Original Research Article

**Molecular characterization of multidrug-resistant *Enterobacteriaceae*-associated urinary tract infections in a geriatric population in Port Harcourt metropolis, Rivers State, Nigeria**

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ABSTRACT

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| **Background**: Bacteria belonging to the *Enterobacteriaceae* family are responsible for most of the urinary tract infection globally with disproportionate levels of burden among vulnerable populations. **Aim**: This study evaluated the burden and also characterized the species of bacteria associated with a disadvantaged population, geriatric group, in Port Harcourt metropolis of Rivers State in Nigeria. **Study Design**: This study was a cross-sectional study which employed a simple random sampling method. **Methods**: Conventional techniques were used to isolate the pathogen. Molecular methods were used to confirm the 16S rRNA, NDM-1, KPC and QnrA genes. Gene sequencing was performed on the 16S rRNA gene and an evolutionary relationship tree was plotted. **Results**: The prevalence of urinary tract infection (UTI) among the elderly was 33.3% with the females having (32.6%) higher prevalence than the males (31.3%). *Escherichia coli* had the highest prevalence (38%) while *Proteus mirabilis* showed the lowest level of prevalence. Cephalosporin class of antibiotics demonstrated the highest resistance profile (100%). NDM-1 targeted gene (550 bp) was detected in one of the *Klebsiella pneumoniae* while the KPC targeted gene (600 bp) was detected in another *Klebsiella pneumoniae* and *Escherichia coli* isolates. The 16S rRNA of the isolates confirmed the isolates to be closely related *to Klebsiella pneumoniae, Escherichia coli* and *Proteus mirabilis.* **Conclusion**: This study demonstrated the presence of recently discovered resistance gene (NDM-1) among the geriatric populations. |

*Keywords: Geriatric population, Antibiotic resistance, Uropathogens, Betalactamase*

1. INTRODUCTION

“Urinary tract infection (UTI) is one of the most common widespread infections. It has been incriminated as one of the major causes of illness that leads to individuals seeking medical care in medical facilities. Urinary tract infection is one of the illnesses implicated in the morbidity amongst older adults. Among the population at high risks of urinary tract infections including older adults. This may be due to the following factors: long-term/prolonged catheterization in elderly home care facilities, misuse of antibiotics or other underlying factors such as poor immunity of ageing individuals, poverty, lack of adequate access to health care facilities etc. All these factors may contribute to predispose the older adult’s population to UTIs” (Robichaud *et al.,* 2008).

“Urinary tract infections are mainly caused by uropathogens*,* especially *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, enterococcus spp., Pseudomonas spp.* and so on. Urinary tract infection has been reported to be the second most common infection among older adults living in the community setting, as well as the leading site of infection in older adults in long-term care facilities” (Robichaud *et al.,* 2008). “*Escherichia coli* has been reported as the most prevalent pathogen causing urinary tract infections” (Drews *et al.,* 2005). “However, a research report has also indicated that *Staphylococcus aureus* is gaining a high prevalence as one of the most common organisms in causing UTI in both hospitalized and outpatients” (Omoregie *et al.,* 2010). “Recently, research has revealed that these uropathogens have become resistant to the most conventional antibiotics and there is an increase in the emergence of multi-drug resistant uropathogens among the older adult’s population suffering from urinary tract infection” (Robichaud *et al.,* 2008).

“Extended-spectrum β-lactamases (ESBLs) are enzymes that hydrolyze oxyimino-cephalosporins and confer resistance to broad-spectrum cephalosporins and aztreonam which are mostly found in *Enterobacteriaceae* harboring ESBLs such as *E. coli*, *Klebsiella* strains as well as the recent significant rise in methicillin-resistance *Staphylococcus aureus* (MRSA) in UTI” (Fadhil et al., 2016; Badiger et al., 2021). “MRSA has been connected with modifications in the penicillin binding proteins (PBPs) subsequently generating an additional penicillin-binding protein, PBP2a or PBP2’. Penicillin Binding Protein-2a is encoded by the mecA gene which is carried on a large genomic island labeled SCCmec” (Ray *et al.,* 2016). “Both ESBL-producing *Enterobacteriaceae* and methicillin-resistant *Staphylococcus aureus* (MRSA) are majorly related with urinary tract infections in older adults, and this may be a consequence of bad clinical facilities, inappropriate antibiotics therapy, long term care facilities and high cost of hospital bills thus poses global medical health problems with limited available treatment options” (Kumar *et al.,* 2006; Ray et al., 2016). “According to research, there has been an increase in the widespread of both the methicillin-resistance *Staphylococcus aureus* and β-lactamase-mediated resistance *Enterobacteriaceae* accounting for high significance in the prevalence of UTI” (Paterson *et al.,* 2004).

