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Antibiotic resistance characterization and detection of BLAIMP gene among uropathogenic *Escherichia coli* and *klebsiella pneumoniae* isolates in Okada, Edo State, Nigeria

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Abstract

Antibiotic resistance is a major public health issue worldwide and became one of the principal international healthcare crises of the 21st century. The production of MBL is one of the resistance mechanisms in *Enterobacteriaceae*, and they are increasingly detected in *Escherichia coli* and *Klebsiella pneumoniae* globally. Therefore, the aim of this study was to determine the phenotypic and molecular characteristics of MBL-producing *E. coli* and *K. pneumoniae* among Okada patients in IUTH with UTIs. A total of 300 urine samples were obtained from Igbinedion Univeristy Teaching Hospital in Okada between January 2022 to December 2023. *E. coli* and *K. pneumoniae* isolates were culturally and biochemically identified. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion technique. Metallo beta-lactamase production was detected by combined disc assay using meropenem and meropenem/EDTA discs. Bacteria showing resistance to at least three different classes of antibiotics were considered multidrug resistant (MDR). In order to spot blaIMP), polymerase chain reaction was conducted. Of the total 300 urine samples processed, 93(31%) samples were culture positive, among which E. coli and *K. pneumoniae* samples were 75 (80.6%) and 18 (19.3%) respectively. Among the 75 *E. coli* and the18 K. pneumoniae, 67 (89.3%) *E. coli* and 18(100%) *K. pneumoniae* were multidrug resistant. The lowest rates of resistance were seen toward imipenem (5.3%) and meropenem (5.3%) followed by nitrofurantoin (60%) and ofloxacin (69%). Four of the 5 MBL phenotypically screen positive *E. coli* were PCR positive for blaIMP gene. The study showed high prevalence of drug-resistant genes harbored by the uropathogens. Imipenem and meropenem are the most effective drugs to treat ESBL producers. It is therefore recommended that antibiotic stewardship programs should be implemented immediately to combat antibiotic resistance.

Keywords: Antibiotic Resistance; Uropathogenic Escherichia Coli; *Klebsiella Pneumonia* Metallo; Extended Spectrum Beta-Lactamase Producing; Okada

1. Introduction

Today, the prevalence of antimicrobial resistance is a major public health issue worldwide and became one of the principal international global healthcare crises of the 21st century. Approximately 150-250 million cases are seen globally per year (Shaikh *et al*. 2015). It is estimated that 40% of women and 12% of men experience minimum one symptomatic urinary tract infection (UTI) during their lifetime. By the year 2050, multidrug resistance infections may cause 10 million deaths annually with about 90 % predicted deaths in Asia and Africa. (Wilson and Torok 2018. Moreover, nearly 27-48% of affected women suffer from recurrent UTIs (Wilson (Wilson and Torok (2018). Among all

