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MOLECULAR CHARACTERIZATION OF CARBAPENEM RESISTANT *Klebsiella pneumoniae* FROM WOUND SURFACES OF PATIENTS ATTENDING GENERAL HOSPITAL MINNA, NIGERIA

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ABSTRACT

The spread of carbapenem-resistant bacteria constitutes a global public healthcare problem due to their rate of spread and limited therapeutic options against them. The purpose of this study was to isolate and characterize carbapenem-resistant *Klebsiella pneumoniae* (CRKP) from patient wound surfaces at General Hospital Minna, Nigeria. One hundred wound specimens were obtained and screened for *K. pneumoniae*. Using the Kirby-Bauer disk diffusion technique, isolates of *K. pneumoniae* were tested for carbapenem resistance using imipenem and meropenem discs. Carbapenem-resistant *K. pneumoniae* were screened for carbapenemase production using a modified carbapenem-inactivation method. Using polymerase chain reaction, carbapenemase-encoding genes were also tested for in carbapenem-resistant isolates. Results revealed that 63.0% of the samples were bacteria culture positive and 67 Gram negative bacteria including; *Klebsiella pneumoniae* 14(20.9%), *Escherichia coli* 33 (49.2%) and *Pseudomonas aeruginosa* 20 (29.9%) were isolated. Infection rate was higher in females (52.2%) than males and in patients within the age group of 21-40 years (49.3%). Nine out of the 14 *Klebsiella pneumoniae* isolates were resistant to carbapenems, and they exhibited high-level of resistance to Trimethoprime-sulfamethoxazole, Ciprofloxacin and Amoxicillin+clavulanate and high level of susceptibility to Fosfomycin and Colistin. None of the CRKP produced carbapenemase production and 55.6% were indeterminate. Molecular identification also confirmed that genes encoding carbapenem resistance (blaOXA-48, blaKPC and blaNDM) were not present in the five indeterminate, carbapenem resistance *Klebsiella pneumoniae* isolates. High prevalence of CRKP was recorded in the study area and efforts should be intensify towards limiting their spread.

Keywords: Carbapenem-resistant, Carbapenemases, Imipenem, *Klebsiella pneumoniae*, Meropenem, Nosocomial

INTRODUCTION

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is an important member of Carbapenem Resistant *Enterobacteriaceae* (CRE). Carbapenem Resistant *Enterobacteriaceae* (CRE) are members of the *Enterobacteriaceae* family that are resistant to carbapenems and most antibiotics (Codjoe and Donkor, 2018). They are regarded as nosocomial infectious agents that produces carbapenemase (an enzyme that hydrolyses the beta-lactam ring of many drugs such as carbapenem, monobactams, penicillin and cephalosporins (Tzouvelekis et al., 2014; Perez et al., 2016; Codjoe and Donkor, 2018).

There emergence is an important challenge in healthcare and its increasing prevalence is a growing concern worldwide (Tilahun et al., 2021). The World Health Organization (WHO) has ranked CRE among the top tier critical-priority-pathogens that pose significant risk to human's health (WHO, 2017; Jalowiecki et al., 2022; Odewale et al., 2024). The incidence rate of Carbapenem-resistant *K. pneumoniae* has risen significantly for the past twenty years (Alicino et al., 2015; Han et al., 2017; Logan et al., 2017; Gomides et al., 2022). Its prevalence has increased in both community and hospital settings and it is now considered a major problem in healthcare sector (Papadimitriou-Olivgeris et al., 2014; Lombardi et al., 2015). Diseases caused by CRKP increase the morbidity and mortality rates of patients with limited therapeutic options for treatment, especially in those that undergo surgical procedures or with open wounds (Papadimitriou-Olivgeris et al., 2014; Lombardi et al., 2015; Codjoe and Donkor, 2018).

Resistance acquired by CRKP due to the presence of carbapenemase such as KPC (*K. pneumoniae* Carbapenemase) and OXA-48 (Oxacillinase-48) is really concerning, as therapeutic options for their treatment is limited (Nordmann et al., 2012). The expansion of this resistance might weaken the healthcare system and make it more difficult to provide immune-suppressive medication and perform effective invasive procedures (European Centre for Disease Prevention and Control, 2018).

