SAMPLES OF PATIENTS ATTENDING SELECTED GENERAL HOSPITALS WITHIN KADUNA METROPOLIS

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ABSTRACT
Carbapenem-resistant Enterobacteriaceae(CRE) is a growing concern worldwide. The study was conducted to determine the prevalence of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* from urine of suspected urinary tract infected patients from (5) General Hospitals within Kaduna metropolis. Following the collection of (350) urine samples, *Escherichia coli* and *Klebsiella pneumoniae* were isolated, identified and characterized using selective media (Eosin methylene blue and Macconkey agar), biochemical test and microgene kit respectively. Antibiotic susceptibility test was carried out for all the isolates using Kirby-Bauer disc diffusion technique. The isolates that are resistant to morepenem and ertapenem were amplified to detect the resistance genes. The PCR amplified DNA products were examined for the common ESBL-encoding genes (bla TEM and bla SHV) and carbapenem resistance genes (blaKPC, blaNDM and blaVIM). In all the hospitals, female respondents recorded the highest prevalence (74%) than the male (25%). Among the study population, *Escherichia coli* was the most prevalent (28%) followed by *Klebsiella pneumoniae* (13%).Among the five age groups, (≤10, 11-20, 21-30, 31-40 and 41years and above), (31-40) recorded the highest prevalence (5.4%) of *Escherichia coli* and *Klebsiella pneumoniae*. Among the twelve antibiotics used, *Klebsiella pneumoniae* recorded the highest number of resistant drugs. On the prevalence of *Escherichia coli* and *Klebsiella pneumoniae* resistance to meropenem and ertapenem, (6.1%) was recorded for *Escherichia coli* and (6.5%) for *Klebsiella pneumoniae*, which is slightly higher when compared with the previous report of (5.2%) and (2.5%) for *Escherichia coli*, and (7.8%) and (5.5%) for *Klebsiella pneumoniae*. Following the examination of PCR Amplified DNA products, the ESBL genes (bla TEM and blaSHV) were detected while for the carbapenemase, only blaVIM gene was detected, with the absence of blaKPC and blaNDM genes. Detection of these genes constitutes an alarming threat, and have been the cause of country-wide epidemics of healthcare associated infections. There should be regular and strict monitoring of Carbapenem resistance among the Enterobacteriaceae from time to time in all general and teaching hospitals, as to curtail the rising incidence along with the public health burden associated with these resistance genes.

Keywords: Carbapenemase, *Escherichia coli*, *Klebsiella pneumoniae*, Kaduna, Resistance

INTRODUCTION
Urinary tract infections (UTIs) are one of the most common infectious diseases ranking next to upper respiratory tract infection (Rubab *et al*., 2017). It is often associated with significant morbidity and mortality (Melaku *et al*., 2012). UTIs are primarily caused by Gram-negative bacteria, but Gram-positive pathogens may also be involved. More than 95% of uncomplicated UTIs are monobacterial (Sobel 2014). The most common pathogen for uncomplicated UTIs is *Escherichia coli* (75%–95%), followed by *Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis*, group B Streptococci, and *Proteus mirabilis*(Grude *et al*., 2001).However, *E. coli* can cause both complicated and uncomplicated UTIs (Sobel, 2014). Niranjan *et al.* (2018) reported a prevalence of (44%) among Gram negative Enterobacteriaceae, associated with UTI. However, Matar *et al.* (2008) and Matar *et al.* (2010), who in a study conducted among carbapenem resistant isolates recorded a prevalence of (2.5%) for *E. coli* and (7.8%) for *K. pneumoniae.* Oshun & Ogunsola (2012) reported prevalence of (5.2%) at the Lagos University Teaching Hospital, Lagos, Nigeria, while Ogbolu & Webber (2014) recorded a lower prevalence of (5.5%)from Tertiary Hospitals in Nigeria.

In recent years, the global spread of extended spectrum beta-lactamase (ESBL)-producing *E. coli* such as CTX-M-15 has emerged as a significant cause of community-associated UTIs (Sobel, 2014; CDC, 2017). Highly antibiotic- resistant uropathogens, including AmpC β-lactamase- or carbapenemase-producing Enterobacteriaceae (e.g., New Delhi metallo-β-lactamase (NDM) and *Acinetobacter* spp., are increasingly being reported among health care associated complicated UTIs (Sobel, 2014). Carbapenem-resistant Enterobacteriaceae (CRE) is a growing concern worldwide (Nordmann 2011). An isolate is considered a CRE if it's resistant to imipenem, meropenem, doripenem, or ertapenem by susceptibility testing or if it is identified to have a carbapenemase gene (CDC, 2017). The CDC is tracking CRE types such as *K. Pneumoniae* carbapenemase (KPC), NDM, IMP-1, and OXA β-lactamases. Among these, KPC is the most prevalent type in the United States, and NDM is the most antibiotic resistant type, often resistant to new cephalosporin/β-lactamase inhibitor combinations (Yusuf *et al.,* 2015; CDC, 2017).

Globally, multidrug resistance has reached an alarming stage and WHO has described it as one of the three most serious problems confronting human health (WHO, 2014). In Nigeria there have been reports of carbapenemase producing clinical isolates of enteric bacteria particularly among *E. coli* and *Klebsiella* sp (Akinduti *et al*.,

2012). Mathers *et al.* (2013) reported two cases of orthotopic liver transplant recipients that died as a result of infections caused by KPC-producing *K. pneumoniae.*

Carbapenems are a class of β-lactam antibiotic and exhibit a bactericidal activity. They act by binding to penicillin binding proteins (PBP), thus preventing the linking of peptidoglycan strands and further synthesis of the bacterial cell wall (Kimbery, 2008). They are known to be most effective against Gram negative bacterial infections (Freeman *et al*., 2015).