“Urinary tract infections are a global health challenge, most strains of uropathogens have proven antimicrobial resistance (AMR) and as such pose public health threat to many parts of the world. *Enterobacteriaceae* have been highly incriminated on several research as the common cause of urinary tract infections especially *E. coli* followed by Gram-positive cocci bacteria especially *S. aureus* and the recent increase in the emergence of uropathogens antimicrobial resistant strains isolated from adults’ individuals is alarming” (Robichaud, 2008). “The emergence of multidrug-resistant strains of *Enterobacteriaceae* have resulted in increased mortality rate and economic burden exponentially increased globally. Urinary tract infections in the general population have been reported as the second most common infections, especially those in the community settings and as well as in long-term care facilities” (Robichaud, 2008). “*Escherichia coli* has been reported as the most common cause of urinary tract infections in both adults and older adults’ population” (Orr *et al*., 1996). “Also, followed by *Klebsiella* species. *Proteus mirabilis* and *Staphylococcus* species are causative agents of UTIs” (Foxman, 2002). “The misuse and overuse of broad-spectrum antibiotics, mainly cephalosporins, must be contributive to selection and wide spread of multidrug resistant bacteria associated with UTI” (Nwana et al., 2020). “These bacteria produce enzymes that develops mechanisms for acquiring and disseminating plasmids, transposons, phages and other genetic determinants which aids in antibiotic resistance. Though, the use of different classes of antibiotics in UTIs treatments has been widely discussed and subjected to constructive criticisms by the scientific community since the inception of UTI. The development and emergence of multidrug resistance among bacteria associated with UTI have become a major health challenge. However, it is important to note that poverty is a risk factor that could lead to poor sanitation, poor education/communication, malnutrition due to starvation, immunodeficiency and poor health management due to lack of resources, all of which may propagate multidrug resistance”. (Byarugaba, 2004). All these flashpoints highlighted above, if not properly monitored and managed, could lead to an increase in the morbidity and mortality rate of UTIs amongst the geriatric population as emergence of multi-drug-resistant strains of uropathogens are on the rise. Therefore, this study will not only focus on the susceptibility pattern of the UTI causative organisms but also more on the molecular analysis outcome of the geriatric population.

“Resistance to antibiotics is one of the main public health problems of the near future” (Bush, 2018). “Resistance is an ancient phenomenon related to many factors including the excessive use of antibiotics and alternative medicine in humans and cross transmission of resistant strains from humans to humans” (Monsi et al., 2017). The emergence and spread of extended-spectrum beta-lactamases among members of *Enterobacteriaceae* family of uropathogens is a major concern in the health sector and has posed public health problems worldwide. In the treatment of UTIs, the use of cephalosporins, monobactams, penicillin as prophylaxis treatment to prevent UTIs has been regarded as an important risk factor, contributing to the selection and spread of ESBL-producing *Enterobacteriaceae*. Multidrug resistant strains of uropathogens are reported worldwide. The misuse or abuse of antibiotics in handling UTIs inpatients has also promoted the development of highly specific antibiotic resistance mechanisms. Based on previous studies, resistance genes such as TEM, SHV, OXA, CTX-M, NDM, KPC, QnrA etc. in bacteria uropathogens have been identified. These genes are clinically relevant and contributive to difficulties in the treatment of urinary tract infections caused by these bacterial strains. Globally, there are several studies on urinary tract infections on the pediatric, adults and geriatric population but scanty information exists on the geriatric (elderly) population in our country especially, Port Harcourt metropolis, Rivers State. Therefore, a need to investigate these uropathogens and determine the prevalence, susceptibility pattern and molecular basis of the multi-drug resistance would be of great importance in the management of the UTI geriatric population in Port Harcourt, Rivers State.

Certain questions surrounding the occurrence, antibiotics susceptibility patterns and presence of multidrug resistance biomarkers in uropathogens need to be resolved. The aim of this study was to carry out molecular characterization of multidrug resistant *Enterobacteriaceae*-associated UTI in a geriatric population in Port Harcourt metropolis in Rivers State of Nigeria.

