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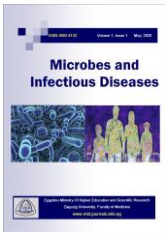

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Original article

Characterization of multidrug-resistant genes of *Klebsiella pneumoniae* from clinical isolates in Asaba, Delta State, Nigeria

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ABSTRACT

Background: The awakening of multidrug-resistant (MDR) *Klebsiella pneumoniae* strains is a major public health issue. Thus, this study aims to characterize MDR genes of *K. pneumoniae* isolates from clinical samples. **Methods**: This is a descriptive cross-sectional study conducted at the Federal Medical Centre, Asaba. Cultural and biomedical methods were employed in identifying *K. pneumoniae*. The modified Kirby-Bauer disc diffusion method was used for the antibiotic susceptibility. The double-disc synergy test revealed ESBL production, Imipenem disc phenotypically assessed carbapenemase production using a modified Hodge test, and multiplex PCR was used to identify resistant genes. **Results:** Out of 365 specimens, 51 (13.97%) isolates were positive for *Klebsiella* species and 21 (41.18%) *K. pneumoniae*, having been identified using API 20E. The resistant patterns of *K. pneumoniae* isolates were amoxicillin 100%, augmentin 38.1%, gentamicin 38.1%, ciprofloxacin 38.1%, nitrofurantoin 100%, cefotaxime 100%, ceftazidime 52.3%, ceftriaxone 38.1%, cefpodoxime 100%, cefuroxime 38.1%, ofloxacin 48.0%, tetracycline 52.3%, imipenem 4.80%, levofloxacin 33.3%, and meropenem 100%, respectively. 90% of *K. pneumoniae* samples had ESBL-producing properties. The genotypical ESBL genes detected were SHV 61.9% (13) CTX-M 76.2% (16), and QnrB 23.8% (5), while mixed genes of SHV and CTX-M 52.4% (11), SHV, CTX-M and QNRB 19.1% (4). Carbapenem-producing *K. pneumoniae* was detected in 4.76% (1) phenotypically, and genotypically, KPC was identified. **Conclusion:** The study emphasizes the widespread nature of MDR *K. pneumoniae* strains in clinical settings.
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Introduction

Gram-negative *Klebsiella pneumoniae* bacteria, are naturally occurring in the mouth, skin, and intestines, as well as in soil and water. A non-motile rod-shaped bacterium called *K. pneumoniae* is responsible for several hospital illnesses [1]. This microbe can cause septicemia, meningitis, pneumonia, burns, and urinary tract infections (UTI). Nosocomial infections are prevalent in hospitalized patients nationwide (8.7%), and immunocompromised individuals are more likely to suffer from complications [2]. Multi-drug resistant (MDR) enteric bacterial infections are a global problem, especially in low-and middle-income countries (LMICs). These regions have the world's greatest antimicrobial-resistant (AMR) infection burden, with 14.8 deaths per 100,000 people. Despite this, there is a scarcity of medical

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literature addressing this pressing issue in these places. Carbapenem-resistant Enterobacteriaceae (CRE) resistance patterns frequently extend beyond beta-lactam antibiotics [3,4]. Antimicrobial-resistance is a prominent cause of gastrointestinal illnesses, particularly in MDR, and extensively drug-resistant (XDR) bacteria. Antibiotic overuse and misuse cause selective pressure, which promotes resistance and increases the burden of MDR enteric bacteria among healthy people [5]. Horizontal gene transfer also aids in the sustenance and spread of AMR because plasmids and transposons harboring AMR genes are easily shared across intestinal bacteria [6].

Currently, the care of infectious diseases focuses on those with symptoms while neglecting those without any symptoms, particularly in LMICs where enteric infections are prevalent, and transmission is high because of poor hygiene. Although asymptomatic carriers of pathogenic enteric bacteria play a crucial role in the persistence and transmission of disease, they are often overlooked in research investigations [7]. Antibiotic-resistant genes (ARGs) are primarily found in a healthy human microbiome and can spread to other dangerous bacteria. MDR enteric infections disproportionately impact children, and AMR enteric bacterial infections are a leading cause of newborn sepsis in Nigeria [8]. Despite the significant burden caused by MDR organisms, there is inadequate research addressing MDR-E strains in Nigeria, Africa's largest populated country [9]. This lack of research limits our capacity to properly deploy targeted antimicrobial stewardship and epidemiological control approaches [4]. To address these essential knowledge lapses, we conducted a thorough study to examine MDR genes found in *Klebsiella pneumoniae* isolates from clinical isolates obtained from patients at the Federal Medical Centre in Asaba, Delta State, Nigeria.

Our research seeks to provide crucial knowledge for designing effective interventions, optimizing treatment techniques, and directing infection control practices in LMICs by clarifying resistance mechanisms and gaining insight into the present strain landscape. Thus, this investigation aims to characterize multidrug-resistant genes in *Klebsiella pneumoniae* isolates from clinical samples of patients visiting the Federal Medical Centre, Asaba, Delta State, Nigeria.

Materials and Methods

Study design, location, and sample size

This study was a cross-sectional descriptive research project, conducted at the Federal Medical Center in Asaba, specifically at the Medical Microbiology/Parasitology Laboratory Division of the Department of Medical Laboratory Services, Delta State, Nigeria. This Medical Facility provides a comprehensive range of medical and healthcare services not only to the residents of Asaba but also as a referral facility for numerous hospitals across a wide geographical area. Building upon previous research, we utilize Cochran's formula for cross-sectional sample size determination [10]. While ensuring a 95% confidence interval, and a 5% allowable error, the prevalence rate of 34% for *K. pneumoniae* was taken into consideration in calculating the sample size [11], and a total sample size of 345 was obtained.

Inclusion criteria and exclusion criteria

The study included all isolates of Klebsiella spp. recovered from a variety of clinical samples, such as sputum, urethral swabs, blood cultures, urine, stool, peritoneal fluid, wound swabs, high vaginal swabs, pleural fluid/aspirate, and nasal swabs. Between December 2022 and April 2023, samples were taken from patients receiving care at the Asaba Specialist Hospital and the Federal Medical Centre, Asaba, from in and outpatients. Any duplicate isolates from the same patient were not included in the analysis unless obtained from different specimens with discernible susceptibility patterns.

Sample Processing

From growth media, bacterial colonies that exhibited traits suggesting they were Klebsiella spp. were extracted. These traits included big size, mucoid appearance, and red pigment that seeped into the surrounding agar due to lactose fermentation and acid generation. Following inoculation onto trypticase soy agar slants, these colonies were cultured for 18 to 24 hours at 37 °C before being stored at 4 °C.