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the nosocomial infections, UTIs comprise about 40-50% of the infections that contribute to increased morbidity and prolonged hospitalization (Oli *et al.* 2019). UTI is also an economic problem because each year about 150 million people are treated (treated of UTIs (Slam et al. 2010; Rahman et al. 2017; Otokunefor *et al.* 2019). Therefore, the presence of numerous bacterial cells in the urine (105 CFU/mL) without the clinical symptoms is called asymptomatic bacteriuria. UTIs are mainly caused by *E. coli*, which are responsible for 70-95% of UTI cases (Oladeinde *et a*l. 20211). Other etiological agents of UTIs are *Klebsiella pneumoniae* (about 7%), Proteus mirabilis (about 5%), Staphylococcus aureus, *S. saprophyticus*, and *Enterococcus faecalis* (Ahmed *et al.* 2012). Although UTI is caused by a wide array of pathogens, Escherichia coli is responsible for the majority of the cause of infections. UTIs is nearly recognized to occur in all ages group, but some group such as neonates, pregnant women or the elderly patient are more vulnerable to it. (Shrestha, 2013). However, the development of UTIs depends on anatomical factors, the integrity of host defense mechanisms, and the virulence of the infecting organisms. UTIs are classified into disease categories according to the site of infection: cystitis (the bladder), pyelonephritis (the kidney) and bacteriuria (the urine) (Shrestha, 2013). Successful establishment of infection by bacterial pathogens requires adhesion to host cells, colonization of tissues, and, in certain cases, cellular invasion, followed by intracellular multiplication, dissemination to other tissues, or persistence. Colonization of the urine in the absence of the clinical symptoms is called asymptomatic bacteriuria (ABU). Most patients with ABU do not need treatment, and in many cases the colonizing by the ABU strains may help to prevent infection by other more virulent bacteria. The primary causative agents responsible for more than 80% of all UTIs, including both ABU and symptomatic UTIs, are strains of uropathogenic *E. coli*. The resistance of pathogenic organisms to antibiotics has become a worldwide problem with serious consequences on the treatment of infectious diseases (Shaikh, et al., 2015), like that of the *E. coli*. The heightened use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to the phenomenon (Shaikh, et al., 2015). According to Shaikh, et al., (2015) in the research on antibiotics resistance Extended B-lactamase producing *E. coli*, there is already an alarming increase of antibiotic resistance in bacteria that cause either community infections or hospital acquired infections (Shaikh, et al., 2015). They further reported that the most interesting one in particular are the multidrug resistant pathogens, e.g. *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii*, methicillin-resistant Staphylococcus aureus, penicillin-resistant *Streptococcus pneumoniae,* vancomycin-resistant Enterococcus, and extensively drug-resistant Mycobacterium tuberculosis (Shaikh, et al., 2015). Similarly, based on the reports of Bartoletti *et al.,* (2016), the efficacy of antibiotic treatment depends on the identification and antimicrobial resistance pattern of uropathogens responsible for UTI, and as such the practice of prescribing antibiotics to treat UTI without bacterial characterization led to increased resistance among uropathogens and to decreased effectiveness of oral therapies ((Haberecht *et al*. 2019). . Antimicrobial therapy usually is the first strategy in treating a UTI. Antimicrobial therapy usually is the first strategy in treating a UTI. The choice of antimicrobial compound depends on the patient's health condition the type and susceptibility profile of bacteria causing the UTI. However, due to misuse of antibiotics, antimicrobial resistance (AMR) has greatly expanded among bacteria (Anene et al 2021). Many *E. coli* strains, especially UPEC have become multi-extensively-or pan-drug resistant (MDR, XDR, or PDR). This poses a great challenge in the treatment of UTI infections. Therefore, monitoring the emergence and dissemination of new AMR is necessary to continuously refine guidelines for empiric antimicrobial therapy. Extended Spectrum Beta lactamases (ESBLs) enzymes are commonly found in the *Enterobacteriaceae* family, which includes more than 120 species. ESBL is commonly acquired by horizontal gene transfer and confers resistance to oxyimino cephalosporins. Some of these are mutant derivatives of established plasmid-mediated Beta-lactamases (TEM/SHV) or those recruited from environmental bacteria (CTX-M). These enzymes hydrolyze penicillins, broad-spectrum cephalosporin, and monobactams. However, they do not affect cephamycins or carbapenems and are inhibited by clavulanic acid. The emergence of Metallo Beta Lactamase (MBLs) which have activity against carbapenems (e.g. the VIM and IMP families of enzymes) impairs the clinical utility of this class of antibiotics. Consequently, there is a pressing need to explore and develop innovative strategies to combat ESBL infections, as reliance on carbapenem is no longer a sustainable solution. Typically, the activity of these enzymes can be counteracted by beta lactamase inhibitors (BLIs) like clavulanic acid, sulbactam, and tazobactam, which neutralize their effect and restore the efficacy of beta lactam antibiotics. In this present study we aimed to investigate antibiotic resistance pattern and detection of Extended and Metallo Beta Lactamase Producing Uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* in Igbinedion University Teaching Hospital IUTH, Okada, and Edo State.