Carbapenem in combination with other agents, remain a mainstay of therapy in patients with serious hospital acquired infections. The introduction of carbapenem into clinical practice represents a great advancement for the treatment of β-lactam resistant bacteria. This is due to their broad spectrum of activity and stability to hydrolysis by most β-lactamases, the carbapenem have been the drug of choice for treatment of infections caused by penicillin or cephalosporin resistant Gram negative bacilli (Jesudason *et al*., 2009). However, Bacterial resistance to carbapenems is due to the production of carbapenem hydrolyzing enzymes called carbapenemases, and have the potential to spread rapidly across continents (Maletis *et al.,*2012). Carbapenem resistance is produced via 3 mechanisms, namely: reduced permeability, efflux, and synthesis of carbapenem β-lactamases (James, 2008; Evan *et al*., 2012).

The aim of this study was to determine the prevalence of Carbapenem resistant *E. coli* and *K. pneumoniae* in urine samples from patients suspected with urinary tract infection from selected general hospital within Kaduna metropolis.

MATERIALS AND METHODS

Study Area.

The study was conducted in Kaduna metropolis, Kaduna State, located in North Western Nigeria at latitude 10°31'35.1" N longitude 7°26'19.6" E (Ashafa, 2004).Its metropolis comprises of Kaduna North, Kaduna South and some part of Chikum and Igabi local Government. Selected Hospitals are; General Hospital Kawo, Yusuf Dantsoho Memorial Hospital Tudun Wada, Barau Dikko Teaching Hospital Kaduna State University (KASU), Gwamna Awan General Hospital Kakuri and General Hospital Sabon Tasha.

Sample Size

Using the Cochran's formula, $\boldsymbol{n_0} = \frac{\boldsymbol{z^2pq}}{\boldsymbol{e^2}}$(Cochran, 1977). Where, n_o is the sample size, *z* is the selected critical value of desired confidence level, *p* is the estimated proportion of an attribute that is present in the population, *q* = 1 – *p* and *e* is the desired level of precision. The sample of the study was determined using the prevalence rate of carbapenem resistance of 9.3% (Motayo *et. Al*., 2013). Sample size of (196) was calculated, but was rounded up (350) samples

p =0.5
q = 1-0.5 = 0.5 e = 0.05 z = 1.96, and
n = (1,96)

Inclusion Criteria

Patients sent to the Microbiology laboratory with a suspected cases of Urinary Tract Infection were included in this study.

Exclusion Criteria

Patients sent to the Microbiology laboratory for suspected cases other than Urinary Tract Infection and Patients that did not want to participate were excluded.

Ethical Approval

Ethical approval was obtained from the Ethical Committee, Kaduna State Ministry of Health and Human Services, and Ethical Committee of Barau Dikko Teaching Hospital Kaduna State University.

Administration of Questionnaire

Semi-structured questionnaires were administered to the patients that fulfilled the inclusion criteria, to capture their demographic information, such as sex, age, sexual contact and medical history.

Sample Collection and Processing

A total of 350 urine samples were collected from patients sent to Microbiology laboratory of the selected hospitals within Kaduna metropolis. All the samples collected were processed according to standard operating procedure. The samples were inoculated on selective and differential agar and incubated overnight at 37ºC.

Cultural and Microscopic Characteristics

Isolates were identified by their morphological characteristics on MacConkey agar and Eosin Methylene blue agar. Pink mucoid colonies and flat dry circle pink colonies on MacConkey and colonies with green metallic sheen on Eosin methylene blue agar after incubation at 37^0C for 24hours were processed for Gram staining.

Gram Staining

Thin Smears were prepared from a 24h culture, air-dried and heat-fixed by passing the slide over a Bunsen flame for three quick successions. The smears were Gram stained and examined microscopically under oil immersion objective lens following the addition of oil immersion (Cowans & Still, 2004).

Biochemical test

For further reconfirmation, suspected colonies of *E. coli* and *K. pneumoniae* were subjected to a number of biochemical tests which includes; Indole test, Methyl Red - Voges-Proskauer test, citrate utilizations test, urease test, Catalase test, motility test, hydrogen Sulphide Production along with carbohydrate fermentation test (Glucose, Lactose, Sucrose, and Mannitol fermentation) (Cheesbrough, 2000).

Antibiotic Susceptibility test

Biochemically characterized isolates of *E. coli* and *K. pneumonia* were standardized by inoculating the isolates into a nutrient broth. The broth was incubated at 37°C until the visible turbidity matches that of 0.5 McFarland standard. Using the modified Kirby-Bauer disc diffusion technique, the standardized isolates were inoculated in an already prepared Mueller Hinton agar, and the sensitivity disc were gently placed on the agar surface. The antibiotic discs used were Meropenem (10ug), Artapenem (10ug), Ciprofloxacin (30ug), Septrin(30ug), Chloropenicol (30ug), Gentamycin (30ug), Streptomycin (30ug), Pefloxacin (30ug), Augmentin (10ug), Amoxicillin (30ug), Sparfloxacin (10ug) and Tarivid (10ug) (CLSI, 2009). Isolates that are resistant to Meropenem and Ertapenem were investigated to detect for the resistance gene using Multiplex

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PCR.

Molecular Detection of Resistance Genes
Genomic DNA extraction
An overnight suspension of the bacterial isolateswas prepared in a sterile 2.0mL ependorf tube containing 1.8 mL of brain heart infusion broth. The tubes were centrifuged at 14000 rpm for 1 minute. The supernatant was discarded and the cells were resuspended in 300 uL of nuclease free water. DNA was extracted from the suspension using qiagen DNA extraction kit (QIAamp DNA minikit of 50), following the manufacturer's instructions.