The use of carbapenems has become mandatory for the management of *Enterobacteriaceae* that produce extended spectrum βlactamases (ESBL) (Paterson and Bonomo, 2005; Nordmann et al., 2011). Bacterial infections occurring in critically ill patients undergoing transplantation or other surgical procedures, as well as those admitted to intensive care units are usually treated using carbapenems (Nordmann et al., 2011).

Antibiotic resistance has evolved as a result of irrational antibiotic usage, resulting in increased morbidity and death, higher health-care costs and longer hospital stays (Fair and Tor, 2014). When patients are immunocompromised, undergoing surgery, or receiving an organ transplant, early action is required to prevent mortality by giving effective empirical antibiotics in clinical settings where CRKP is a significant concern.

High rate of carbapenem resistance in K. pneumoniae has been reported in some part of Nigeria, Ibrahim et al., 2017; Odewale et al., 2023) reported however not much study has been carried out on CRKP in the study area (This study was carried out investigate the prevalence of carbapenem-resistant

K. pneumoniae in wound as well as the genes that encode carbapenem resistance. Hence, this study provides data on the occurrence and antibiotic susceptibility patterns of CRKP isolated from wound surfaces of patients attending General Hospital Minna, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in General Hospital, Minna, Nigeria. General Hospital, Minna is the hospital attended by most of the populace in Minna. The hospital is a tertiary health care institution that provides clinical services for about a 100, 000 people within and outside Minna metropolis.

Sample Size Determination

The Cochran's formular was used to determine the sample size for this study (Uchendu, 2018). Based on the prevalence (6.8%) of *K. pneumoniae* isolated from wound samples in the study conducted by Ogba et al. (2014), the sample size was obtained. One hundred (100) samples were collected for the study.

$$N = \frac{Z^2 pq}{d^2}$$

Where, N = Sample size; Z = Confidence interval (1.96); p = the probability (0.068) q= 1-p which is 0.059; and d= margin of error (5%) = 0.05

Study Population and Sampling Techniques

One hundred (100) wound samples were taken from patients hospitalized to various wards of General Hospital Minna, Nigeria, using a stratified random sampling approach. This included patients who had accidents, surgical wounds and those with acute or chronic wounds. This approach ensured that the various units of the hospitals with wounds patients were included in the study.

Inclusion and Exclusion Criteria

Patients on admission, accident patients, patients who have surgical wound, those with acute or chronic wounds and lacerations who were on antibiotics treatment for at least three weeks prior to sample collection were included in this study. Patients with abrasion, those whose clinical records could not be obtained and those who have no knowledge of antibiotic usage were excluded from the study.

Sample Collection

The surface of the skin around the wound of each patient, was gently wiped using a sterile guaze pad already moistened with normal saline. Sterile wound swab stick was used to swab the surface of the wound by gently rotating the swab stick between fingers, swabbing the wound from margin to margin and using adequate pressure within the wound tissue (Jill, 2007). The swab sticks were stored in a sterile ice pack and then sent to CGEB (Centre for Genetic Engineering and Biotechnology) Laboratory, Federal University of Technology Minna, Nigeria where they were processed within 8 hours using standard microbiological procedures.

Isolation and Identification of Bacteria

Samples were inoculated by streaking method on MacConkey Agar (MCA) and Eosin Methylene Blue Agar (EMB), then incubated at 37 ºC for 24 hours. Pure culture of colonies obtained after incubation were identified as *Klebsiella pneumoniae* and other Gram-negative bacteria using colony morphology, Gram staining and conventional biochemical tests such as oxidase test, methyl red test, indole test, citrate utilization, Voges Proskauer, urease hydrolysis and motility test (Cheesbrough, 2010; Agarwal et al., 2019).

Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion technique was utilized to evaluate the antimicrobial susceptibility of the *K. pneumoniae* isolates that were obtained. The suspension of the test organism was prepared in sterile normal-saline and turbidity was adjusted to 0.5 McFarland standards. Mueller-Hinton agar lawn cultures were produced and left to dry. Antibiotic discs (Meropenem (10µg), Imipenem (10µg), Fosfomycin (50µg), Amoxycillin+clavulanate (30µg), Ciprofloxacin (10µg), Trimethoprim-sulfamethoxazole (25µg) and Colistin (10µg)) were placed on the Mueller-Hinton agar surface. The plates were incubated at 37 ºC for 24 hours after which the diameter of zones of inhibition was read and interpreted as: sensitive (S), resistant (R) or intermediate (I) according to the standard criteria recommended by the Clinical Laboratory Standards Institute (CLSI, 2018). The ratio of the number of antibiotics to which an isolate is resistant to the total number of antibiotics to which the isolate is exposed was used to calculate multi-antibiotic resistant (MAR) index.