2. Materials and Methods

2.1. Sample collection and processing

Patients with complaints or diagnosis of urinary tract infection (UTIs) in Okada, Edo State were recruited for the study.. A total of 300 sample patients was use for this study. However, positive urine culture result of 300 patients with the diagnosis of urinary tract infection (UTIs) in Okada, Edo State were aseptically evaluated. After examination of the hospital laboratory records, the age, gender, and culture results of the patients as well as the susceptibility and resistance to antibiotics were evaluated.

2.2. Inclusion Criteria

Patients included in this study were patients showing symptoms including fever, dysuria, and/or loss of bladder control, and/or lower back pain, and/or cloudy or foul-smelling urine. Patients with a positive culture, i.e., (≥105 colony forming units per milliliter (CFU/mL).

2.3. Exclusion Criteria

Repeated samples from the same patient, patients currently on antibiotic and those not fulfilling the criteria of the American Society for Microbiology were excluded from the study.

2.4. Isolation and Identification of isolates

Midstream voided urine specimens were collected using the clean-catch method in sterile disposable tube, the samples were analyzed immediately within 2hours of collection in the laboratory. All patients were well instructed by trained personnel on how to collect clean catch midstream urine. A total of 300 urine samples were collected between January 2022 and January 2023. The urine samples were cultured on 5% blood agar and MacConkey agar using standard quantitative 10 µL loops and incubated aerobically for 24 hrs. At 37°C. UTI is defined as the presence of single organism in the urine in concentration of ≥105 CFU/mL. A total of 93 non-duplicate *E. coli and Klebsiella pneumoniae* isolates were isolated from clean-catch midstream urine of patient. The bacteria were identified and confirmed using standard microbiological / biochemical tests including Gram staining, and reactions on MacConkey agar, triple sugar iron agar, SIM medium, Simmons' citrate agar, MR-VP medium (Cheesbrough, 2016).

2.5. Maintenance of bacteria stock culture

Each of the isolated *E. coli and klebsiella pneumoniae* isolates were maintained as stock cultures for further studies on nutrient agar slants in Bijou bottles. This was done by streaking the isolates on the agar slants, and incubated at 30oC for 18-24 hrs. After incubation, the inoculated slants were stored in the refrigerator at ambient temperature and these stock cultures served as source of E. coli and *Klebsiella pneumoniae* isolates for further bacteriological studies.

2.6. Antimicrobial Susceptibility Testing

Susceptibility and resistance of isolates to antimicrobials were determined using Kirby-Bauer disc diffusion method recommended by Clinical and Laboratory Standards Institute 2020 18 (CLSI-202018). The antibiotic disc manufactured by Celtech diagnostic containing the following antibiotics were used for this study: Ceftriaxone/Sulbactam, Cefuroxime (30µg), Gentamicin (30g), Ofloxacin (5µg), Amoxicillin/clavunalic acid (30µg), Nitrofurantoin (300µg), Cefotaxime (30µg), Levofloxacin (30µg), Nalidixic Acid, Cefixime, Imipenem/ Cilastatin (10µg), Meropenem (10µg) were used . *E. coli* ATCC 25922 was used as positive control. The zone of inhibition produced by the antibiotics was recorded and interpreted as per CLSI guidelines. The isolates non-susceptible to at least three of antibiotic categories were defined as MDR. (Magiorakos *et al.* 2019).