Identifying Isolates of *Klebsiella pneumoniae*

Several factors were used to identify *Klebsiella pneumoniae*, including the colony morphology on MacConkey Agar, the Gram staining reaction, and a number of biochemical tests, including those that measure gas production, motility, citrate utilization, indole test, catalase, oxidase, and urease. To guarantee the accuracy of

the isolated strains, a control strain was obtained from the Microbiology laboratory and used in culture, biochemical assays, and other phenotypic assessments. Using API 20E (LiofichemsrlRosto d. Abruzzi (TE), Italy, Enterosystem 18 R) and profiles 344577, the isolates of *K. pneumoniae* were confirmed in accordance with the manufacturer's instructions.

Antimicrobial susceptibility test

Using Mueller-Hinton (MH) agar (HiMedia, Mumbai, India) and the agar disc diffusion method (Oxoid Ltd., Wade Road, Basingstoke, Hants, RG24 8PW, UK and Mast Group Ltd., Mast House, Derby Road, Bootle, Merseyside, L20 IEA, UK), antimicrobial susceptibility testing for *K. pneumoniae* was carried out. The investigation evaluated K. *pneumoniae's* resistance to a range of antimicrobial agents, such as imipenem, cefpodoxime (30 µg), levofloxacin (5ug), ofloxacin (5ug), meropenem (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), amoxicillin (AX) (25 ug), amoxicillin/clavulanate (20/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), and gentamicin (10 µg). Peptone broth was used to cultivate the organisms, and the suspension's turbidity was adjusted to meet the 0.5 McFarland criterion. The inhibitory zone diameters were calculated and compared to the interpretation criteria suggested by CLSI guidelines (2022) after overnight incubation at 37˚C. The characteristic of MDR *Klebsiella pneumoniae* is its acquired resistance to one or more antimicrobial agents across three or more categories. These categories include penicillins, aminoglycosides, first and second-generation cephalosporins, extended-spectrum cephalosporins, carbapenems (third and fourth-generation cephalosporins), β-lactamase inhibitors, and fluoroquinolones [12]. ESBL-positive *K. pneumoniae* and ESBL-negative *Escherichia coli* ATCC 25922 control strains were employed to identify ESBLs. To identify carbapenemase, the strain of *E. coli* ATCC 25922 was used as a negative control.

Drug resistance pattern detection phenotypically

DDST for ESBL Identification

The initial screening for ESBL-producing pathogens involved evaluating the profile of antibiotic susceptibility testing (AST). Strains exhibiting decreased susceptibilities to CAZ (ceftazidime) and/or CTX (cefotaxime) were provisionally classified as ESBL producers. They were then verified in compliance with the CLSI's (Clinical and Laboratory Standards Institute) requirements. Mueller Hinton Agar (MHA) was used in the DDST, which was carried out as a typical disc diffusion assay. Third-generation cephalosporin discs (ceftazidime, ceftriaxone, and cefpodoxime) were positioned 20 mm apart from the center of the amoxicillin/clavulanic acid disc, which contained amoxicillin/clavulanic acid (20/10 µg) on it. Based on phenotypic traits, an increase in the inhibition zone towards the amoxicillin/clavulanic acid disc was seen as a favorable outcome for the ESBL (12M) development [13].

Modified Hodge test for carbapenemase detection

The surface of a Mueller-Hinton agar plate was uniformly inoculated with an overnight culture suspension of *Escherichia coli* control stain, ATCC 25922, cultured in peptone broth, using a cotton swab to conduct the carbapenem hydrolysis test. The suspension was tuned to a tenth of the McFarland standard turbidity of 0.5. Following inoculation, the plate was left to remain at room temperature for fifteen minutes to allow for a quick drying period. A disc containing 10 µg imipenem was then positioned in the middle of the plate. Extracted from the overnight culture plates, the test strains were widely streaked from the disc's edge toward its perimeter. After that, the plate was incubated at 37 °C for the entire night. The next day, the existence of a deformed zone of inhibition around the streaking region was regarded as a positive result demonstrating carbapenem hydrolysis, especially at the intersection of the streak and the zone of inhibition [14].

Genetic analysis of MDR *Klebsiella pneumoniae* isolates

Detection of antibiotic resistance genes (ESBLs encoding genes)

The PCR analysis was performed at the Nucleometrix Research Laboratory, located off Imiringi Road in Yenagoa, Bayelsa State, Nigeria. The specific genes targeted in this study were SHV, CTX-M, KPC, and QnrB. Inqaba Biotech provided the primers used for the amplification procedure (525 Justice Mahomed St, Muckleneuk, Pretoria, 0002, South Africa). *Klebsiella pneumoniae* DNA was extracted using the boiling technique. Isolates were subcultured for analysis and then purged with PCR after an overnight incubation at 37°C. Gel electrophoresis was used to document the amplified products [15].

SHV gene amplification

The SHV F primer (5' CGCCTGTGTATTATCTCCCT-3') and SHV R primer (5'-CGAGTAGTCCACCAGATCCT-3') were used to amplify the SHV genes from the isolates. The PCR reactions were carried out using the ABI 9700 Applied Biosystems thermal cycler for 35 cycles in a final volume of 30 microliters. Taq polymerase, DNTPs, and MgCl were included in the X2 Dream taq Master mix, which was provided by Inqaba, South Africa, for the PCR mix. 50ng of the isolated DNA was utilized as the template, and 0.4M of primers were employed. The following were the conditions for the PCR: 5 minutes of initial denaturation at 95ºC, 30 seconds of denaturation at 95ºC, 40 seconds of annealing at 56ºC, 35 cycles of 50 seconds of extension at 72ºC, and 5 minutes of final extension at 72ºC. After that, the PCR product was separated on a 1% agarose gel and exposed to 120 volts for 25 minutes. A UV transilluminator was used to determine the 281 bp product size.

CTX-M genes amplification

Using the CTX-M F: primer (5'-CGCTTTGCGATGTGCAG-3') and CTX-M R: primer (5'-ACCGCGATATCGTTGGT-3'), the isolates' CTX-M genes were amplified. An ABI 9700 Applied Biosystems thermal cycler was used to perform the amplification procedure, with a final volume of 40 microliters and 35 cycles. Taq polymerase, DNTPs, and MgCl were included in the X2 Dream taq Master mix, which was provided by Inqaba, South Africa, for the PCR mix. 50ng of the isolated DNA was utilized as the template, and 0.4M of primers were employed. The following were the conditions for the PCR: 5 minutes of initial denaturation at 95ºC, 30 seconds of denaturation at 95ºC, 30 seconds of annealing at 52ºC, 30 seconds of extension at 72ºC for 35 cycles, and 5 minutes of final extension at 72ºC. Following 25 minutes of resolution on a 1% agarose gel at 120V, the PCR product was seen with a UV transilluminator to reveal a 550 bp product size.