2. materials and methods

**2.1 Study Area**

This study was carried out in Port Harcourt metropolis, the capital of Rivers State, Nigeria. It is located between latitude 4055’N, and latitude 4055’ N and longitude 60 55’E and longitude 70 05’E in Rivers State.

**2.2 Study Design**

This is a cross-sectional study with convenient sampling carried out among geriatric populations (elderly population) in Port Harcourt, Nigeria. The study was carried out in the Department of Medical Microbiology of Rivers State University, Port Harcourt, Nigeria. This entailed sample collection, processing and identification of isolates using conventional and biochemical methods. The molecular analysis was carried out at the Nucleo-Metrix Molecular Laboratory, Bayelsa State.

**2.3 Sample Size Determination**

Sample size was determined using the equation as described by (Charan & Biswas, 2013) with precision or absolute error of 5% and 95% confidence interval. Sample size = **Z2 P(1-P)/d2**. Where **Z** = Standard normal variation = 1.96, **p**= Expected proportion of infections in the population based on previous study, **d**= absolute error at 95% confidence interval = 0.05. Using the prevalence observed amongst the elderly population as 11.03% as reported by (Omoregie *et al.,* 2010), approximately one hundred and fifty (150) urine specimens were recruited for this study from elderly subjects indicating signs and symptoms of urinary tract infection in Port Harcourt metropolis based on the aforementioned sample size formula.

**2.4 Exclusion and Inclusion Criteria**

Those elderly individuals who are ≥65 years and above did not give their consent to the study were excluded. Those elderly individuals who are ≥65 years and above give their consent to the study but were on antibiotics treatment/medication were excluded. Indications of signs and symptoms of urinary tract infection in elderly individuals who are ≥65 years and above but had not commenced antibiotics treatment/medication. Only elderly subjects who are≥65 years and above and willing to provide at least verbal and written consent were included in the study.

**2.5 Specimen Collection and Processing**

Ten milliliters (10 ml) clean-catch midstream urine specimens were collected in a sterile universal bottle from one hundred and fifty (150) geriatric patients at homes of the elderly in Port Harcourt, Rivers State University, inpatients and outpatients of Rivers State University Teaching Hospital (RSUTH) comprising 67 males and 89 females whose age ranged from ≥65 years and above. The urine samples collected in a sterile universal container was properly labeled with the age, sex, serially designated laboratory numbers and was immediately transported within 30 minutes to the medical microbiology laboratory in Rivers State University for urine examination and culture.

**2.6 Urine Examination**

**2.6.1 Macroscopic Examination**

The colour of the urine was observed and recorded as pale amber and clear, amber and slightly cloudy, colourless and cloudy etc urine is sometimes coloured red which may be an indication of presence of blood, a deep amber or deep brownish green with pile pigments and may also be coloured by drugs however, normal urine colours pale yellow. The urine specimens were spun at 3000 revolution per minute (rpm) for 15 minutes and the sediment for the presence of pus cells, epithelial cells, crystals, red blood cells and bacteria present. A count of pus cells ≥ 5 under high power field (× 40 objective) was considered to indicate infection.

**2.6.2 Bacteriological Examination**

A calibrated wire loop to deliver 0.001 ml was used to streak a clean-catched midstream urine samples onto each of the agar plates (CLED agar and MacConkey agar) and was incubated aerobically for 24 hours at 370C and counts were expressed in a colony forming unit (cfu) per milliliter (ml). A count of ≥ 105 cfu/ml was considered significant to indicate UTI.

**2.7 Conventional Isolation of Bacteria**

The isolates were distinguished by their growth characteristics on MacConkey agar and cysteine lactose electrolyte deficient (CLED) agar, incubated at 370C and examined after 24 hours of incubation.