2.7. Screening test for Metallo beta lactamase (MBL)

MBL enzyme-producing *E. coli* and *Klebsiella pneumoniae* isolates were suspected when the test organism(s) was resistant to any of the carbapenems including imipenem and meropenem as was described previously (Ejikeugwu *et al*, 2017). Bacterial isolates showing inhibition zone diameter IZD) of ≤ 23mm to any of the carbapenems were suspected to produce MBL enzyme and these isolates were subjected to phenotypic confirmation test

2.8. Phenotypic detection of metallo-beta lactamase (MBL)

2.8.1. Combined disc (CD) and double disc synergy (DDS) tests

Phenotypic CD and DDS test were performed on meropenem- and imipenem resistant isolates using meropenem to detect MBLs (Ranjan *et al.* 2016 &, Sfeir *et al*. 2019). Practically, for CD test a bacterial suspension of the 0.5 McFarland standard was cultured on the Mueller–Hinton agar, and then two of imipenem (10 µg) and imipenem –EDTA(10µg-1460µg) disc were added to the Mueller–Hinton agar. After a 24-h incubation at 35 °C, the inhibition zone diameter was measured. The isolates were considered MBL-producer when the diameter of inhibition zone around the imipenem-EDTA disc increased by ≥ 7 mm compared to imipenem disc alone. (Ugwu *et al*., 2020).

2.9. Molecular studies

2.9.1. DNA extraction

A 50-100mg (wet weight) bacterial cells that have been suspended in up to a 200µl of isotonic buffer (e.g., PBS) were added to a ZRBashing TM Lysis Tube. 750µl Lysis Solution was also added to the tube which was secured in a bead fitted with 2 ml tube holder assembly and was process at maximum speed for> 5 minutes. The ZR Bashing Bead TM Lysis Tube was centrifuged in a microcentirifuge at > 10,000 x g for 1 minute. Then 400 µl supernatant was transferred to a Zymo-Spin TM IV Spin Filter (orange top) in a Collection Tube and centrifuge at 7,000 x g for 1 minute. 1,200µl of Bacterial DNA Binding Buffer were added to the filterate in the Collection Tube. 800µl of the bacterial DNA binding buffer was transferred to a Zymo-Spin TM IIC Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute. A 200µl DNA Pre-Wash Buffer were added to the Zymo-Spin TM IIC Column in new Collection Tube and centrifuge at 10,000 x g for 1 minute. 500 µl of Bacterial DNA Wash Buffer were added to the Zymo-Spin TM IIC Column and centrifuge at 10,000 x g for 1 minute. The Zymo-Spin TM IIC Column were transferred to a clean 1.5 ml microcentrifuge tube, 100ul (35 µl minimum) of DNA Elution Buffer was added directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA. DNA is now suitable for PCR and other downstream applications

2.10. PCR amplification of MBL resistance genes

The PCR cocktail mix consists of 2.5ul of 10x PCR buffer,1ul of 25mM MgCl2, 1ul each of forward primer and reverse primer, 1ul of DMSO, 2ul of 2.5mMDNTPs, 0.1ul of 5u/ul Taq DNA polymerase, and 3ul of 10ng/ul DNA. The total reaction volume was made up to 25ul using 13.4ul Nuclease free water.

A Touchdown PCR program was used for PCR amplification; it consists of an initial denaturation step at 94°C for 5 mins, followed by 10 cycles of 94°C for 40 s, 55°C for 45 s and 72°C for 40 s, with decreasing 1 °C per cycle of the annealing temperature from 55°C to a touchdown at 48°C, and 30 cycles of 94°C for 40 s, 48°C for 45 s and 72°C for 40 s, finished at 72°C for 10 min.

Table 1 Oligonucleotide sequence of the primers used in multiplex PCR

TARGET GENE	NUCLEOT1DE	
BlaIMP F	5-GGA GCA TTT TCA GGA TGC-3	
R	5 –TGG TGG TTT GTT GAT GTG 3	429

2.11. Agarose gel electrophoresis

The PCR amplification products were separated on a 1.5% agarose gel. Electrophoresis was carried out at 80V for 1 hour and 30 minutes. A 50 base pairs DNA ladder (Solis Biodyne) was used as the DNA molecular weight marker. At the end of electrophoresis, DNA bands were visualized by ethidium bromide staining, and the detected genes were photographed in an ultraviolet transilluminator.