Primers
Table 1: Primer sets for amplification of carbapenemase and extended spectrum betalactamase genes

Gene	Primer sequence (5// 3//)	Amplicons size (bp)	References
blaVIM	F- GATGGTGTTTGGTCGCATA R- CGAATGCGCAGCACCAG	390	(Poirel *et al.,* 2011)
blaNDM	F- GGTTTGGCGATCTGGTTTTC R- CGGAATGGCTCATCACGAT	621	(Poirel *et al.,* 2011)
blaKPC	F- CGTCTAGTTCTGCTGTCTTG R- CTTGTCATCCTTAGGCG	798	(Poirel *et al.,* 2011)
blaTEM	F- TCCAACATTTTGTCGTCG R- CTGACAGTTACCAATGCTTA	293	(Schlesinger *et al.,* 2005)
blaSHV	F- TTTATCGGCCTTCACTCAAGG R- GCTGCGGGGCCGGATAACG	403	(Schlesinger *et al.,* 2005)

Key:
VIM: Verona integron-encoded metallo-β-lactamase, NDM: New Delhi metallo-β-lactamase, KPC: *Klebsiellapneumoniae* carbapenemase, TEM-1, Temoniera-1; SHV -1, sulphydry1 variable-1

Multiplex Polymerase Chain Reaction (PCR).
The primers were used to amplify internal fragments with sizes from 232 to 798 bp, as presented in Table 1.For easy interpretation, to avoid similar-in-size amplicons for a given PCR tube, and to maintain clinical relevance of the screening (combining together the most widely distributed genes in clinical isolates), 2 multiplex reactions were defined, with no. 1 including detection of TEM and SHV with the expected amplicon size of 293-bp and 403-bp respectively. The second multiplex PCR including detection of blaVIM, blaNDM, blaKPC with expected amplicon size of 390-bp, 621-bp and 798-bp respectively. Amplification was carried out with the following thermal cycling conditions: 10 min at 94°C and 36 cycles of amplification consisting of 30 s at 94°C, 40 s at 52°C, and 50 s at 72°C, with 5 min at 72°C for The final extension (Poirel *et al*., 2011).

Agarose Gel electrophoresis
Amplified products were analyzed by electrophoresis in a 2% agarose gel at 100 V for 60 min 1× TAE (40 mmol/L Tris–HCl [pH8.3], 2 mmol/L acetate, 1 mmol/L EDTA) containing 0.05 mg/L ethidium bromide. A 100-bp from Dongsheng Biotech, China was used as DNA ladder. Following electrophoresis, the gel was transferred to a Biorad- gel documentation device and viewed using the U.V Trans illuminator (Poirel *et. Al.*, 2011).

Statistical Analysis
Results obtained were presented in charts, tables, and graphs where applicable. The result was further analyzed statistically using χ^2 at 95% confidence interval.

RESULT
Table 2 shows the occurrences of *Escherichia coli* and *Klebsiella pneumoniae* isolates from the urine samples. Out of 350 subjects analysed, 96 *Escherichia coli* and 46 *Klebsiella pneumoniae* were isolated.

Table 2: Occurrences of *Escherichiacoli* and *Klebsiella pneumoniae* in urine Samples collected from (5) General hospital within Kaduna metropolis

Hospitals	*E. coli* (%)		*K. Pneumoniae* (%)		Total	(Male and female) (%)
	Male	Female	Male	Female	*E. coli*	*K. Pneumoniae*
GHK	3(3.06)	13 (13.26)	1(2.17)	9(19.56)	16(16.56)	10(21.73)
BDTH	9(9.18)	16(16.32)	3(6.52)	6(13.04)	25(25.50)	9(19.56)
YDMH	6(6.12)	15(15.30)	1(2.17)	4(8.69)	21(21.42)	5(10.86)
GAGH	7(7.14)	11(11.22)	2(4.35)	10(21.74)	18(18.36)	12(26.09)
GHST	4(4.08)	14(14.28)	1(2.17)	9(19.56)	18(18.36)	10(21.73)
Total	29(29.6%)	69(70.4%)	8(17.3%)	38(82.6%)	98(100%)	46(100%)

Key:
GHK General Hospital Kawo, BDTH: Barau Dikko Teaching Hospital, YMDH:
Yusuf Dantsoho Memorial Hospital, GAGH: General Hospital Sabon Tasha**.**

Table 3 shows the percentage distribution of *Escherichia coli* and *Klebsiella pneumoniae* among male and female patients from the selected General Hospitals within Kaduna metropolis. Out of 98 *Escherichia coli* isolated 29 where from male patients and 69 from female patients, while *Klebsiella pneumoniae* out of 46, 8 for male patients and 38 for female patients.

Table 3: Distribution of *E. coli* and *K. pneumoniae* among Male and female patients from selected general hospitals within Kaduna metropolis

Hospital	***Escherichia coli***	***Klebsiella pneumoniae***
General Hospital Kawo	16(4.5%)	10(2.8%)
Barau Dikko Teaching Hospital	25(7.1%)	09(2.5%)
Yusuf Dantsoho Memorial Hospital	21(6.0%)	05(1.4%)
Gwamna Awan General Hospital	18(5.1%)	12(3.4%)
General Hospital Sabon Tasha	18(5.1%)	10(2.8%)
Total	98(28.0%)	46(13.1%)

Table 4 shows the distribution of *Escherichia coli* and *Klebsiella pneumoniae* among male and female patients of different age groups.