QnrB gene amplification

Using the QNRB F: primer (5'-GATCGTGAAAGCCAGAAAGG-3') and QNRB R: primer (5'-CGATGCCTGGTAGTTGTCC-3'), the isolates' QNRB genes were amplified. An ABI 9700 Applied Biosystems thermal cycler was used to carry out the amplification process, with a final volume of 40 microliters used for 35 cycles. The X2 Dream taq Master mix, which included taq polymerase, DNTPs, and MgCl, was provided by Inqaba, South Africa, and made up the PCR mixture. A 0.4M concentration of primers was employed, and 50ng of the isolated DNA was used as the template. The annealing temperature was set to 58 °C for 30 seconds, the extension temperature was set to 72°C for 30 seconds for 35 cycles, and the final extension temperature was set to 72°C for five minutes. These were the settings for the PCR. The 400 bp product size of the PCR result was then observed using a UV transilluminator after it had been resolved on a 1% agarose gel at 120V for 25 minutes.

KPC genes amplification

Using the KPC F: primer (5'-GCTCAGGCGCAACTGTAAG-3') and KPC R: primer (5'-AGCACAGCGGCAGCAAGAAAG-3'), the isolates' KPC genes were amplified. An ABI 9700 Applied Biosystems thermal cycler was used to carry out the amplification process, with a final volume of 40 microliters used for 35 cycles. Taq polymerase, DNTPs, and MgCl were included in the X2 Dream Taq Master mix, which was provided by Inqaba, South Africa, for the PCR mix.A 0.4M concentration of primers was employed, and 50ng of the isolated DNA was used as the template. The annealing temperature was set to 58 °C for 30 seconds, the extension temperature was set to 72°C for 30 seconds for 35 cycles, and the final extension temperature was set to 72°C for five minutes. These were the settings for the PCR. The 400 bp product size of the PCR result was then observed using a UV transilluminator after it had been resolved on a 1% agarose gel at 120V for 25 minutes.

Data analysis

SPSS software Version 23 (SPSS Inc., Chicago, IL, USA) was used to conduct descriptive statistics to summarize the data. The numerical data were displayed as percentages (%) and counts (n). Inferential statistics were done to investigate relationships between various variables like patient demographics, antibiotic resistance genes, and antimicrobial susceptibility patterns. Furthermore, cross-tabulations and Chi-square tests were conducted to identify any statistically significant relationships between the variables under investigation, with the significance level set at 0.05.

Ethical approval

Ethical approval was obtained from the Ethics Committee, Federal Medical Centre, Asaba, Delta State, Nigeria, with the reference number FMC/ASB/A81VOL.XII/313.

Results

This research was to characterize the MDR genes of *K. pneumoniae* isolated from clinical isolates in Asaba. 184(50.41%) urine specimens were collected for this study, of which 22(41.13%) yielded *Klebsiella spp.* and 9(42.9%) *K. pneumoniae*. 65(17.81%) sputum samples were cultured for this study, which yielded 13(25.49%) *Klebsiella spp* and 7(33.3%) *K. pneumoniae* isolates. 60(16.45%) wound swab specimens were collected, of which 7(13.72%) *Klebsiella spp* and 2(9.52%) *K. pneumoniae* were isolated. Out of 3(0.82%) blood cultured samples, 3(5.89%) *Klebsiella* species and 2(9.52%) *K. pneumoniae* were isolated. No *Klebsiella* species or *K. pneumoniae* were found in the 2(0.54%) eye swab samples collected, 2(0.54%) urethra samples, and 3(0.82%) throat swab samples collected. In respect of the 36(9.87%) high vaginal swabs obtained for this study, 5(9.81%) *Klebsiella* species were isolated without *K. pneumoniae* isolates. 10(2.74%) ear swabs were cultured in this study, which yielded 1(1.96%) *Klebsiella* species and 1(4.76%) *K. pneumoniae* isolates. A statistical correlation between various study samples and positive isolates showed no statistical significance at $p>0.05$.

The antimicrobial susceptibility pattern of confirmed isolated *K. pneumoniae* to various antibiotics was reported. Amoxillin+Clavulanate (20/10ug) expressed a sensitivity level of 5(23.8%), an intermediate level of 8(38.1%), and a resistant level of 8(38.1%) on *K. pneumoniae* isolates. Gentamicin (10ug) exhibited a sensitivity level of 13(61.9%) and a resistance level of 8(38.1%). Nitrofurantoin (30ug) showed no sensitivity or intermediate levels, with only a resistance level of 9(100%). Tetracycline expressed a sensitivity level of 9(42.9%), an intermediate level of 1(4.80%), and a resistance level of 11(52.3%). Ciprofloxacin (5ug) showed a sensitivity level of 5(23.8%), an intermediate level of 8(38.1%), and a resistance level of 8(38.1%). A sensitivity level of 7(33.3%), an intermediate level of 4(19.1%), and a resistance level of 10(48.0%) of Ofloxacin were observed. Levofloxacin (20/10ug) expressed a sensitivity level of 13(61.9%), an intermediate level of 1(4.80%) and a resistance level of 7(33.3%). Cefuroxime expressed a sensitivity level of 6 (28.6%), an intermediate level of 7 (33.3%), and a resistance level of 8(38.1%). Ceftazidime (30ug) showed a sensitivity level of 6(28.6%), an intermediate level of 4(19.1%), and a resistance level of 11(52.3%) on *K. pneumoniae* isolates. Cefotaxime, cefpodoxime, and meropenem showed no sensitivity or intermediate levels, with only a resistance level of 21(100%) on *K. pneumoniae* isolates. Ceftriaxone (30ug) had a sensitivity effect of 10(47.6%), an intermediate effect of 13(14.3%), and a resistance effect of 8(38.1%) on *K. pneumoniae* isolates. Imipenem (10ug) exhibited a sensitivity level of 20(95.2%) and a resistance level of 1(4.80%). It is important to note that Imipenem (10ug) possesses the highest level of sensitivity 20(95.2%) and *K. pneumoniae* isolates expressed the highest level 21(100%)) of resistance to amoxicillin, cefotaxime, cefodoxime, and meropenem antibiotics. In patient samples between the ages of 0-15 years, the samples yielded *K. pneumonia* isolates in male 1(12.5%), and female 1(7.69%) samples. Among patients within the age group of 31-45 years, 3(37.5%) *K. pneumoniae* were isolated from the male samples, while 4(30.76%) *K. pneumoniae* were isolated from the female samples. 4 (50.00%) *K. pneumoniae* were isolated from male patients with an age greater than 60, and 1(7.69%) *K. pneumoniae* was isolated from female patients of the same age group. There were no *K. pneumoniae* isolates in male individuals within the age groups of 16-30 and 45-60 years; however, 5(38.46%) and 2(15.4%) *K. pneumoniae* isolates, respectively, were found in the female counterparts of these age groups. There was no statistically significant association between age groups, gender, and resistant isolates at $p>0.05$. Based on gender differences, 8(38.1%) *K. pneumoniae* and 8(38.1%) MDR *K. pneumoniae* isolates were found in the male sample; on the other hand, 13(61.9%) *K. pneumoniae* and 13(61.9%) MDR *K. pneumoniae* isolates were found in female samples. Distribution of *K. pneumoniae* and MDR *K. pneumoniae* isolates based on the study site presented as Asaba Specialist Hospital, Asaba with 4(19.0%) *K. pneumoniae* and MDR *K. pneumoniae* isolates and Federal Medical Center, Asaba with 17(81.0%) *K. pneumoniae* and 17(81.0%) MDR *K. pneumoniae* isolates. A cross-tabulation between gender, study site, and resistant isolates (*K. pneumoniae* and MDR *K. pneumoniae*) showed no statistical significance at $p>0.05$. Urine and aspirate samples were collected from both inpatients and out-patients for this study. No *K. pneumoniae* strains were found in both samples collected from in-patients however, 9(56.25%) and 1(6.25%) *K. pneumoniae* strains were isolated from both samples collected from out-patients respectively. From the