**2.8 Antimicrobial Susceptibility Testing**

This was performed using the modified Kirby-Bauer disk diffusion method. The bacteria suspension in physiological saline solution, with a turbidity matched to 0.5 on McFarland standard, was prepared by picking up 1-2 colonies from pure culture. The suspension was spread using a sterile swab stick to make for uniformity on a Mueller-Hinton agar (Oxoid, UK).The following sixteen (16) Antimicrobial-impregnated (Oxoid, United Kingdom) agents comprising (μg/ disk) cefoxitin (30 μg), ceftazidime (10 μg), cefotaxime (30 μg), cefuroxime (30 μg), cefepime (30 μg) gentamicin (10 μg), clindamycin (2 μg),azithromycin (15 μg), amoxicillin-clavulanic acid (30 μg), ofloxacin (5 μg), oxacillin (1 μg), levofloxacin (5 μg), imipenem (10 μg), meropenem (10 μg), aztreonam (30 μg), and colistin (10 μg) were used by the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2017). These disks were placed at the culture medium surface using sterilized forceps. The plates were incubated at 370C for 24 hours. After incubation, the antimicrobials efficacy was determined by measuring the diameter of the zone of inhibition or clearance using meter rule and interpreted as per Clinical and Laboratory Standard Institution (CLSI, 2017). The bacteria strains were classified as Susceptible (S), Intermediate (I) or Resistance (R) based on the zone of inhibition after the outcome of the measurement (mm) according to (CLSI, 2017) guidelines. Hence, those *Enterobacteriaceae* isolate(s) that were resistant to three (3) or more different classes of antimicrobial agents were confirmed multidrug resistant (MDR) based on the pattern on Mueller Hinton agar (UK) after 24 hours of incubation with a measured (mm) zone of inhibition according to (CLSI, 2017) guidelines.

**2.9 Molecular Methods**

A total number of ten (10) representative of the multidrug resistant *Enterobacteriaceae* which includes; two pure colony of *Escherichia coli*, six of *Klebsiella pneumoniae* and two of *Proteus mirabilis* were inoculated into a sterile 60% glycerol in an Eppendorf tube and stored in a refrigerator at temperature of 2-40C before proceeding to Nucleo-Metrix Molecular Laboratory, Bayelsa State, where it was subcultured and pure colonies were picked up for the molecular analysis and detection of some resistance genes (NDM, KPC and QnrA).

**2.9.1 DNA extraction**

The bacterial genes were isolated according to the extraction protocol of ZR Fungal/Bacterial DNA MiniPrep™ (Catalog No. D6005). The ultra-pure DNA was quantified using the Nanodrop 1000 spectrophotometer and stored at -20 degree for other downstream reactions.

**2.9.2 Polymerase Chain Reaction**

The polymerase chain reaction (PCR) method was utilized for the 16S rRNA, NDM-1, KPC and QnrA genes of some genes responsible for resistance in *Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis.* The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 56ºC for 40 seconds; extension, 72ºC for 50 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator. The primers used for the research are tabulated in Table 1.

**Table 1. Oligonucleotide Sequence of Primers used**

|  |  |
| --- | --- |
| **Gene**  | **Primers** |
| 16S rRNA | 27F: 5'-AGAGTTTGATCMTGGCTCAG-3’ 1492R: 5'-CGGTTACCTTGTTACGACTT-3’ |
| NDM | F: 5' CGTTTGGCGATCTGGTTTTC-3’R: 5'-CGGAATGGCTCATCACGATC-3' |
| KPC | F: 5’-GCTCAGGCGCAACTGTAAG-3’ R: 5’-AGCACAGCGGCAGCAAGAAAG-3’ |
| QnrA | F: 5’-AGAGGATTTCTCACGCCAGG-3’ 5’-GCAGCACTATKACTCCCAAGG-3’ |

**2.9.3 DNA Sequencing**

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 μl BigDye® terminator v1.1/v3.1, 2.25 μl of 5 x BigDye sequencing buffer, 10 μM primer, and 2-10 ng PCR template per 100 bp. The sequencing conditions were as follows 32 cycles of 96°C for 10 seconds, 55°C for 5 seconds and 60°C for 4 minutes.

**2.9.4 Phylogenetic Analysis**

Obtained sequences were edited using the bioinformatics algorithm Ridom Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method as explained in Monsi et al. (2019).

**2.10 Data Analyses**

Statistical analysis was done using GraphPad Prism (version 8.02). Results were presented as Mean ±SD. Chi-square, One-way ANOVA, P-value of ≤0.05 were accepted as significant results (with turkey’s post) were the tools used. DNA sequencing was carried out using a targeted resequencing design on the MiSeq platform (Illumina, San Diego, California).

3. results and discussion

A total of 150 urine specimens were collected from geriatric patients aged ≥ 65 and screened for urinary tract infection. Out of these patients, 67 were male patients and 89 were female patients. The prevalence of urinary tract infection (UTI) among the elderly was 33.3%. The elderly females had (32.6%) higher than the males (31.3%) respectively as shown in Table 1.