Table 4: Distribution of *E coli* and *K. pneumoniae* among Male and Female of different age groups

Age	*E. coli*		*K. pneumoniae*		Total (male and female)	*E. coli K. pneumoniae*
	Male	Female	Male	Female		
	0	0	0	0	0	0
11-20	3	8	0	2	11	2
21-30	4	13	1	7	17	9
31-40	11	28	0	19	39	19
41-above	10	21	5	11	31	16

Table 5 shows the antimicrobial susceptibility patterns of Escherichia *coli* from urine samples of patients in selected general hospital within Kaduna metropolis. The various zone of inhibitions were measured and compared with the new standard guidelines of clinical and laboratory standards institute (CLSI) 2018. Meropenem and ertapenem having the highest sensitivity profile of *Escherichia coli* 95.92% and 97.96%.

Table 5: Antimicrobial susceptibility profile of *E. coli* from urine samples of patients in selected General Hospital within Kaduna metropolis

Antibiotics (ug)	% Susceptibility of *Escherichia coli* (n=98)	
	Resistant	Susceptible
MEM (10)	4 (4.08)	94 (95.92)
ETP (10)	2 (2.04)	96 (97.96)
SXT (30)	29 (29.59)	69 (70.41)
CH (30)	72 (73.47)	26 (26.53)
SP (10)	56 (57.14)	42 (42.86)
CPX (30)	81 (82.65)	17 (17.35)
AM (30)	66 (67.35)	32 (32.65)
AU (10)	59 (60.20)	39 (39.80)
CN (30)	42.(4286)	56 (57.14)
PEF (30)	81 (82.65)	17 (17.35)
OFX (30)	54 (55.10)	44 (44.90)
S (30)	30 (30.61)	68 (69.399)

Key:
MEM= Meropenem, ETP= Ertapenem, SXT= Septrin, CH=Chloramphenicol, SP= Sparfloxacin, OFX= Tarivid, S= Streptomycin.

Table 6 shows the antimicrobial susceptibility patterns of *Klebsiella pneumonia* from urine samples of patients in selected general hospital within Kaduna metropolis. The various zone of inhibitions were measured and compared with the new standard guidelines of clinical and laboratory standards institute (CLSI) 2018. Meropenem and ertapenem having the highest sensitivity profile of Klebsiella *pneumoniae* with 97.83% and 95.65%.

Table 6: Antimicrobial susceptibility profile of *K*. pneumoniae from urine samples of patients in selected General Hospitals within Kaduna metropolis.

Antibiotics (ug)	% Susceptibility of *Klebsiella pneumoniae* (n=46)	
	Resident	Susceptible
MEM (10)	1 (2.17)	45 (97.83)
ETP (10)	2 (4.35)	44 (95.65)
SXT (30)	39 (84.78)	7 (15.22)
CH (30)	33 (71.74)	13 (28.26)
SP (10)	34 (73.91)	12 (26.09)
CPX (30)	26 (56.52)	20 (43.48)
AM (30)	37 (80.43)	9 (19.57)
AU (10)	34 (73.91)	12 (26.09)
CN (30)	28 (60.87)	18 (39.13)
PEF (30)	30 (65.22)	16 (34.78)
OFX (30)	29 (63.04)	17 (36.96)
S (30)	19 (41.30)	27 (58.70)

Key:
MEM= Meropenem, ETP= Ertapenem, SXT= Septrin, CH=Chloramphenicol, SP= Sparfloxacin, OFX= Tarivid, S= Streptomycin.

Table 7 shows the prevalence of meropenem and ertapenem resistant *E.coli* and *Klebsiella pneumoniae* from the sample analysed.

Table 7: Prevalence of Meropenem and Ertapenem resistant *E. coli* and *K. pneumoniae* from the Samples Analysed.

Bacteria	Meropenem and Ertapenem resistance	Meropenem and Ertepenem Susceptibility
Esherichiacoli (n=98)	6 (6.12)	92 (93.88)
Klebsiellapneumoniae (n=46)	3 (6.52)	43 (93.48)

Plate 1 shows the agarose gel electrophoresis detection of the resistant gene blaKPC, blaNDM and blaVIM from the resistant isolates. The lane M is the DNA marker leader of 100bp, lane 2 to 7 shows the wells of multiplex PCR products and lane 8 shows the negative control.

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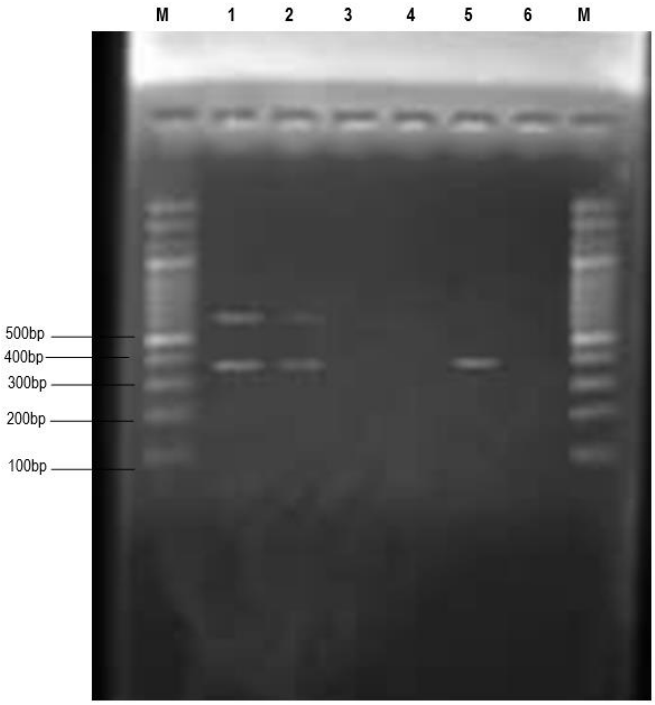

Plate 1: Agarose gel electrophoresis of blaKPC, blaNDM, and blaVIM (multiplex PCR) of the resistant genes of bacteria recovered from urine samples analysed. Where well M (DNA marker, 100bp, Biolabs New England)), 1,2,3,4 and 5 are sample Wells and 6 Negative control.