sputum samples collected from patients, 1(20.0%) *K. pneumoniae* strain was isolated from the in-patient sample, while the out-patient samples yielded 6(37.5%) *K. pneumoniae* isolates. Wound swabs and blood culture samples collected from patients yielded 1(20.0%) and 3(60.0%) confirmed *K. pneumoniae* isolated from in-patient samples, with no *K. pneumoniae* found in out-patient samples. A statistical correlation between various study samples, in and out-patients, and resistant isolates showed no statistical significance at $p>0.05$.

In the GOPC (General Outpatient Clinics), 13(61.91%) MDR *K. pneumoniae* were isolated from patient samples. 2(9.53%) MDR *K. pneumoniae* were isolated from patients in the male surgical ward; 1(4.76%) MDR *K. pneumonia* was isolated from patients in the children's outpatient clinic, the National Health Insurance Scheme (NHIS), Accident and Emergency, Dentistry Male Ward and the Children's Ward. The phenotypic detection of ESBLs and carbapenemase production among *K. pneumoniae* isolates in patient samples reveal that 6(37.5%) ESBLs and 1(4.76%) carbapenemase production were detected in *K. pneumoniae* isolates in urine samples. 6(37.5%) ESBL production was detected in sputum samples, while 1(6.25%) ESBL production was observed in blood culture, ear swab, aspirate, and wound swab; however, no carbapenemase production was observed among these *K. pneumoniae* isolates. In urine samples in which the presence of ESBLs was detected, *K. pneumoniae* isolates expressed resistance to multiple antibiotic drugs such as amoxicillin (AX) (25ug), amoxicillin + clavulanic acid (AMC) (20ug + 10ug), ceftazidime (CAZ) (30ug), ceftriaxone (CRO) (30ug), cefpodoxime (CPD), tetracycline (TE) (30ug), meropenem (30ug) (ME), gentimicin (CN) (10ug), ciprofloxacin (CIP) (5ug), nitrofurantoin (F) (300ug), cefuroxime (CXM), levofloxacin (LEV) (5ug), cefotaxime (CTX) (30ug), ofloxacin (OFL) (5ug). The highest number of *K. pneumoniae* was isolated in the urine sample, and the presence of ESBL resistance was observed in 1 urine sample. Extended-spectrum beta-lactamases caused *K. pneumoniae* to exhibit a certain phenotypic resistance pattern to amoxicillin (AX) (25ug), ceftazidime (CAZ) (30ug), ceftriaxone (CRO) (30ug), cefpodoxime (CPD), meropenem (ME) (30ug), gentamicin (CN) (10ug), cefotaxime (CTX) (30ug), amoxicillin + clavulanic acid (AMC) (20ug + 10ug), ciprofloxacin (CIP) (5ug), tetracycline (TE) (30ug), levofloxacin (LEV) (5ug), cefuroxime (CXM) antibiotics with the highest amount of antibiotics. The resistance observed in the sputum samples were 10 isolates. Upon examination of the phenotypic resistance pattern of isolates, *K. pneumoniae* isolated from aspirate and ear swab samples showed resistance to amoxicillin (AX) (25ug), amoxicillin + clavulanic acid (AMC) (20ug + 10ug), cefpodoxime (CPD), cefotaxime (CTX) (30ug), meropenem (ME) (30ug) antibiotics, respectively. *K. pneumoniae* isolated from patient wounds (wound swab) showed resistance to amoxicillin (AX) (25ug), amoxicillin + clavulanic acid (AMC) (20ug + 10ug), ceftriaxone (CRO) (30ug), imipenem (IMP) (10ug), meropenem (ME) (30ug), cefotaxime (CTX) (30ug), ceftazidime (CAZ) (30ug), cefpodoxime (CPD), ciprofloxacin (CIP) (5ug). The *K. pneumoniae* isolated from the patient's blood culture exhibited resistance to amoxicillin (AX) (25ug), cefpodoxime (CPD), meropenem (30ug).

The presence of isolated *K. pneumoniae* genes among various samples in this study was noted. QnrB gene was found in 5(23.81%) isolates. The KPC gene was found in 1(4.76%) only. The presence of the CTX-M gene was detected in 16(76.2%) isolates. The SHV gene was found in 13(61.91%) isolates. From the result, 16 isolates of *K. pneumoniae* were confirmed to contain the CTX-M gene, making it the highest-occurring gene of this study, followed by the SHV gene, which was found in 13 isolates of *K. pneumoniae*, and the KPC-positive gene had the least number of individual isolates having been isolated in sample number 16. However, it is important to note that while some genes were isolated separately, some samples yielded multiple gene isolations. QnrB, CTXM, and SHV-positive genes were detected in 4(19.04%) isolates. CTX-M and SHV-positive genes were detected in 11(52.4%) isolates. SHV and QnrB coexisted in 1(4.76%) of the isolates. All four genes (QnrB, KPC, CTXM, and SHV) were detected in 1(4.76%) isolate.