Phenotypic Testing for Carbapenemase Production

Modified carbapenem inactivation method (mCIM) was used in determining carbapenemases production by *K. pneumoniae* isolates showing resistance (zone of inhibition diameter ≤19 mm) to carbapenems (Imipenem and Meropenem). A loop full (approximately 10 µg) of bacterial culture was taken from the Muller Hinton agar plate and suspended in 2 mL Tryptic Soy Broth, according to a procedure adapted from another study (Van der Zwaluw, et al., 2015). Antibiotics disc (Meropenem, 10µg) was immersed in the suspension and incubated for four hours at 35°C. Using the direct colony suspension technique, a 0.5 McFarland suspension of susceptible indicator organism (*E. coli*) was prepared and a lawn culture of the *E. coli* was made on a Mueller-Hinton agar plate. The Meropenem was removed from the suspension with the aid of an inoculation loop and placed on a Muller-Hinton agar plate previously inoculated with *E. coli* indicator strain. The plates were incubated at 35°C for 18-24 hours. After 24 hours of incubation, the diameter of zones of inhibition was interpreted according CLSI, 2018; Jing et al., 2018). Inhibition zone diameters at 6-15 mm were considered positive, 16-18 mm inhibition zone diameter were indeterminate, while zone of inhibition ≥ 19 mm were considered to be negative result.

Molecular detection of carbapenemase encoding genes

Isolates that showed indeterminate carbapenemase production from phenotypic testing were screened for the most commonly found carbapenemase encoding genes; blaKPC, blaNDM and blaOXA-48 by multiplex PCR assay using appropriate primers (Poirel et al., 2010; Van der Zee et al., 2014).

DNA extraction

Following the manufacturer's instructions, genomic DNA was extracted using a columnbased JENA Bioscience Bacteria DNA Preparation Kit. Bacteria cells were extracted from a 500 µL aliquot of bacteria broth culture by centrifuging it for one minute at 10,000 rpm. The residual pellet was re-suspended in 300 µL of re-suspension buffer and 2 µL of Lysozyme solution. After inverting numerous times to homogenize the mixture, it was incubated at 37 °C for one hour. Centrifugation was used to collect re-suspended cells, which were then lysed using 300 µL of Lysis buffer, 2 µL RNase A, and 8 µL proteinase K solution, and then incubated

at 60 °C for ten minutes. The tube was cooled on ice for five minutes before adding 300 µL binding buffer and vortexing momentarily; the mixture was then cooled on ice for five minutes before centrifuging at 10,000 rpm for five minutes. To trap the DNA, the supernatant was directly placed into the spin column and spun at 10,000 rpm for one minute. The trapped DNA was washed twice with washing solution before being eluted into a clean Eppendorf tube with 50 µL elution buffer (Poirel et al., 2010; Van der Zee et al., 2014).

Primer information

Primers were synthesized by Macrogen Europe, Netherlands and the sequences are presented below: blaKPC (**Forward**: 5^1 GCG GAA CCA TTC GCT AAA CT 3^1 **Reverse**: 5^1 GGC GGC GTT ATC ACT GTA TT 3^1); blaNDM (**Forward**: 5^1 GTT TGA TCG TCA GGG ATG GC 3^1 **Reverse**: 5^1 CCG TAT GAG TGA TTG CGG C 3^1); blaOXA-48 (**Forward**: 5^1 GGG CGT GGT TAA GGA TGA AC 3^1 **Reverse**: 5^1 CAT CTT GCT CAT ACG TGC CT 3^1).