Figure 1: Prevalence of Bacterial isolates that cause Urinary tract infection (UTI) among Geriatric population

Antimicrobial Susceptibility Profile of Bacterial Isolates

The antimicrobial susceptibility pattern showed the characteristics of the different antibiotics, their potency and pathogen resistance. To identify organisms with multi-drug resistance (MDR), the multiple antibiotic resistance (MAR) indices were calculated. MAR index values greater than 0.2 indicate high risk of resistant infections caused by misuse or abuse of antibiotics. However, the result revealed that only three (3 out of the 14) antibiotics showed significantly high sensitivity to all the bacterial isolates, these antibiotics include meropenem (58%), amoxicillin-clavulanic acid (66%), and imipenem (78%). The antimicrobial susceptibility pattern of bacterial isolates was expressed in percentage (%) as is shown in Table 2.

**Table 2 Antibiotics Susceptibility Profile of Bacterial Isolates**

|  |  |  |
| --- | --- | --- |
| **Class of antibiotics** | **Antibiotics tested** | **Resistance** |
| Cephalosporin | Cefoxitin (FOX) 30 μg | 16 (100) |
| Cephalosporin  | Ceftazidime (CAZ) 10 μg | 48 (96.0) |
| Cephalosporin | Cefotaxime (CTX) 30 μg | 49 (98.0) |
| Cephalosporin  | Cefuroxime Sodium (CXM) 30 μg | 43 (86.0) |
| Cephalosporin | Cefepime (FEP) 30 μg | 43 (86.0) |
| Floroquinolone | Levofloxacin (LEV) 5 μg | 41 (82.0) |
| Floroquinolone | Ofloxacin (OFX) 5 μg | 43 (86.0) |
| Carbapenem | Meropenem (MEM) 10 μg | 21 (42.0) |
| Carbapenem | Imipenem (IMI) 10 μg | 11 (22.0) |
| Aminoglycoside | Gentamycin (GM) 10 μg | 25 (50.0) |
| Lincosamide | Clindamycin (DA) 2 μg | 47 (94.0) |
| Monobactam  | Aztreonam (ATM) 30 μg | 42 (84.0) |
| Lipopeptide | Colistin (CT)10 μg | 40 (80.0) |
| Β-Lactam combination agent | Amoxicillin-clavulanic acid (AMC) 30 µg | 17 (34.0) |
| Macrolide | Azithromycin (AZM) 15 μg | 39 (78.0) |
| Penicillinase-stable | Oxacillin (OX) 1 μg | 50 (100.0) |

With respect to the ten (10) multidrug resistant *Enterobacteriaceae* isolates that were subjected to molecular assay for the detection of targeted resistant genes of KPC, NDM-1 and QnrA revealed that NDM-1 targeted gene was detected in one of the *Klebsiella pneumoniae* while the KPC targeted gene was detected in another *Klebsiella pneumoniae* and *Escherichia coli* isolates as shown in Table 2 respectively. With respect to the Agarose Gel Electrophoresis showing the Amplified KPC gene, lane 6 and 7 represents the KPC gene band at 600 bp while lanes 1, 2, 3, 4, 5, 8, 9 and 10 were negative for KPC and lane C represents the 100 bp Molecular ladder as shown in Figure 2. With respect to the agarose gel electrophoresis showing the amplified NDM-1 gene, lane 1 represents the NDM-1 gene band at 550 bp while lanes 2, 3, 4, 5, 6, 7, 8, 9 and 10 were negative for NDM-1 and lane C represents the 100 bp Molecular ladder as shown in Figure 3.

The obtained 16S rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Klebsiella* speciesand *Escherichia coli and Proteus* species and revealed a close relatedness *to Klebsiella pneumoniae, Escherichia coli* and *Proteus mirabilis.* In (Figure 4) *Escherichia coli* isolates, M-4 and M-6 showed a close relatedness to *E. coli* strain (CP102379), the *Proteus mirabilis*, P-5 and P-2 showed a closely relatedness to *P. mirabilis* (OP247569) while the *Klebsiella pneumoniae,* K-11 was close related to *K. pneumoniae* strain (OP102086), M-10 was close related to *K. pneumoniae* strain (CP104678) and P-15, K-4, E-2 and E-7 were close related to *K. pneumoniae* strain (CP104659).