Table 1. Total number of *Klebsiella pneumoniae* isolated from clinical specimens

Specimen types	Number of specimens (%)	Number of *Kleb*. Species isolates (%)	Total confirmed *K. pneumoniae* (%)
Urine	184 (50.41)	22 (43.13)	9 (42.9)
Sputum	65 (17.81)	13 (25.49)	7 (33.3)
Wound swab	60 (16.45)	7 (13.72)	2 (9.52)
Blood culture	3 (0.82)	3 (5.89)	2 (9.52)
Eye swab	2 (0.54)	-	-
High vaginal swab	36 (9.87)	5 (9.81)	-
Urethral swab	2 (0.54)	-	-
Ear swab	10 (2.74)	1 (1.96)	1 (4.76)
Throat swab	3 (0.82)	- *p*–value:0.0365	- *p*-value: 0.0949
Total	365	51	21

Table 2. Antimicrobial susceptibility pattern of *K. pneumoniae* isolate (N-21).

Antibiotics	Sensitive %	Intermediate %	Resistant %
Amoxillin+clavulanate(20/10ug)	5 (23.8)	8(38.1)	8(38.1)
Gentamicin(10ug)	13(61.9)	-	8 (38.I)
Nitrofurantoin(300ug)	-	-	9 (100)
Tetracycline (30ug)	9 (42.9)	1 (4.81)	11(52.3)
Ciprofloxacin(5ug)	5 (23.8)	8 (38.1)	8 (38.1)
Ofloxcilin (5ug)	7 (33.33)	4 (19.1)	10 (48.0)
Levofloxacin (5ug)	13 (61.9)	1 (4.80)	7 (33.3)
Cefuroxime(30ug)	6 (28.6)	7 (33.33)	8 (38.1)
Ceftazidime (30ug)	6 (28.6)	4 (19.1)	11 (52.3)
Cefotaxime(30ug)	-	-	21 (100)
Cefpodoxime (30ug)	-	-	21 (100)
Ceftriaxone (30ug)	10 (47.6)	3(14.3)	8(38.1)
Imipenem (10ug)	20 (95.2)	-	1 (4.80)
Meropenem (30ug)	-	-	21 (100)
Amoxicillin (25ug)	-	-	21 (100)

Table 3. Distribution of *Klebsiella pneumoniae* by age

Age Years	Culture positive male N=8	R	NR	Culture positive female N=13	R	NR
0-15	1 (12.5)	1(20.00)	0	1 (7.69)	0	1(16.66)
16-30	-	0	0	5 (38.46)	2(28.6)	3(50.00)
31-45	3 (37.5)	1(20.00)	2(66.66)	4 (30,76)	2(28.6)	2(33.34)
46-60	-	0	0	2 (15.4)	2(28.6)	0
>60	4 (50.00)	3 (60.0)	1(33.34)	1(7.69)	1(14.2)	0
	X = 2.917 *p*- value: 0.233			X= 6.667 *p*- value: 0.353		

Keys
R= Resistant NR= Non-resistant N= Total number

Table 4. Distribution of isolate among gender and study site.

Characteristics	**K. *pneumoniae***	**MDR K. *pneumoniae***	**p-value**
	n=21 (%)	**n=21 (%)**	
Sex			
Male	8 (38.1)	8 (38.1)	0.157
Female	13 (61.9)	13 (61.9)	
Total	21	21	
Study site			
Asaba Specialist Hospital, Asaba	4 (19.0)	4 (19.0)	0.157
Federal Medical Center, Asaba	17 (81.0)	17 (81.0)	

Table 5. Distribution of confirmed *K. pneumoniae* strains isolated from in-patients and out-patients among specimens

Site of isolates	**In patients N =5 (%)**	**R**	**NR**	**Outpatients N=16 (%)**	**R**	**NR**
Urine	-	0	0	9 (56.25)	4 (50.00)	5 (62.5)
Sputum	1 (20)	1 (33.33)	0	6 (37.50)	3 (37.5)	3 (37.5)
Wound swab	1 (20)	1 (33.33)	0	-	0	0
Blood culture	3 (60)	1 (33.34)	2 (100.0)	-	0	0
Aspirate	-	0	0	1 (6.25)	1 (12.5)	0
Total	5	3	2	16	8	8
		X= 0.833 *p* value = 0.361			X= 10.00 p value =0.125	

Table 6. Distribution of MDR K. *pneumoniae* isolate among clinics and wards (N=21)

Clinic/wards	No of MDR K. *pneumoniae* isolate (%)
GOPC (general outpatient clinics)	13 61.9
Male surgical ward	2 9.53
Children's ward	1 4.76
Children outpatient clinic	1 4.76
NHIS	1 4.76
Accident and emergency	1 4.76
Dentistry	1 4.76
Male ward	1 4.76
Total	21 100

NHIS: National Health Insurance Scheme (NHIS)

Table 7. Phenotypic detection of ESBL and carbapenemase production among isolate (N=16)

S/N	Sample	No of ESBL	No of carbapenemase producer
1	Urine	6 (37.5)	6.25% (1)
2	Sputum	6 (37.5)	-
3	Blood culture	1 (6.25)	-
4	Ear swab	1 (6.25)	-
5	Aspirate	1 (6.25)	-
6	Wound swab	1 (6.25)	-
	Total	16	1

Table 8. The phenotypic resistance pattern of *K. pneumoniae* isolates among samples.

S/n	Source of isolate	Phenotypic resistance pattern	MDR	ESBL
1	Urine	AX,AMC,CAZ,CRO,CPD,CXM,TE,ME,CN,CIP,F,LEV,CTX	MDR	+
2	Urine	AX,AMC,CAZ,CRO,CPD,CXM,CTX,CIP,F,TE,LEV,ME	MDR	+
3	Urine	AX,CTX,CPD,ME,TE,F	MDR	+
4	Urine	AX,CTX,CPD,ME,TE,F,LEV,CIP,OFL	MDR	+
5	Urine	AX,AMC,CXM,ME,CPD,TE,CIP,CN,LEV,OFL,CTX	MDR	+
6	Urine	AX,AMC,CAZ,CPD,CTX,CXM,ME,LEV,CIP,TE,F	MDR	+
7	Urine	AX,CPD,ME,TE,F,CTX,CN,CIP	MDR	+
8	Urine	AX,CPD,ME,CTX,F,TE,AMC,CAZ	MDR	+
9	Urine	AX,CAZ,CXM,CRO,CPD,ME,F,TE,CN,CTX	MDR	+
10	Sputum	AX,CAZ,CRO,CPD,ME,CXM,CN,CTX	MDR	+
11	Sputum	AX,CPD,ME,CTX,AMC	MDR	+
12	Sputum	AX,CPD,ME,CTX	MDR	+
13	Sputum	AX,CPD,ME,CTX,CN,CIP	MDR	+
14	Sputum	AX,CAZ,CPD,ME,TE,CTX	MDR	+
15	Sputum	AX,CRO,CXM,CTX,ME,LEV,CIO	MDR	+
16	Aspirate	AX,AMC,CPD,CTX,ME	MDR	+
17	Ear swab	AX,AMC,CTX,CPD,ME	MDR	+
18	Wound swab	AX,AMC, CXM, CRO,IMP,ME,CTX,CAZ, CPD, and CIP	MDR	+
19	Blood culture	AX,CPD,ME	MDR	+
20	Blood culture	AX,CPD,ME	MDR	+
21	Wound swab	AX,CRO,CTX,CIP,CN,ME	MDR	+