Plate 2 shows the agarose gel electrophoresis detection of the resistant gene blaTEM and blaSHV from the resistant isolates. The lane M is the molecular marker leader of 100bp, lane 2-6 sample wells and 7 negative control.

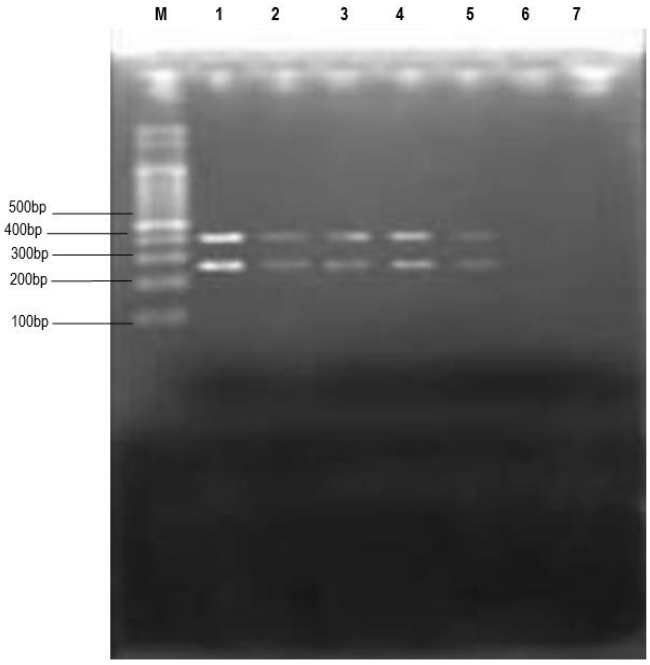

Plate2: Agarose gel electrophoresis of blaTEM and blaSHV (multiplex PCR) of the resistant gene of the bacteria recovered from urine samples analysed. Well M (DNA marker (100bp, Biolabs New England), 1,2,3,4,5 and 6 are the sample wells and well 7, Negative control.

DISCUSSION

Out of the 350 samples collected, 144(41%) positive samples were recorded, with *E. coli* having 98 (28%) and *K. pneumoniae* having 46 (13%), making *E. coli* the most prevalent organism among all the entire study population. From all the hospitals, Barau Dikko Teaching Hospital recorded the highest number of positive samples 34(9%), while General Hospital Kawo and Yusuf Dantsoho Memorial Hospital recorded the lowest number of negative samples 26 (7%).

However, from all the hospitals, Barau Dikko Teaching Hospital and Gwamna Awan General Hospital had the highest occurrence of *E. coli* 25 (7%),and *K. Pneumoniae* 12 (3%)respectively. General Hospital Kawo and Yusuf Dantsoho Memorial Hospital had the lowest occurrence of *E. coli* 16 (4%), and *K. pneumoniae* 05(1%)respectively (Table 1).

The higher number of *E. coli* (98) and *K. pneumoniae* (46) recorded in the urine samples analysed agrees with the report of May *et al.* (2015) who stated that *E. coli* and *K. pneumoniae* are the most frequent Enterobacteriaceae associated with urinary tract infections.

The result is similar with the findings of Aswani *et al.* (2014) who also reported a varying occurrence of different isolates among different study area in Nigeria. However, the variations in the number of positive samples, along with the variation in the isolates occurrence from among the different hospitals might be due to the fact that a number of species of Enterobacteriaceae are responsible for urinary tract infections (Niranjan *et al.,* 2018; Mohamed *et al*., 2019).

Niranjan *et al.* (2018) and May *et al*. (2015) also reported a higher occurrence of *E. coli* and *K. pneumoniae* among females, compared to their male counterparts. The higher occurrence of these isolates among females as presented in (Table 2) might be attributed to; the organisms easy passage to the bladder, their shorter urethra compared to their male counterparts along with sexual contact, which may cause UTI, as the bacteria can be pushed into their urethra. However, Niranjan *et al.* (2018) reported a prevalence of (44%) among Gram negative Enterobacteriaceae, which is a bit higher than the prevalence reported for *E. coli* (29.6%) and lower than that reported for *K. pneumoniae* (70%) in this study. The high number of these isolates among patients of different age groups (≤10, 11-20, 21-30, 31-40 and 41years and above), presented with UTI (Table 3), can be attributed to the fact that *E. coli* and *K. pneumoniae* are the commonest isolates implicated among patients with (UTI) (Melaku *et al.,* 2012; Sharma & Paul, 2012). The high occurrence of the isolates among females, is in line with the findings of May *et al*. (2015) who reported that females, compared to their male counterparts experience much occurrence of urinary tract infections (UTI's).

Out of a total number of (98) *E. coli* isolates tested against various antibiotics (Table 4), meropenem, ertapenem, septrin, gentamycin, and streptomycin recorded a higher number of susceptible isolates, while chloramphenicol, sparfloxacin, ciprofloxacin, amoxicillin, Augmentin, pefloxacin, and tarivid have a higher number of resistant isolates.