**Table 3. Distribution of KPC, NDM and QnrA Genes in *Enterobacteriaceae* Isolates**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S/N** | **Isolates** | **KPC** | **NDM** | **QnrA** |
| 1 | *K. pneumoniae* | - | + | - |
| 2 | *K. pneumoniae* | - | - | - |
| 3 | *K. pneumoniae* | - | - | - |
| 4 | *K. pneumoniae* | - | - | - |
| 5 | *K. pneumoniae* | - | - | - |
| 6 | *K. pneumoniae* | + | - | - |
| 7 | *E. coli* | + | - | - |
| 8 | *E. coli* | - | - | - |
| 9 | *P. mirabilis* | - | - | - |
| 10 | *P. mirabilis* | - | - | - |

Key: + = Presence of Genes, - = Absence of Genes

 

 1 2 3 4 5 C 6 7 8 9 10

600bp

1500bp

Figure 2. Agarose Gel Electrophoresis showing the Amplified KPC gene. Lane 6 and 7 represents the KPC gene band at 600bp while 1, 2, 3, 4, 5, 8, 9 and 10 were negative for KPC. Lane C represents the 100bp Molecular ladder.

 

550bp

 1 2 3 4 5 C 6 7 8 9 10

**Figure 3. PCR-Amplified NDM gene**. Lane 1 represents the NDM gene band at 550 bp while 2, 3, 4, 5, 6, 7, 8, 9 and 10 were negative for NDM. Lane C represents the 100 bp Molecular ladder.



**Figure 4. Evolutionary Relatedness between the Isolated *Enterobacteriaceae* from Geriatric subjects in Port Harcourt Metropolis**.

**Table 4. Some Molecular Profile of 10 Multidrug resistant *Enterobacteriaceae* Isolates**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gender** | **Age** | **Code** | **Sample** | **CT** | **CTX** | **LEV** | **GM** | **IMI** | **ATM** | **CXM** | **AZM** | **AMC** | **MEM** | **FEP** | **OFX** | **OX** | **CAZ** | **DA** | **KPC** | **NDM** | **QNR** |
| Male | 70 | K11 | *K. pneumoniae* | R | R | R | S | R | R | R | R | R | S | R | S | S | S | R | NEG | POS | NEG |
| Female | 74 | K10 | *K. pneumoniae* | R | R | R | R | R | R | R | R | R | R | R | S | R | S | R | NEG | NEG | NEG |
| Female | 65 | K2 | *K. pneumoniae*  | R | R | R | R | R | S | R | R | R | R | R | S | R | S | R | NEG | NEG | NEG |
| Female | 81 | K4 | *K. pneumoniae*  | R | R | R | R | R | R | R | R | R | R | R | S | R | R | R | NEG | NEG | NEG |
| Male | 71 | K5 | *K. pneumoniae*  | R | R | S | S | R | S | R | R | R | R | R | R | R | S | R | NEG | NEG | NEG |
| Female | 68 | K7 | *K. pneumoniae* | R | R | S | S | R | R | R | R | R | R | R | S | R | S | S | POS | NEG | NEG |
| Female | 80 | C4 | *E. coli* | R | R | S | R | R | R | R | R | R | R | R | R | R | S | R | POS | NEG | NEG |
| Male | 77 | C6 | *E. coli* | R | R | S | R | R | R | R | R | R | R | R | S | R | S | S | NEG | NEG | NEG |
| Female | 75 | P2 | *P. mirabilis* | S | R | R | S | S | S | R | R | R | R | R | S | S | S | R | NEG | NEG | NEG |
| Male | 86 | P5 | *P. mirabilis* | R | R | R | R | R | R | R | R | R | S | R | R | R | R | R | NEG | NEG | NEG |

4. discussion

This study investigated and determined the prevalence of UTI in geriatric patients, etiological agents, antimicrobial susceptibility pattern and molecular characterization of some multi-drug resistant Enterobacteriaceae isolates in the elderly patients in Port Harcourt metropolis. In this study, a total of 150 urine specimens were collected from subjects aged ≥ 65 and screened for urinary tract infections, out of the total patients, 67 were male patients and 89 were female patients. This study revealed that 50 patients had UTI with a prevalence of 33.3%. out of which 21 males had UTI (31.3%) while 29 females had UTI (32.6%) respectively. This implies that the female patients had a higher prevalence of UTI than male patients and this may be due to the close proximity of their urethra in the urinary tract, this report is in agreement with a research carried out by Manges *et al.,* (2008) which reported that females are of high risk of urinary tract infection due to their short urethra and lifestyle factors which includes delay in micturition, sexual activity and the use of spermicides which promote colonization of the periurethral area. This study prevalence with respect to age range showed that the chance of contracting urinary tract infection increases with increase in age as elderly people who are 80years and above had the highest cases of UTI (35.9%) compared to 65–69years that had (30.6%) and this finding is in agreement with report by (Aiyegoro *et al.,* 2007; Robichaud *et al*., 2008) and disagrees with the research report of Omeregie *et al.,* (2010). This study revealed that the female patients harbored more *Enterobacteriaceae* isolates than male patients especially the *E. coli* and this is in an agreement with a research report by Manges *et al.,* (2008) which noted that *E. coli* are the most common cause of urinary tract infections in women, and this could be probably because of the anatomical structure of women reproductive system.