AX: amoxicillin (25ug) AMC: amoxicillin + clavulanic acid (20ug + 10ug) CN: gentimicin (10ug) CPD: cefpodoxime (30ug) LEV:levofloxacin (5ug) CXM: Cefuroxime (30ug CTX: cefotaxime (30ug) CRO: ceftriaxone (30ug) CAZ: ceftazidime (30ug) IMP: imipenem (10ug) TE: tetracycline (30ug) F: nitrofurantoin (300ug) CIP: ciprofloxacin(5ug) OFL: ofloxacin (5ug) ME:meropenem (30ug)

Table 9. Presence of *K. pneumoniae* isolated genes among samples.

Samples	QnrB positive gene	KPC positive gene	CTXM positive gene	SHV positive gene
1				
2	+		+	+
3				
4			+	+
5			+	+
6			+	
7				
8			+	+
9				
10			+	+
11				
12				
13			+	
14				
15				
16	+	+	+	+
17				
18				
19				
20			+	
21				
22				
23			+	
24	+		+	+
25	+		+	+
26			+	
27				
28				
29			+	+
30			+	+
31			+	+
32				
33				
34				
35	+			+

Plate 1. Agarose gel electrophoresis of some selected bacterial isolates. Lane 2, 16, 24, 25, 35 represents QnrB gene bands (400bp). Lane M represents the 100bp DNA ladder.

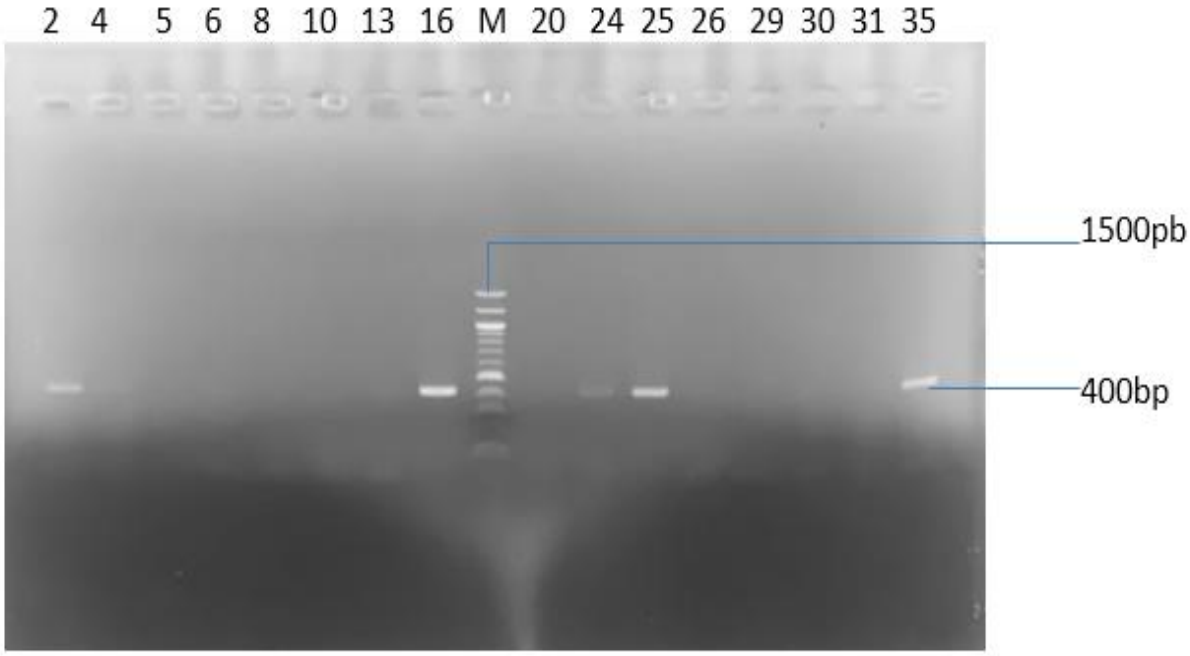

Plate 2. Agarose gel electrophoresis of some selected bacterial isolates. Lane 16 represents KPC gene bands (550bp). Lane M represents the 100bp DNA ladder.

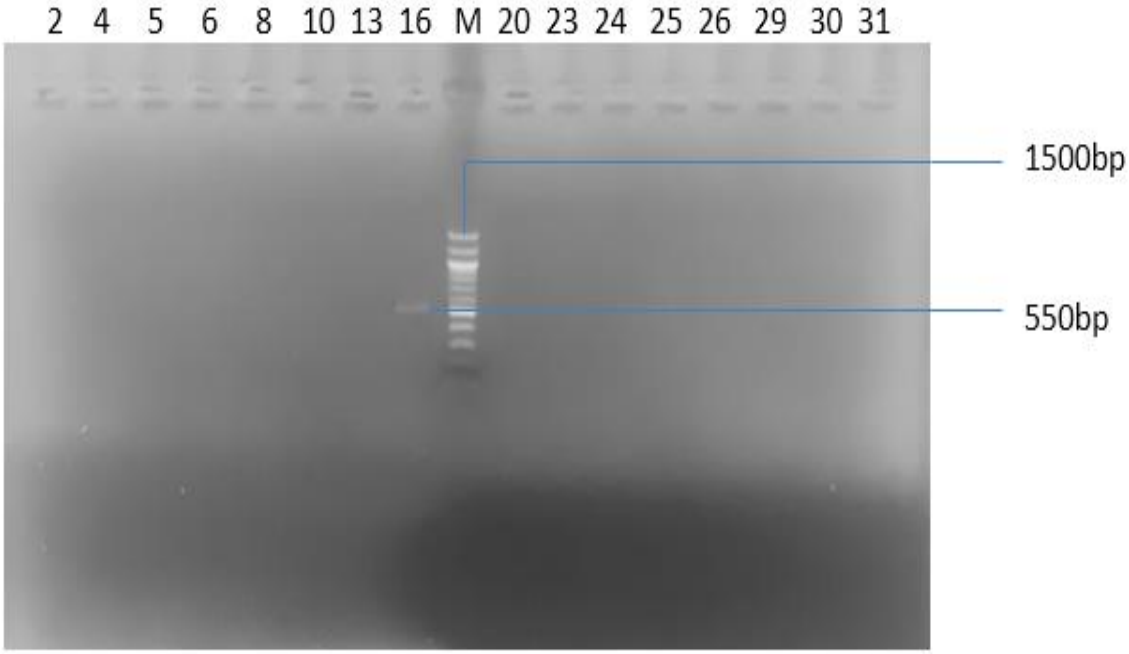

Plate 3. Agarose gel electrophoresis of some selected bacterial isolates. Lane 1-16 represents CTX-M gene bands (500bp). Lane M represents the 100bp DNA ladder.