Among the *K. pneumoniae,* out of total of (46) positive isolates

tested each among the various antibiotics (Table 5), a higher number of resistant isolates were recorded among; septrin, chloramphenicol, sparfloxacin, ciprofloxacin, amoxicillin, Augmentin, gentamycin, pefloxacin, and tarivid antibiotics, while meropenem, ertapenem and streptomycin recorded a higher number of susceptible isolates. The high level of susceptibility demonstrated to (chloramphenicol, sparfloxacin, ciprofloxacin, amoxicillin, Augmentin, pefloxacin, and tarivid, in this study agrees with the study of Chikwendu *et al.* (2010) Osundiya *et al.* (2013).

Among the twelve different antibiotics tested against the two isolates (Table 4 and 5), *E. coli* recorded the highest number of drugs (5 antibiotics) been susceptible to the isolates with *K. pneumoniae* having the least (3 antibiotics). Furthermore, *K. pneumoniae* recorded the highest number of drugs (9 antibiotics) been resistant to the isolates with *E. coli* having the least (7 antibiotics). The result is in line with the findings of Eshetie *et al.* (2015), who reported that the emergences of multi drug resistance among enterobacteriaceae were mainly due to the production of enzymes, such as carbapenemases and that this carbapenemase production is one of the main mechanisms in the occurrence of drug resistance in the family of Enterobacteriaceae.

On the prevalence rate of meropenem and ertapenem among *E. coli* isolates (Table 6), out of a total number of (98), higher number of susceptible isolates were recorded with a prevalence rate of (93.8%) representing (92) samples and a lower prevalence rate on the resistant isolates having (6.1%) representing (6) isolates.

However, on the prevalence of meropenem and ertapenem among *K. pneumoniae* (Table 6), out of a total number of (46) samples, (n=46), higher number of susceptible isolates were recorded, with a prevalence rate of (93%) representing (43) isolates and a lower prevalence rate on the resistant isolates, having (6.5%) representing (3) isolates. The prevalence rate of meropenem and ertapenem resistant *E. coli* and *K. pneumoniae* having (6%) and (6.5%), is slightly higher when compared with the report of Ogbolu & Webber (2014) who recorded a prevalence rate of (5.5%).

However, the prevalence of carbapenems recorded in this study varies with the findings of Matar *et al.* (2010), who in a study conducted among carbapenem resistant isolates recorded a prevalence of (2.5%) for *E. coli* and (7.8%) for *K. pneumoniae.* Similarly, the results also vary with the findings of Oshun & Ogunsola (2012), who reported a carbapenem prevalence rate of (5.2%), and Eshetie *et al.* (2015) reported a higher prevalence of (61.2%) for *E. coli* and a lower prevalence of (15.8%) for *K. pneumoniae.* Similar variation was also observed upon comparison with the findings of Niranjan *et al.* (2018) who reported a higher prevalence of (49.8%) and (37.4%) for both *E. coli* and *K. pneumoniae* respectively.

This shows that the carbapenemase resistance is on rapid increase in Nigeria, and this may be as a result of unawareness of their occurrence, effects but only the consequences is being felt as a number of treatment failures have been reported when antibiotics such as cephalosporins, penicillins, quinolones are used (Yusuf *et al.,* 2012).

Currently, the increased burden of Multidrug resistant Enterobacteriaceae causing (UTI) compounded by harboring carbapenem resistance genes mainly among *E. coli* and *K. pneumoniae* have become a serious threat to public health, as they are associated with high mortality rates and have the potential to spread widely (Matar *et al.,* 2010). Carbapenem such as imipenem, meropenem, ertapenem, and doripenem are considered as the last resort antibiotics to treat ESBL producing Enterobacteriaceae (Habte *et al.,* 2009).

However, the high level usage of carbapenems both as prescribes by clinicians, along with their indiscriminately use, may have contributed to this higher prevalence rate of resistance recorded in Kaduna State general hospitals (Codjoe, 2016). *Klebsiella pneumoniae* carbapenemase production is an important mechanism of resistance for an increasingly wide range of Gram-negative bacteria and is no longer limited to *K. pneumoniae* (Hussaini *et al.,* 2017).

At present, infections caused by Carbapenamases producing Enterobacteriaceae (CPE) are difficult, and in some cases impossible to treat and have been associated with mortality rates up to (50%) (Eshetie *et al.,* 2015). Due to the movement of patients throughout the health care system, if (CPE) is a problem in one facility, then typically, they are a problem in other facilities in a State as well. Carbapenemase producing enterobacteriaceae are mostly endemic in specific geographical regions, but reports of their rapid spread into other geographical locations are indeed a point of grave concern these days (Nordmann *et al.,* 2011).

The PCR Amplified DNA products were examined for the common ESBL encoding genes (bla TEM and blaSHV) and carbapenem resistance genes (blaKPC, blaNDM and blaVIM). However, all the ESBL genes were detected while for the carbapenemase, only blaVIM was detected, with the absence of blaKPC and blaNDM genes.

The presence of bla-VIM, bla TEM (Plate 1) and blaSHV genes (Plate 2), among *K. pneumoniae* and *E. coli* corresponds with the findings of Carrer *et al.* (2010), Lopez *et al.* (2011), and Yusuf *et al.* (2015), who reported the presence of these genes among hospitalized patients, and other clinical isolates, but varies with the findings of Mohamed *et al.* (2019), who reported the presence of blaKPC and blaNDM genes among the isolates. The absence of any active control plans to curtail the rising spread of carbapenems resistance in Nigeria shows that attentions are not been paid to these problems, which is the likely reason for the low level of awareness among the medical practitioners in the country (Yusuf *et al.,* 2015).

The detection of ESBL and carbapenem resistant genes among *E. coli* and *K. pneumoniae* have been reported in Nigeria, and it constitutes an alarming threat (Yusuf *et al.,*2011*,* Yusuf *et al.,*2015).