3. Results

3.1. Prevalence of *E. coli* and *Klebsiella pneumoniae* isolates from the urine

A total of 93 (31%) isolates of which *E. coli* were 75(81%) and *Klebsiella pneumoniae* 18(19%) respectively, were identified by colonial/cultural morphology and biochemical tests from the urine samples of 300 patient recruited and bacteriologically investigated in this study. The distribution of the isolates according to their Age Brackets is shown in Table 2. It was observed that the prevalence and incidence of UTI is higher in females than males. The female samples were 30% more than male samples collected. Of the 93 samples, 60(65%) were of females and 33 (35%) of males. Regarding patient age, 17% of the participants were aged 18-30 years, 24% were aged 31-40 years, 16% were aged 41-50, 16% were aged 41-50, 18% were aged 51-60, 13% were aged 61-70 and 12% were aged >70 as shown in Table 1and 2. Of the 75 Escherichia coli samples, 52 (69%) were from female and 23(31 %) were from male while of the 18 *Klebsiella pneumoniae* 8(44) were from female and 10(56) were from male

3.2. Antibiotic susceptibility pattern of the isolated uropathogens.

The isolated organisms were tested against 12 different antibiotics (CETECH Gram-negative multi-disk). The antimicrobial susceptibility pattern revealed varied levels of sensitivity and resistance of the isolates to the tested

antibiotics (Table 3). All the isolates were 100% resistant to cefuroxime, Cefotaxine, Nalidixic acid, and Amoxicillin +clavulanic. A higher percentage of isolates showed resistant to Ampiclox, Ceftriaxone, and Levofloxacin. The lowest percentage of *E. coli* (8%) isolates was resistant to Carbapenems, followed by Nitrofurantoin (60%), Ofloxacin (69%), and Gentamicin (76%).(17%) of Klebsiella spp isolates were resistant to Carbapenems and 89% showed resistance to Nitrofurantoin, 83% to Ofloxacin and 94% resistance to Gentamicin. Out of the 8 isolates of *E. coli* that were resistant to carbapenem, only 5(6.6%) *E. coli* were phenotypically confirmed to produce MBL (Table 4) using combined disc (CD) test. The other isolates did not produce MBL. The MARI profile of the 93 isolates is shown in Table 4. All the isolates were resistant to at least one antibiotic. Most of the isolates 93(100%) were MAR isolates with MAR indices greater than 0.2 2. The commonest MAR index was 0.8(59%) This implies that the resistant isolates recovered from the urine samples are multidrug-resistant and shows resistance to antibiotics in at least four different classes.

Table 2 Antimicrobial sensitivity patterns of *E. coli*

Antimicrobial Group	**Antibiotics**	**S (%)**
Penicillin	Amoxicillin/ clavulanate	0
Cephalosporin	Cefuroxime Cefotaxime Ceftriaxone/sulbactam Cefixime	0 0 0 1
Carbapenems	Imipenem Meropenem	85 88
Fluoroquinolone	Levofloxacin Ofloxacin	10 16
Aminoglycosides	Gentamicin	12
Nitrofuran	Nitrofuranton Nalidixic acid	40 0

Table 3 Antimicrobial intermediate patterns of *E. coli*

Antimicrobial Group	**Antibiotics**	***I (%)***
Penicillin	Amoxicillin/ clavulanate	0
Cephalosporin	Cefuroxime Cefotaxime Ceftriaxone/sulbactam Cefixime	0 0 1 2
Carbapenems	Imipenem Meropenem	7 4
Fluoroquinolone	Levofloxacin Ofloxacin	3 15
Aminoglycosides	Gentamicin	12
Nitrofuran	Nitrofuranton Nalidixic acid	12 0

Table 4 Antimicrobial resistance patterns of *E. coli*

Antimicrobial Group	**Antibiotics**	***R (%)***
Penicillin	Amoxicillin/ clavulanate	100
Cephalosporin	Cefuroxime Cefotaxime Ceftriaxone/sulbactam Cefixime	100 100 99 97
Carbapenems	Imipenem Meropenem	8 8
Fluoroquinolone	Levofloxacin Ofloxacin	87 69
Aminoglycosides	Gentamicin	76
Nitrofuran	Nitrofuranton Nalidixic acid	48 100