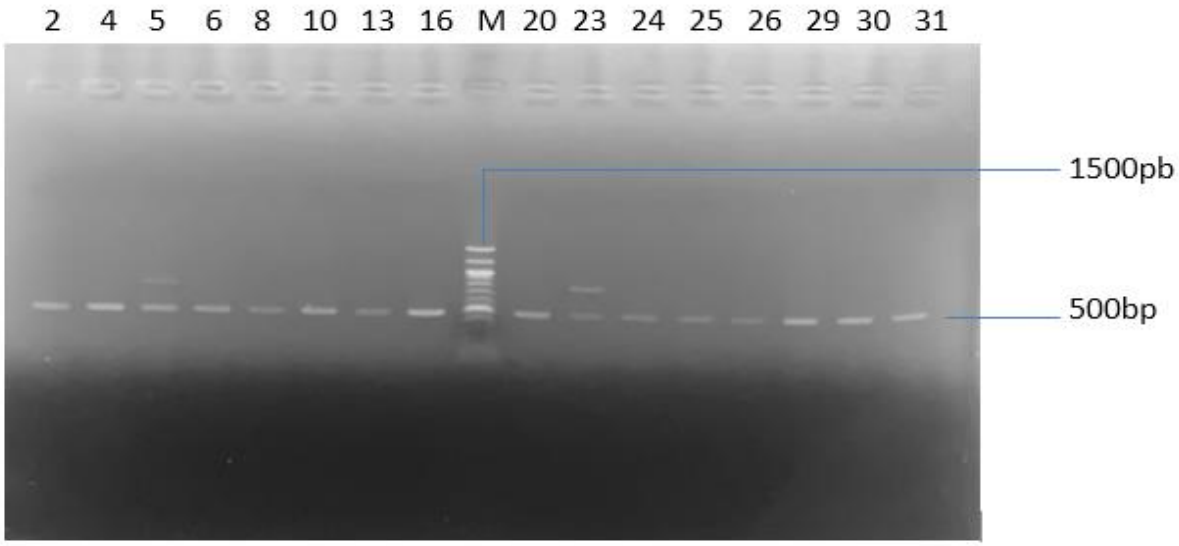

Plate 4. Agarose gel electrophoresis of some selected bacterial isolates. Lane 1-16 represents SHV gene bands (401bp). Lane M represents the 100bp DNA ladder.

Discussion

This research investigates the existence and characterization of an MDR gene in *K. pneumoniae* clinical isolates from various clinical specimens in Asaba, Delta State, Nigeria. The study included 365 clinical isolates, which yielded a prevalence of 21(5.75%) *K. pneumoniae* isolates. Of these isolates, 16/21(76.2%) are ESBL-producing *K. pneumoniae*, while 1/21(4.76%) of the isolates yielded carbapenemase. The prevalence of *K. pneumoniae* in this study is lower than the prevalence of 12.8% in Kaduna [16], 16.2% in Gabon [17], 17.9% in Anyigba, Nigeria [18], 30.5% in Southwestern Nigeria [19], and 34% in Lagos Hospitals, Nigeria [11]. The prevalence of ESBL-producing *K. pneumoniae* is similar to 69.8% in Lagos, Nigeria [11], 75.8% in Egypt [20], 76% in Malaysia [21], but higher than 31% in Anyigba,

Nigeria [18], and 42% in East Africa [22]; however, this prevalence is less than 84% recorded in Cote d'Ivoire [23]. The prevalence of carbapenemase *K. pneumoniae* in this study is also in agreement with 6.5% reported in Northwest Nigeria [16], while it was lower than 28% in the year after in Northwest Nigeria [24]. The prevalence of *K. pneumoniae*, including its ESBL and carbapenemase-producing strains, differs by region and country, as noted in this study. This could be due to several factors, including individual and agricultural antibiotic usage patterns, healthcare infrastructure, economic development levels, agricultural practices, international travel and trade, the bacteria's genetic characteristics, insufficient infection control measures, international travel, and genetic factors, all of which contribute to the rise and prevalence of antibiotic-resistant *K. pneumoniae* strains across various geographical locations. These intricate connections highlight the necessity for comprehensive, region-specific measures to effectively tackle AMR [25,26].

This study demonstrates the antimicrobial susceptibility pattern of the confirmed *K. pneumoniae* isolates, with key findings including an alarming incidence of resistance, notably to antibiotics such as nitrofurantoin, cefotaxime, and cefpodoxime, with all isolates demonstrating resistance (100%). Amoxicillin-clavulanate and ciprofloxacin also show high rates of resistance. In contrast, medications such as imipenem and meropenem have high sensitivity rates, indicating that they are effective against *K. pneumoniae* infections. The results of this research are in tandem with the study by Ugwu *et al*. in Nigeria, who reported 55%, 50%, and 20% resistance of *K. pneumoniae* to ceftriaxone, ciprofloxacin, and meropenem, respectively [27], and a study by **Mofolunsho et al.** reporting 83.3%, 75%, 62.5%, and 66.7% resistance to amoxicillin clavulanic, ciprofloxacin, cefoxitin, and gentamicin, as well as 100% resistance to cefotaxime and imipenem, respectively [18].