Polymerase chain reaction (PCR)

Each PCR reaction mixture consisted of 12.5µL mastermix (2x JENA Ruby hot start pol), 1µL (10 pmol) each of forward and backward primers of the gene of interest, 1 µL DNA template and 9.5µL sterile nuclease free water to make up a total reaction volume of 25µL. An Applied Biosystem 2720 Thermocycler was used for PCR amplification. An initial denaturation of the mixture at 94 degrees Celsius for 3 minutes was followed by 35 cycles of denaturation at 94 degrees Celsius for 45 seconds, annealing at 55 degrees Celsius for 60 seconds, and extension at 72 degrees Celsius for 60 seconds; and a final extension at 72 degrees Celsius for 10 minutes (Poirel et al., 2010; Van der Zee et al., 2014). The PCR products were visualized on a 2% agarose gel containing ethidium bromide in 0.5x Tris-borate buffer (pH 8.0). A molecular ladder marker (Jena Bioscience, 200bp) was run simultaneously to determine the size of the amplicons. The bands that appeared were compared with that of the ladder and the observation were recorded.

Data Analysis

The antibiotic susceptibility results were analysed with IBM SPSS version 25 software. Age, gender, wound type and ward of patient comparison with the prevalence of Gram negative bacteria were performed using the Pearson Chi-square test and a p-value of less or equal to 0.05 was considered significant.

RESULTS AND DISCUSSION

Prevalence of Gram-negative bacteria in wound samples

Out of the 100 samples analyzed, 63 (63.0%) were culture positive and 67 Gram negative bacteria were isolated. *Escherichia coli* 33 (49.2%) was the most prominent isolate especially in post-surgical wounds, followed by *Pseudomonas aeruginosa* 20 (29.9%) and *K. pneumoniae* 14 (20.9%). The most infected wound type with Gram negative isolates (*E. coli, P. aeruginosa and K. pneumoniae*) was post-surgical wound (40.3%), followed by accident wound (23.9%), abscess (14.9%), diabetic wound (8.9%), boil (3.0%) and the least (1.5%) infected wound type was nail puncture and bedsore.

The highest prevalence (62.7%) of Gram-negative bacteria was isolated from wound of patients in accident and casualty ward followed by female ward (20.9%) and B-ward (14.9%) while the least prevalence (1.5%) of Gram- negative isolates (only *E. coli*) was isolated from wound of patients in A-ward. Females (52.2%) were more infected with Gram negative bacteria than males (47.8%). The highest prevalence of *K. pneumoniae* (57.1%) and *P. aeruginosa* (65.0%) was isolated from female, while the highest prevalence of *E. coli* isolates was obtained from male (57.6%).

The distribution of Gram-negative bacteria in accordance to age showed that patient within the age group 21 – 40 years had the highest prevalence rate (49.3%) of Gram- negative bacteria wound infection, followed by age group 41-60 years (34.3%), 0-20 years (10.5%) while the least prevalence of infection was recorded in age group 61-80 years as shown in the Table 1

Table 1: Prevalence of Gram-negative bacteria in clinical samples

Factors	*K. pneumoniae*	*P. aeruginosa*	*E. coli*	Total (%)
Wound type				
Abscess	2(14.3)	2(10.0)	6(18.2)	10(14.9)
Abrasion	0 (0.0)	4(20.0)	0 (0.0)	4(6.0)
Accident wound	2(14.3)	5(25.0)	9(27.3)	16(23.9)
Boil	0 (0.0)	2(10.0)	0 (0.0)	2(3.0)
Bed sore	0 (0.0)	0 (0.0)	1(3.1)	1(1.5)
Nail puncture	0 (0.0)	0 (0.0)	1(3.1)	1(1.5)
Post-surgical wound	7(50.0)	6(30.0)	14(42.2)	27(40.3)
Diabetic wound	3(21.4)	1(5.0)	2(6.1)	6(8.9)
Total	**14(20.9%)**	**20(20.9%)**	**33(49.2%)**	**67**
Ward				
Accident and casualty theatre	9(64.3)	16(80.0)	17(51.5)	42(62.7)
Male ward	0 (0.0)	0 (0.0)	1(3.1)	1(1.5)
Male surgical	3(21.4)	0 (0.0)	7(21.2)	10(14.9)
Female surgical	2(14.3)	4(20.0)	8(24.2)	14(20.9)
Total	**14(20.9%)**	**20(20.9%)**	**33(49.2%)**	**67**
Gender				
Male	6(42.9)	7(35.0)	19(57.6)	32(47.8)
Female	8(57.1)	13(65.0)	14(42.4)	35(52.2)
Total	**14(20.9%)**	**20(20.9%)**	**33(49.2%)**	**67**