Table 5 Antimicrobial sensitivity patterns of *Klebsiella pneumonia*e Isolates

Antimicrobial Group	**Antibiotics**	**S (%)**
Penicillin	Amoxicillin/ clavulanate	0
Cephalosporin	Cefuroxime Cefotaxime Ceftriaxone/sulbactam Cefixime	0 0 6 0
Carbapenems	Imipenem Meropenem	83 83
Fluoroquinolone	Levofloxacin Ofloxacin	6 17
Aminoglycosides	Gentamicin	6
Nitrofuran	Nitrofuranton Nalidixic acid	11 0

Table 6 Antimicrobial intermediate patterns of *Klebsiella pneumonia*e Isolates

Antimicrobial Group	**Antibiotics**	**I (%)**
Penicillin	Amoxicillin/ clavulanate	0
Cephalosporin	Cefuroxime Cefotaxime Ceftriaxone/sulbactam Cefixime	0 0 0 0
Carbapenems	Imipenem Meropenem	0 0

Fluoroquinolone	Levofloxacin Ofloxacin	0 0
Aminoglycosides	Gentamicin	0
Nitrofuran	Nitrofuranton Nalidixic acid	0 6

Table 7 Antimicrobial resistance patterns of *Klebsiella pneumonia*e Isolates

Antimicrobial Group	**Antibiotics**	**R (%)**
Penicillin	Amoxicillin/ clavulanate	100
Cephalosporin	Cefuroxime Cefotaxime Ceftriaxone/sulbactam Cefixime	100 100 94 100
Carbapenems	Imipenem Meropenem	17 17
Fluoroquinolone	Levofloxacin Ofloxacin	94 83
Aminoglycosides	Gentamicin	94
Nitrofuran	Nitrofuranton Nalidixic acid	89 94

Table 8 Distribution of Multiple Antibiotic-Resistant Index of the isolates

MARI	**NO**	**%**
0.5	3	3.2
0.6	11	11.8
0.7	19	20.4
0.8	55	59
1	5	5.3

Table 9 Phenotypic detection of Metallo beta lactamase enzyme (MBL)

Total number of MBL isolates	**Total number (%)** ***Escherichia coli***	***Klebsiella pneumoniae***
MBL Producer	5(6.6%)	0(0%)
MBL non-producer	70(93%)	18(100%)
Total	75	18

Out of the 8 isolates of *E. coli* that were resistant to carbapenem, only 5(6.6%) *E.coli* were phenotypically confirmed to produce MBL (Table 9) using combined disc (CD) test. The other isolates did not produce MBL

3.3. Results of Genotypic detection

PCR was performed on the 5 confirmed MBL producing isolates and MBL genes were detected in 4 *E. coli* isolates. (Table 6, Figure 1).