Klebsiella pneumoniae was isolated in all age groups of females, which is consistent with a study that found *K. pneumoniae* in samples from all age groups examined [11]. In contrast, *K. pneumonia* was isolated in only three age groups of males, which is inconsistent with **Jalal et al.** [28], which found an increase in *K. pneumonia* cases in all age groups with an increasing age. The higher female preponderance of *K pneumoniae* in this study is consistent with that seen in studies by **Osman et al.** [29], however, this is not the same as in the studies of **Jalal et al.** and **Akinyemi et al.** [11,28], respectively. These comparisons highlight the variation in *K. pneumoniae* occurrence among various demographic groups and highlight how crucial it is to take these details into account when comprehending and treating the infection. According to the study site-based distribution of *K. pneumoniae* isolates, the Asaba Specialist Hospital in Asaba had 4 isolates, while 17 cases were isolated from the Federal Medical Center in Asaba with an equal number of MDR *K. pneumoniae* isolates. This is in line with a study that found that the prevalence of *K. pneumoniae* varied among the study site's health institutions [29]. This highlights the need for more comprehensive surveillance and management approaches to effectively handle the problem of *K. pneumoniae* infections in any setting [30]. On the other hand, out-patients showed a significant presence of *K. pneumoniae* across different specimen types, with urine samples being particularly prominent. In-patients showed a decreased prevalence of *K. pneumoniae* strains across different specimen types. This research bears similarities to the study of **Pantha et al. [**31]. Notably, the General Outpatient Clinics (GOPC) included the bulk of MDR *K. pneumoniae* isolates; however, other clinics and wards also showed variable degrees of MDR *K. pneumoniae* prevalence, albeit at lower rates, in line with the findings of the **Awoke et al.** [32]. This distribution highlights how common MDR *K. pneumoniae* is in a variety of clinical contexts, with GOPC standing out in particular due to its increased prevalence. The prevalence of MDR genes in *K. pneumoniae* is a concern since this might result in treatment failure and the spread of infections that are resistant to antibiotics. This study identified 5(23.81%) QnrB genes as a unique multidrug resistance gene in *K. pneumoniae*; this is higher than 14% and 6.0%, respectively, detected in the USA [33,34], and 6.1% found in pediatric hospitals in China [35]. The findings in this study are quite lower than 62.5% in China [36], 50%, and 55.9% in Korea, respectively [37,38]. Infections as a result of Gram-negative bacteria are frequently treated with fluoroquinolones, a family of medicines that can easily give resistance to quinolones since QnrB is a plasmid-mediated quinolone resistance gene [38]. The presence of the *K. pneumonia* carbapenemase (KPC) gene in Africa has drawn attention, although

there has been little research on this subject in Africa. In this study, the KPC gene was found in 4.76% of isolates, this is lower than 93% of KPC reported between 2005 and 2006 in Israeli hospitals [39], 67.4% of KPC reported in Indian hospitals [40], 33.7% detected in Brazil [41]. The prevalence of CTX-M in *K. pneumoniae* isolates has been the subject of recent investigations. For instance, the presence of the CTX-M gene was detected in 76.2% of the isolates, this agrees with approximately 76.8% of CTX-M genes reported in Southern China [42], however, this is lower than 96.2% and 84.8% in China Hospitals [43,44], 90.6% in Dar es Salaam, Tanzania [45]. Our findings agree with other studies that reported a predominance of CTX-M genes [43,46–48]. The extensive use and overuse of cephalosporin antibiotics such as ceftriaxone and cefotaxime in clinical, veterinary, and agricultural instances, combined with horizontal gene transfer pathways, contribute to the rapid introduction and spread of CTX-M genes among bacterial pathogens [49]. Addressing antimicrobial resistance necessitates comprehensive policies that promote responsible antibiotic use, improve infection control measures, and develop novel therapeutic ways to combat resistant illnesses [50,51]. Another typical ESBL is SHV, which is produced by a resistance gene that is present in many gram-negative bacteria, including *K. pneumoniae.* The SHV gene was found in 61.91% of isolates, this is higher than 32.7% in Tehran, Iran [52] 21.7% in Kenya [53], 10.6% in Germany [54], but lower than 82.7% in Kenyan facilities [55], 85.5% in Iran [56], 88.2% in Kilimanjaro, Tanzania [22]. Inappropriate infection control methods and weak antibiotic stewardship policies in healthcare institutions may have led to the proliferation of resistant strains inside hospital environments [57]. The presence of numerous resistance genes in some isolates demonstrates the complexity of antimicrobial resistance pathways in *K. pneumoniae*. The existence of genes like QnrB, which imparts resistance to quinolone antibiotics, together with beta-lactamase genes like CTX-M and SHV, highlights the multifaceted nature of resistance acquisition and the possibility of cross-resistance to several antibiotic classes [58,59].

Comparing these findings to other research conducted in Africa, similar patterns of high CTX-M and SHV gene prevalence in *K. pneumoniae* isolates have been found in several African nations. CTX-M-producing *K. pneumoniae* strains have regularly been identified as substantial contributors to antibiotic resistance in hospital settings in studies conducted in Nigeria, Kenya, and South Africa. Furthermore, the presence of multiple resistance genes in *K. pneumoniae* isolates has been observed in various studies, emphasizing the importance of comprehensive surveillance and control strategies to prevent the spread of multidrug-resistant infections in Africa [60–63]. The results of this study emphasize how critical it is to enhance antimicrobial stewardship initiatives, surveillance tactics, and prevention and control measures in order to slow the emergence and spread of MDR *K. pneumoniae* strains that carry CTX-M, SHV, and other resistance genes in Nigerian and African healthcare settings. Collaboration among healthcare providers, researchers, and policymakers is necessary for successfully tackling this expanding public health problem [9].

Conclusion

In conclusion, the prevalence of MDR *K. pneumoniae* isolates in the study site highlights the need for enhanced programs for antimicrobial stewardship and infection control. The detection of various resistance genes in this study, including ESBLs and carbapenemases, is of particular concern due to the limited therapy options available for infections caused by these strains. In order to stop the spread of MDR *K. pneumoniae* in hospital settings, further research is required to comprehend the causes of resistance and create practical countermeasures. Overall, this study underscores the significance of continuous monitoring of antimicrobial resistance patterns and the need for appropriate and judicious use of antibiotics in clinical practice.

Recommendation

Several significant recommendations come from the characterization investigation of MDR genes in *K. pneumoniae* from clinical isolates in Asaba. First, antibiotic stewardship programs must be implemented to reduce antibiotic abuse and misuse, thereby decreasing the establishment of multi-drug resistance strains of *K. pneumoniae*. Second, constant monitoring of antibiotic resistance patterns and the prevalence of multi-drug resistant bacteria is critical for developing empirical therapy and infection control strategies. Third, finding new medications or other treatments for infections brought on by MDR *K. pneumoniae* strains needs to be done immediately. Furthermore, strong infection control measures such as hand hygiene, thorough

cleaning of medical equipment, and isolation of patients with multi-drug resistant diseases are critical to preventing bacterial transmission and spread. Finally, coordination among healthcare providers, researchers, and policymakers is critical for effectively addressing the growing threat posed by multidrug-resistant *K. pneumoniae* and other antibiotic-resistant bacteria.

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Conflict of interest

The authors declare no conflict of interest.

Availability of data and materials

The dataset used and analyzed during this current study is available from the corresponding author upon reasonable request.

Ethical approval and consent to participate

Ethical approval was obtained from the Ethics Committee, Federal Medical Centre, Asaba, Delta State, Nigeria, with the reference number FMC/ASB/A81VOL.XII/313, and patient consent was obtained from each participants prior to the start of the study

Author contributions

All authors contributed equally to the writing of this paper. All authors have read and approved the final draft.

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