The elderly are at increased risk of UTI, it has been reported as the second most common infection in the community setting and long-term care facilities Aiyegoro et al., (2007). This study showed that the elderly females have an increased risk of developing UTI compared with males, and this is in agreement with previous research reports by Omeregie et al., (2010) and this further agrees with a research carried out by Manges et al., (2008) which reported that females are of high risk to urinary tract infection due to their short urethra and lifestyle factors which include delay in micturition, sexual activity and the use of spermicides which promote colonization of the periurethral area. While chronic urinary retention associated with prostate hypertrophy, bacterial prostatitis, and incontinence could be the principal risk factors for UTI in elderly males. It has been suggested that there is an increased prevalence in comorbidities, a known risk factor for UTI in the elderly, between 60 and 69 years according to Robichaud *et al* (2008). This study found that *E. coli* was the most predominant isolates causing UTI in both sexes of the study population. This is in agreement with the research report by (Ronald, 2002) but it is in contrast with research reports by Omeregie et al.,(2010). This study revealed that there is a significant increase in the emergence of multidrug resistant bacteria (uropathogens) especially among the *Enterobacteriaceae* family and this is in agreement with the recent research report of an increase in the emergence of resistant uropathogens to antimicrobial agents among the geriatric (elderly) population (Robichaud et al., 2008; Omeregie et al., 2010).

This study antimicrobial susceptibility pattern results revealed that the *Escherichia coli* isolated had an average MAR of 0.74, and this index is far above the 0.2 benchmark for multi-drug-resistant organisms. This implies that the said organisms possess very high resistance to antibiotics used. However, the result also revealed that only three (3 out of the 14) antibiotics used showed over 50% sensitivity to all isolates, these antibiotics include Imipenem (73.6%), Amoxicillin (68.4%) and Gentamicin (52.6%). Therefore, these antibiotics are best fit to manage multi-drug-resistant urinary tract infection caused by *Escherichia coli*.

Also, the result of the antimicrobial pattern of the *Klebsiella pneumoniae* isolates showed an average MAR of 0.76 and this index is far above the 0.2 benchmark for multidrug resistant organisms. This implies that the said organisms possess very high resistance to antibiotics used. Although, the result revealed that only two (2 out of the 14) antibiotics showed significantly high sensitivity to all isolates, these antibiotics include Amoxicillin (90%) and Imipenem (80%). Therefore, these antibiotics are recommended as the first line of antibiotics to manage multidrug resistant urinary tract infection caused by *Klebsiella pneumoniae*.

Similarly, the result of the antimicrobial pattern in *Proteus mirabilis* had an average MAR of 0.69 and this index is above the 0.2 benchmark for multi-drug-resistant organisms. This implies that the *Proteus mirabilis* possess high resistance to antibiotics used. However, the result revealed that only two (2 out of the 14) antibiotics showed over 50% sensitivity to all isolates, these antibiotics include Imipenem (60%) and Meropenem (60%). Therefore, these antibiotics are recommended as the first line of antibiotics to manage multi-drug-resistant urinary tract infection caused by *Proteus mirabilis*.

The antimicrobial activity for *Staphylococcus aureus*, the only Gram-positive rod linked with UTI in this study possessed an average MAR of 0.70. The average multiple antibiotic resistance (MAR) index value is greater than 0.2 which implies a high resistance of antibiotics used. Nevertheless, the result revealed that one-third of the antibiotics (5 out of 15) recorded above 50% antimicrobial sensitivity, and they are Imipenem (94%), Meropenem (73%), Amoxicillin (62.5%), Gentamicin (56%), and Azithromycin (50%). Indeed, these antibiotics can be suggested for treatment of multi-drug-resistant urinary tract infection caused by *Staphylococcus aureus*.