Age Group				
0-20	0 (0.0)	4(6.0)	3(4.5)	7(10.5)
21-40	7(10.5)	9(13.4)	17(25.4)	33(49.3)
41-60	6(9.0)	5(7.5)	12(17.9)	23(34.3)
61-80	1(1.5)	2(3.0)	1(1.5)	4(6.0)
Total	**14(20.9%)**	**20(20.9%)**	**33(49.2%)**	**67**

Antibiotic susceptibility pattern of *K. pneumoniae* isolates
Table 2 shows the susceptibility pattern of *K. pneumoniae*. Isolates showed high rate of resistance (64.3%) against Carbapenems (Imipenem and Meropenem), Ciprofloxacin, Trimethoprim-sulfamethoxazole and amoxicillin/clavulanic acid (78.6%). High rate of sensitivity was recorded against Colistin (64.3%) and Fosfomycin (57.1%).

Table 2: Antibiotic susceptibility patterns of *K. pneumoniae*

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Imipenem	5 (35.7)	0(0.0)	9(64.3)
Meropenem	5 (35.7)	0(0.0)	9(64.3)
Ciprofloxacin	4(28.6)	1(7.1)	9(64.3)
Trimethoprim-sulfamethoxazole	4(28.6)	1(7.1)	9(64.3)
Colistin	9(64.3)	5(35.7)	0(0.0)
Fosfomycin	8(57.1)	5(35.7)	1(7.1)
Amoxicillin/clavulanate	2(14.3)	1(7.1)	9(78.6)

Multiple antibiotic resistance index of *K. pneumoniae*
Table 3 shows that out of fourteen (14) *K. pneumoniae* isolates, 3 were fully sensitive to antibiotics tested while 2 out of the 14 isolates, only showed resistance to AMC. Nine (9) isolates were multidrug resistant and the most prevalent phenotype was IPM-MEM-CPX-SXT-AMC with 8 isolates.

Table 3: Multiple antibiotic resistance indices of *K. pneumoniae* isolates

Isolates Code No	Resistance phenotype	MAR Index	Resistance category
1	IPM-MEM-CPX-SXT-AMC	0.7	MDR
2	IPM-MEM-CPX-SXT-AMC	0.7	MDR
23	nr	0	nr
28	IPM-MEM-CPX-SXT-AMC	0.7	MDR
33	IPM-MEM-CPX-SXT-AMC	0.7	MDR
32	AMC	0.1	nr
30	IPM-MEM-CPX-SXT-AMC	0.7	MDR
39	IPM-MEM-CPX-SXT-AMC	0.7	MDR
40	AMC	0.1	nr
41	IPM-MEM-CPX-SXT-AMC	0.7	MDR
51	IPM-MEM-CPX-SXT-AMC	0.7	MDR
52	nr	0	nr
57	IPM-MEM-CPX-SXT-AMC-FOS	0.8	MDR
85	nr	0	nr

Keys: IPM: Imipenem; MEM: Meropenem; CPX: Ciprofloxacin; SXT:Trimethoprime-sulfamethoxazole; AMC: Amoxicillin/Clavulanic acid; FOS: Fosfomycin; nr: no resistance; MDR: multidrug resistance.

Carbapenemase production by carbapenem-resistant *Klebsiella pneumoniae*
Table 4 presented the prevalence of carbapenemase producing CRKP. Out of the 9 CRKP isolates screened for carbapenemase production, 5(55.6%) of the isolates were indeterminate, 4(44.4%) of the isolates were negative while none (0.0%) was positive.

Table 4: Prevalence of carbapenemase producing carbapenem-resistant *Klebsiella pneumoniae*

Carbapenemase Production	Number of isolate (%)
Positive	0 (0.0)
Indeterminate	5(55.6)
Negative	4(44.4)

Prevalence of carbapenemase encoding genes
Polymerase chain reaction confirmed that all the five phenotypically indeterminate carbapenemase-producing isolates examined for the presence of three common carbapenemase encoding genes do not harbour any of the genes; *bla*KPC, *bla*OXA-48 and *bla*NDM (Figure A-C).