Table 10 Summary of blaIMP-PCR-positive isolates

Organisms	IMP
E. coli	4

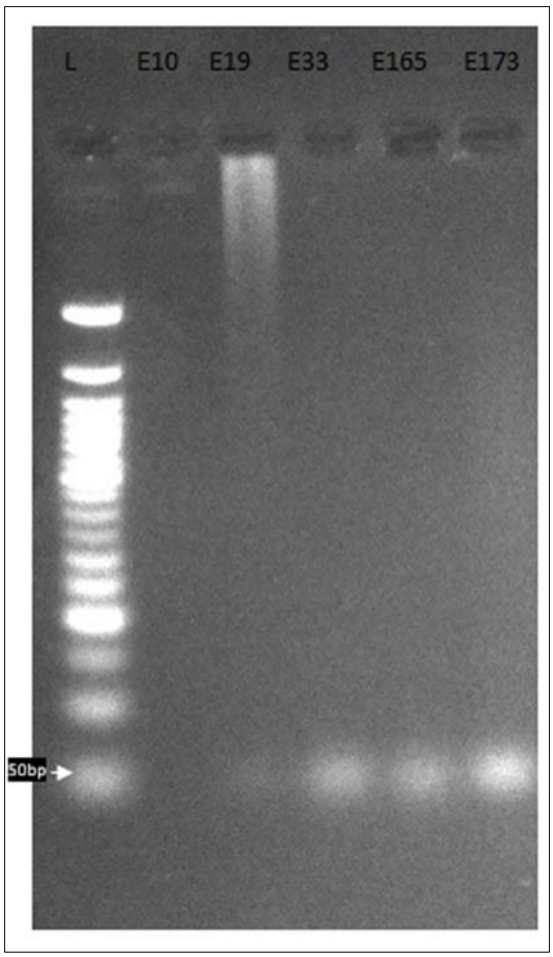

Figure 1 PCR 1image of blaIMP gene

4. Discussion

The effective management of UTI is very crucial, as *Escherichia coli* and *Klebsiella pneumoniae,* which often cause UTIs, can develop resistance to antibiotics. Antimicrobial resistance is a worldwide problem causing serious health threats. While various antibiotics are available, Beta lactam antibiotics are typically prescribed as the first line of treatment for UTIs. However, the overuse and misuse of these antibiotics have led to the emergence of bacteria that produce metallo Beta-Lactamase (MBL) enzymes, which render Beta lactam antibiotics ineffective, posing a significant challenge in treating UTIs (Mirkalantari *et al.,* 2020).

This cross-sectional study was conducted to determine the prevalence and distribution of Metallo β lactamase (MBL)-encoding, gene, and blaIMP among E. coli and *Klebsiella pneumoniae* isolates in Igbinedion University Teaching Hospital IUTH, Okada, and Edo State, Nigeria. In this study, samples of patients between the ages of 18years and above were collected. On the basis of gender male and female samples were segregated in the ratio of 1:1.8. It was observed that the prevalence and incidence of UTI is higher in females than males. The female samples were 30% more than male samples

collected. It is reported worldwide that UTI is more prevalent in females than in males (Ero *et al* 2018). The reason might be an anatomical difference in the male and female urethra. The female urethra is straight, smaller in size and diameter, and not differentiated into parts. In females, it opens to the outside between the clitoris and vagina and is the only urinary system. The invasion and adhesion to epithelial layers of the uropathogens are easy in females as compared to males. Due to the close proximity of the vagina, anus, and urinal openings in females, urinal infections are more prevalent (Masson *et al*. 2019).

All biochemically reported *Escherichia coli and Klebsiella Pneumoniae* showed 100% resistance to cefuroxime, Cefotaxine, Nalidixic acid, and Amoxicillin+clavulanic acid. A higher percentage of isolates showed resistant to Ampiclox, Ceftriaxone, and Levofloxacin. Eigty-nine percent (89%) of Klebsiella *pneumoniae* were resistant to nitrofurantoin, 83% to Ofloxacin and 94% to gentamicin. The lowest percentage of *E. coli (8%)* and Klebsiella *pneumoniae* (17%) isolates was resistant to Carbapenems. Carbapenems are often the final influential therapy that exists for infections resulting from MDR Enterobacteriaceae. (Banerjee *et al* 2017) According to other studies, 100% sensitivity was seen with Imipenem and Meropenem, which has been reported to be the most effective antibiotic including the isolates that produce ESBLs. This is an important result of the present study because many infections can be treated with Carbapenemes. This result can be relevant to the fact that these antibiotics are more expensive and thus used less in this region. Majority of isolates tested were multidrug resistant (89.3%), exhibiting resistance to three or more drug classes. A 3% of the isolates were possible pan drug resistant (PDR) meaning were non-susceptible to all investigated antibiotics categories tested.