This study showed that most of the isolates out of the 50 bacterial spp. associated with the UTI are multidrug resistant (MDR) organisms. Most of the bacterial isolates were resistant to the penicillins, cephalosporins, floroquinolones, this can occur by bacteria production of β-lactamases which decreases the permeability of the antibiotics by changes in porin channels in the cell wall or reduced susceptibility of bacteria cells toward antibiotic through upregulation of efflux pumps and through other mechanisms according to report by Ye *et al*., (2017). This study revealed there was a low level of resistance to Carbapenems (imipenem & meropenem), betalactam combination agent (amoxicillin-clavulanic acid) and Aminoglycoside (gentamycin) among the bacterial organisms. The few carbapenems resistance in this study could be due to production of carbapenemase genes as detected during this study molecular analysis and this could be due to misuse and/or self-medication of carbapenems antibiotics which may have a role in the development of these carbapenemase genes in some of the *Enterobacteriaceae* strains detected during the molecular analysis but was phenotypically susceptible (sensitive). However, this study showed that carbapenems (imipenem and meropenem) remain among the most effective antibiotics for the treatment of UTI caused by *Enterobacteriaceae* isolates in geriatric patients and this is in agreement with a study report by Kamel *et al.,* (2014).

From the multidrug resistant isolates, ten (10) *Enterobacteriaceae* isolates were carefully selected for molecular study, the outcome of the molecular study revealed 30% presence of these targeted genes. The KPC genes had 2 (20%) detected in *Klebsiella pneumoniae* and *Escherichia coli* isolates respectively. This finding is consistent with similar research by (Ajuba *et al*., 2020; Asuquo *et al*., 2022). The NDM resistance gene was only 1 (10%) detected in another *Klebsiella pneumoniae* isolate. This finding is in agreement with a research report by Poirelet al., (2011) that observed only one NDM positive case of bacteria isolate in their research. Whereas, the qnrA were all negative, with no detectable targeted gene among the 10 *Enterobacteriaceae* isolates and this could be due to the scope of this study was limited only to target for qnrA gene whereas there are other types of qnr genes which includes qnrB, qnrS and so on and according to studies, qnrB and qnrS genes have been reported the most prevalent in Africa (Moumouni *et al*., 2017; Salah *et al*., 2019).

Phylogenetic analysis was carried out on ten (10) carefully selected multidrug resistant *Enterobacteriaceae* isolates from the geriatric patients in Port Harcourt Metropolis, Rivers State. Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) database using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method.

The obtained 16S rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolates showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method agreed with the phylogenetic placement of the 16S rRNA of the isolates within the *Klebsiella* and *Escherichia and Proteus* species and revealed a close relatedness *to Klebsiella pneumoniae, Escherichia coli* and *Proteus mirabilis.* Phylogenetics is important as it enriches our understanding of how genes, genomes and species evolve. It gives us a more accurate description of pattern of relatedness than was available before the advent of molecular sequencing. In general, all the *Enterobacteriaceae* isolates of (*Escherichia coli, Proteus mirabilis* and *Klebsiella pneumoniae)* showed a slightly varied phylogenetic group, indicating their heterogeneity.

5. Conclusion

This study revealed high prevalence of UTI among the geriatric patients with the elderly females having a higher prevalence of UTI than the elderly males. The older geriatrics (≥80years and above) have higher prevalence of UTI than younger geriatrics in their 60’s. This study showed that the commonest cause of UTI was *E. coli* followed by *S. aureus*, *K. pneumoniae* and the least was *P. mirabilis*. Also, revealed high number of multidrug resistant bacterial isolates associated with urinary tract infection (UTI). The following antibiotics: imipenem, meropenem, amoxicillin-clavulanic acid and gentamycin were the most sensitive to all the bacterial uropathogens isolated in this study. Multidrug resistant *E. coli, K. pneumoniae, P. mirabilis and S. aureus* isolates were present in the geriatric population and some of the MDR *Enterobacteriaceae* strains (*E. coli, K. pneumoniae* and *P. mirabilis*) isolated from the geriatrics possess some resistant genes of (KPC and NDM-1) which enable them to show some resistance to these classes of antibiotics; cephalosporins, penicillinase-stable and carbapenems. In addition, resistant genes that code for KPC and NDM-1 were detected in this area whereas resistance gene that code for QnrA was not detected.

Consent

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

Ethical approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. An ethical approval was obtained from the Health Research Ethics Committee of the Rivers State Hospitals Management Board, through the Rivers State Ministry of Health with an approval number of RSHMB/RSHREC/2022/028.

A well-structured questionnaire was issued to all the participants to obtain demographic information, medical history and lifestyle after obtaining verbal and written consent of participants prior to enrolment.

Disclaimer (Artificial intelligence)

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1.

2.

3.

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