Discussion
This finding emphasizes the severity of Gram negative bacteria in wound infections. The prevalence rate of wound infections caused by Gram negative bacteria obtained in this study was high (67.0%). This rate was lower than the prevalence rate of 86.1% reported by Pondei *et al*. (2013).

This difference may be due to variances in sample size, study design and study area.

Most of the wound infections (40.3%) were recorded among patients with post-surgical wounds. This tallies with the report of Ogba *et al*. (2014). The infections may have been acquired nosocomially during their admission in the or even from surgical equipment.

Patients admitted in accident and casualty theatre as well as those in female surgical wards, showed the highest prevalence of Gram-negative bacterial infection of wound. This could be explained by the critical nature of the patients' illnesses and their prolonged hospitalization. This result is consistent with the findings of Oluwafolajimi and Hilda (2020) in South West, Nigeria and Thomas and Duse (2018), in South Africa who reported highest prevalence of Gram-negative bacteria infection in surgical wards. To lessen the spread of wound infections caused by Gram-negative bacteria, this information may be utilized to pinpoint hospital areas that need more stringent infection prevention and control procedures.

Patients within the age group 21 - 40 and 41- 60 had the highest prevalence of Gram-negative infections while the least prevalence was recorded among those aged 61 – 80 years. This result is consistent with the findings of other study that recorded the highest proportion (35.9%) of Gram-negative bacteria infection of wound within the age group 40 – 54 years and the lowest proportions within the age group 61-80 years (Pondei *et al*., 2013). High prevalence recorded in middle age (41 - 60 years) and young adult patients (21 - 40 years) could be attributed to co-morbidities such as hypertension, diabetes mellitus and cancer which result in frequent hospitalization, long hospital stay and use of multiple antibiotics which predisposes them to the most severe forms of infections (Patel *et al*., 2008; Idang *et al*., 2020).

Species of Gram- negative bacteria isolated were *E. coli, P. aeruginosa* and *K. pneumoniae*. This was similar to the species of Gram- negative bacteria isolated from wound samples in some previous studies (Sani *et al*., 2012; Enwuru *et al*., 2019). Contamination of wounds by these organisms is a serious problem in hospitals, particularly during surgical procedures, when the sterile operation site can become contaminated and infected, resulting in a high morbidity and death rate (Pondei *et al*., 2013).

High prevalence (20.9%) of *K. pneumoniae* was isolated from wound surfaces, this rate is higher than prevalence of 8.4% *K. pneumoniae* isolated from wound samples in a previous study conducted in Minna (Sani *et al*., 2012). This variation could be attributed to differences in sample size, wound type and antibiotic usage prior to sample collection. Presence of *K. pneumoniae* in wound could cause bacteremia, cellulitis, necrotizing fasciitis, myositis or delayed wound closure and worsened scarring which could be life threatening or cause the patients an increased trauma and treatment cost (Bowler and Duerden, 2001; Brook, 2008).

Most (64.3%) of the *K. pneumoniae* isolated from wound surfaces in this study, were carbapenem-resistant; they showed resistance against Imipenem and Meropenem. This is similar to the prevalence of 64.5% CRKP reported by Silva *et al*. (2017). This was a quite high resistance rate for antibiotics that is used as a last option in the treatment of severe multidrug resistant Gram-negative bacteria infections and in the treatment of *K. pneumoniae* wound infections (Nordmann et al., 2011). One of the most common risk factors for carbapenem-resistant *K. pneumoniae* is prior exposure to any broad-spectrum antibiotic (Thomas and Duse, 2018). Unregulated antibiotic distribution in the study area is a major risk factor as area where antibiotics are procurable over-the-counter without prescription usually have higher prevalence of CRKP infections (Donkor et al., 2011). Carbapenem-resistant *K. pneumoniae* can cause a wide range of infections in hospitals, including wound or surgical site infections, pneumonia, meningitis and urinary tract infections, and is associated with a high mortality rate. During nosocomial epidemics, patients with undiagnosed CRKP infection have served as transmission reservoirs (Snitkin et al., 2012; Han et al., 2017).