Multiple antibiotic resistance index reveals the spread of bacterial resistance in a given population (Joseph et al. 2017). MARI value greater than or equal to 0.2 indicate high-risk source of contamination where antibiotics are often used. About 89.3% of the *E. coli* isolates obtained in this study were multi-drug resistant having MARI above 0.2 which is an indication that a large proportion of the isolates have been previously exposed to several antibiotics with an average index of 0.6. This could be explained by high and uncontrolled use of antibiotics in the study area, these antibiotics are easily procured over the counter, without a proper investigation and prescription (Joseph et al. 2017). Hence, antimicrobial susceptibility testing is imperative in selecting therapeutic options. Similarly, several studies have reported the occurrence of MDR *E. coli* on inanimate hospital surfaces, Ibrahim et al. (2012) and Shams et al. (2014) reported the prevalence of 92.2% and 83% MDR *E. coli* respectively.

5 (37%) were found phenotypically to be MBL producers, which is higher than the earlier study conducted by Mishra *et al.(2021)* in which MBL producers were 1.3%. In contrast with the study conducted by Anjana *et al* (2017) and Shrestha *et al.,* (2020*)* the rate of MBL was 8.0% and 17.43% respectively which is higher. The maximum MBL activity was seen in *E. coli* (62.5%). No MBL activity was seen in *Klebsiella pneumoniae.* The MBL producer show resistance to nearly all the generally used drugs, so it is a matter of great concern. Similar to the findings in this study, many other studies also reveal that generally MBL positive isolates show resistance to even the carbapenems, which are used as the last resort for treatment of MDR Gram- negative bacterial infection. Therefore, there is a need to institute correct antibiotics for patients infected with MBL producers and to prevent the spread of such organisms. As the choice of antibiotics for MBL producers is restricted, it is a great cause of concern

On the molecular level, Polymerase Chain Reaction (PCR) was utilized to amplify the genes from the genomic DNA of *E.coli* and *Klebsiella pneumoniae*. PCR is a robust molecular technique that allows for the detection of specific genetic sequences, making it ideal for identifying resistance genes. Agarose gel electrophoresis was performed to visualize the PCR products. Under UV light, the presence of amplified DNA fragments indicated the existence of various genes within the isolates. The results for bla*IMP* gene were analyzed through agarose gel electrophoresis.

MBLs have been recognized as one of the most notable resistance determinants in *Enterobacteriaceae* (Loganand *et al.2017).* Four (80%) of the MBL phenotypically screened positive *E. coli* had bla_{IMP} gene. There are increasing reports of MBL-producing Gram-negative bacteria in southeastern Nigeria (Ejikeugwu *et al.*2017) Had reported high occurrence of MBL-producing *E. coli and Klebsiella pneumoniae* from an abattoir. Since the genes that code for MBL production in Gram negatives are chromosomally or plasmid mediated, they can easily be transmitted through mobile genetic elements among bacterial population in a community (Ejikeugwu *et al* 2017). The discrepancy in the percentage of phenotypic and genotypic β-lactamase confirmed producers might be because of co expression of more than one ESBL, MBL, and genes in an organism. Occurrence of multiple ESBL types and/or ESBL-AmpC combinations within the same organism has previously been reported which make phenotypic identification of the β-lactamases difficult and not reliable (Padmavathy *et al* 2013).

5. Conclusion

This present study confirmed that *E. coli* (79.5%) is the main uropathogen in patients, although K. *pneumoniae (19.3)* was also frequently isolated from UTI cases. The results suggest that the prevalence of MDR for gram negative bacteria is distressingly high. Antibacterial resistance patterns have to be up dated periodically to confirm proper empiric treatment of UTI. Our results showed that the frequency of antibiotic resistance genes of blaIMP is very high in *E. coli* strains isolated from patients in Igbinedion University teaching hospital.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical Clearance Approval for this study was obtained from the Ethical Committee of the Igbinedion University Teaching Hospital, Okada, and Edo State. Dated 10th January, 2022.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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