In Nigeria, there is report of phenotypic detection of carbapenem resistance among clinical isolates in some hospitals in Kano, North west Nigeria (Yusuf *et al*., in 2011). Nevertheless, carbapenemase-producing strains of *K. pneumoniae* harbouring either blaVIM or blaKPC have been the cause of country-wide epidemics of healthcare associated infections (Nordmann *et al*., 2009; Yusuf *et al.,* 2015).

However, bla-KPC carbapenem resistant gene producing Enterobacteriaceae are often misidentified by routine microbiological susceptibility testing and incorrectly reported as sensitive to carbapenems; however, resistance to the carbapenem antibiotic ertapenem is common and a better indicator of the presence of KPCs (Ryan *et al.,* 2011).The *NDM-1* gene raised global concern since its first recognition in Pakistan and spread to many other countries via the movement of people and other medical tourists (Health Protection Agency, 2009; Kumarasamy *et al.,* 2010). The genes involved in carbapenem resistance among *Enterobacteriaceae* vary greatly among different countries. In Lebanon, these carbapenemases are OXA-48 from both *K. pneumoniae* and *E. coli* (El-Herte *et al.,* 2012).

Conclusion

Out of a total of (350) samples collected, (144) representing (41%) positive samples were recorded, with *E. coli* having (98) representing (28%) and *K. pneumoniae* having (46) representing (13%), making *E. coli* the most prevalent organism among the study population. Out of a total of (98) positive samples of *E. coli,* (29) representing (29.6%) and (69) representing (70%) samples were positive for *E. coli* among the male and female patients respectively. While out of (46) positive samples for *K. pneumoniae,* a total of (8) representing (17%) and (38) representing (82.6%) samples were positive for *K. pneumoniae* among the male and female patients respectively. However, among the five age groups, (≤10, 11-20, 21-30, 31-40 and 41years and above), females have a higher occurrence of both the *E. coli* and *K. pneumoniae* infections. Furthermore, (31-40) age group recorded the highest occurrence of the isolates, having (39) representing (11%) and (19) representing (5.4%) positive samples for *E. coli* and *K. pneumoniae.* Among the twelve antibiotics tested against the isolates, *E. coli* recorded the highest number of susceptible drugs (5 drugs), with *K. pneumoniae* having the least (3 drugs). Furthermore, *K. pneumoniae* recorded the highest number of resistant drugs (9 drugs) with *E. coli* having the least (7 drugs). On the prevalence of meropenem and ertapenem resistance among *E. coli*, a prevalence of (6.1%) (n=98), representing (6) isolates was recorded. While for *K. pneumoniae,* a prevalence of (6.5%) (n=46), representing (3) resistant isolates, was recorded. The prevalence recorded is slightly higher when compared with the previous report of (5.2%) and (2.5%) for *E. coli* and (7.8%) and (5.5%) for *K. pneumoniae.* The PCR Amplified DNA products were examined for the common ESBL encoding genes (bla TEM and blaSHV) and carbapenem resistance genes (blaKPC, blaNDM and blaVIM). However, all the ESBL genes were detected while for the carbapenemase, only blaVIM was detected, with the absence of blaKPC and blaNDM genes. Detection of these genes constitutes an alarming threat, and have been the cause of country-wide epidemics of healthcare associated infections. The absence of any active control plans to curtail the rising spread of carbapenems resistance in Nigeria shows that attentions are not been paid to these problems, which is the likely reason for the low level of awareness among the medical practitioners in the country.

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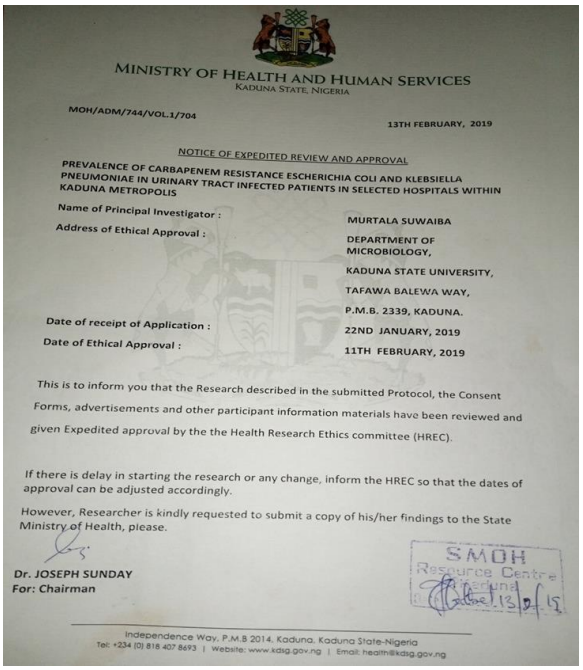

MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.1/704

13TH FEBRUARY, 2019

NOTICE OF EXPEDITED REVIEW AND APPROVAL

PREVALENCE OF CARBAPENEM RESISTANCE ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE IN URINARY TRACT INFECTED PATIENTS IN SELECTED HOSPITALS WITHIN KADUNA METROPOLIS

Name of Principal Investigator : MURTALA SUWAIBA

Address of Ethical Approval : DEPARTMENT OF MICROBIOLOGY, KADUNA STATE UNIVERSITY, TAFAWA BALEWA WAY, P.M.B. 2339, KADUNA.

Date of receipt of Application : 22ND JANUARY, 2019

Date of Ethical Approval : 11TH FEBRUARY, 2019

This is to inform you that the Research described in the submitted Protocol, the Consent Forms, advertisements and other participant information materials have been reviewed and given Expedited approval by the the Health Research Ethics committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

Dr. JOSEPH SUNDAY
For: Chairman

SMOH Resource Centre Kaduna 13/2/19

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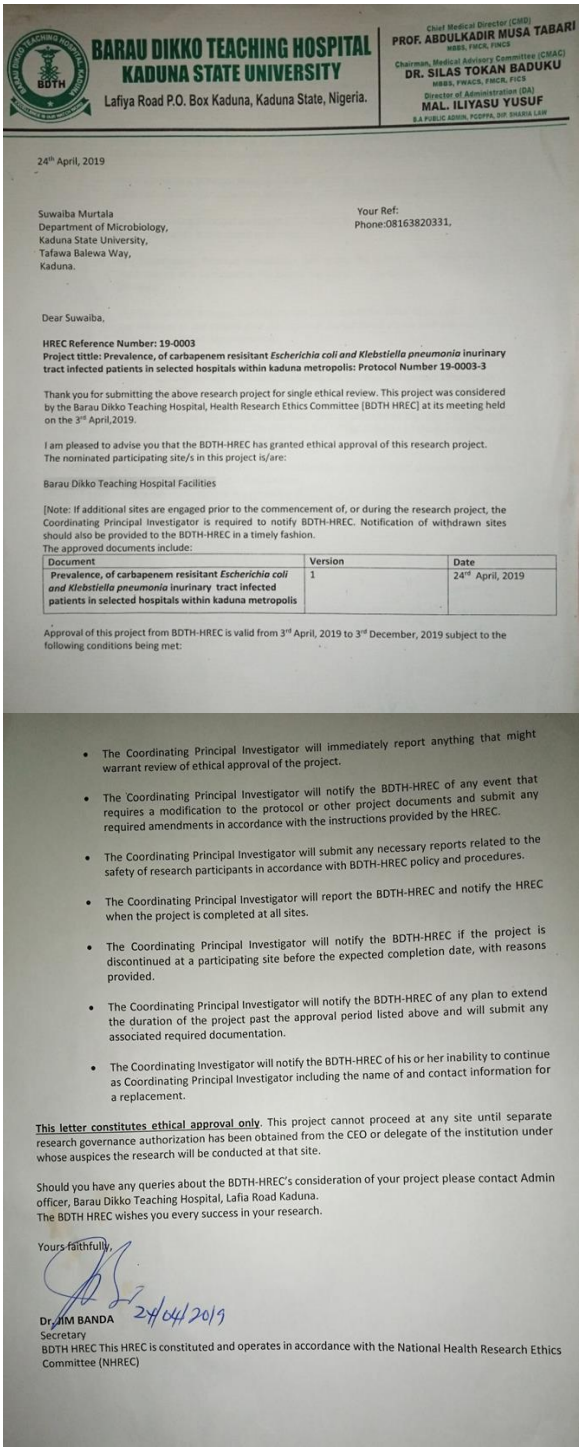

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24th April, 2019

Suwaiba Murtala
Department of Microbiology,
Kaduna State University,
Tafawa Balewa Way,
Kaduna.

Your Ref:
Phone:08163820331,

Dear Suwaiba,

HREC Reference Number: 19-0003
Project tittle: Prevalence, of carbapenem resisitant *Escherichia coli and Klebstiella pneumonia* inurinary tract infected patients in selected hospitals within kaduna metropolis: Protocol Number 19-0003-3

Thank you for submitting the above research project for single ethical review. This project was considered by the Barau Dikko Teaching Hospital, Health Research Ethics Committee [BDTH HREC] at its meeting held on the 3rd April,2019.

I am pleased to advise you that the BDTH-HREC has granted ethical approval of this research project.
The nominated participating site/s in this project is/are:

Barau Dikko Teaching Hospital Facilities

[Note: If additional sites are engaged prior to the commencement of, or during the research project, the Coordinating Principal Investigator is required to notify BDTH-HREC. Notification of withdrawn sites should also be provided to the BDTH-HREC in a timely fashion.
The approved documents include:

Document	Version	Date
Prevalence, of carbapenem resisitant *Escherichia coli and Klebstiella pneumonia* inurinary tract infected patients in selected hospitals within kaduna metropolis	1	24rd April, 2019

Approval of this project from BDTH-HREC is valid from 3rd April, 2019 to 3rd December, 2019 subject to the following conditions being met:

- The Coordinating Principal Investigator will immediately report anything that might warrant review of ethical approval of the project.
- The Coordinating Principal Investigator will notify the BDTH-HREC of any event that requires a modification to the protocol or other project documents and submit any required amendments in accordance with the instructions provided by the HREC.
- The Coordinating Principal Investigator will submit any necessary reports related to the safety of research participants in accordance with BDTH-HREC policy and procedures.
- The Coordinating Principal Investigator will report the BDTH-HREC and notify the HREC when the project is completed at all sites.
- The Coordinating Principal Investigator will notify the BDTH-HREC if the project is discontinued at a participating site before the expected completion date, with reasons provided.
- The Coordinating Principal Investigator will notify the BDTH-HREC of any plan to extend the duration of the project past the approval period listed above and will submit any associated required documentation.
- The Coordinating Investigator will notify the BDTH-HREC of his or her inability to continue as Coordinating Principal Investigator including the name of and contact information for a replacement.

This letter constitutes ethical approval only. This project cannot proceed at any site until separate research governance authorization has been obtained from the CEO or delegate of the institution under whose auspices the research will be conducted at that site.

Should you have any queries about the BDTH-HREC's consideration of your project please contact Admin officer, Barau Dikko Teaching Hospital, Lafia Road Kaduna.
The BDTH HREC wishes you every success in your research.

Yours faithfully,

24/04/2019

Dr. IM BANDA
Secretary
BDTH HREC This HREC is constituted and operates in accordance with the National Health Research Ethics Committee (NHREC)