Carbapenem resistant *Klebsiella pneumoniae* isolates showed total (100.0%) resistance rate against Fluoroquinolone (Ciprofloxacin), Sulfonamides (trimethoprim-sulfamethoxazole) and amoxicillin/clavulanic acid. This was not surprising taking into account the selective pressure due to frequent use of these drugs; This is consistent with the reports of Silva *et al*. (2017) and Bartolini *et al*. (2014) that reported high resistance rate of CRKP isolates against cephalosporins, quinolones and penicillin. The reason for this resistance could be attributed to frequent use and abuse of these drugs in the society and application of empirical approach in initial treatment of wound infection without proper identification of the etiological agent of wound infection.

This study reiterates the fact that CRKP are highly susceptible to Colistin and Fosfomycin, high sensitivity rate was recorded against Colistin and Fosfomycin in this present study. However, this contrast with the findings of Silva *et al*. (2017) that recorded resistance against Colistin. Colistin and Fosfomycin are useful for the treatment of MDR infections caused by CRKP and Colistin can also be last-line therapeutics against CRKP ((Zavascki et al., 2007; Pinto et al., 2014). Multidrug resistance in CRKP is a great challenge in the effective management of wound infections. All the CRKP isolates in this present study were multidrug resistant; the strains were resistant to more than two classes of commonly used antibiotics in the treatment of infection. This species seems to represent a reservoir of resistance transmittable to other Enterobacteria including *E. coli* and *P. aeruginosa* (Tacconelli et al., 2014). The spread of this multidrug resistance by CRKP may incapacitate the health care system and limit the ability to provide successful immune-suppressive therapy and invasive procedures. Furthermore, MAR index of the nine (9) out of the fourteen (14) *K. pneumoniae* isolates were greater than **0.2.** This suggests that the multidrug-resistant *K. pneumoniae* identified in this study came from a high-risk source, possibly human or animal feces, where indiscriminate antibiotic use may have accelerated their development of resistance to several medicines.

The mechanisms underlying resistance in CRKP species generally involves the production of carbapenemases, decrease or loss of outer membrane proteins in addition to the production of ESBL and AmpC (Meletis, 2016). Though, in this study, CRKP isolates were screened for the production of only carbapenemases. The phenotypic test showed that none of the isolates were able to produce carbapenemase, though 55.6% of the CRKP isolates showed indeterminate results. This is similar with the study of Silva *et al*. (2017) that reported that none of the CRKP isolates tested was positive for production of carbapenemase but in contrast to other studies (Oduyebo et al, 2015; Wartiti et al., 2016) that reported production of carbapenemase enzyme by CRKP. This could be an indication of the presence of another type of mechanisms of resistance.

Further amplification of DNA of Carbapenem resistant *Klebsiella pneumoniae* for the detection of gene encoding carbapenemase (*bla*KPC, *bla*OXA-48 and *bla*NDM) showed that CRKP isolates did not harbour genes of interest.

Although, several carbapenemase-encoding genes have been identified so far, blaKPC, blaNDM, and blaOXA-48-like are the most-prevalent determinants of resistance to carbapenems in different part of the world (Hammoudi et al 2014; Viau and Frank, 2016). These genes are the most frequently isolated from *Enterobacteriaceae* including *K. pneumoniae.* This result is however contrary to the report of McMullen *et al.* (2017) that reported presence of *bla*OXA48-like gene in carbapenem resistance *Enterobacteriaceae*. In a study by Danxia *et al.* (2019) six strains of *K. pneumoniae* isolated contained KPC-2 gene. The CRKP isolates examined in this present study may contain other carbapenem resistance encoding genes apart from *bla*KPC, *bla*OXA-48 and *bla*NDM genes.

CONCLUSION

This study revealed that the prevalence of *K. pneumoniae* isolated from wound surface of patients attending General hospital Minna was 20.9%. About 64.3% of the isolates showed high level of resistance to carbapenems (Imipenem and Meropenem), Ciprofloxacin, Trimetoprime-sulphamethoxazole and amoxicillin-clavulanic acid. However, it showed high sensitivity against Fosfomycin and Colistin. Carbapenem resistant *K. pneumoniae* isolates screened for the production of carbapenemase, showed that none were positive for carbapenemase production while 5 (55.6%) were indeterminate. Molecular characterisation of the gene encoding carbapenemase enzyme (blaKPC, blaOXA-48 and blaNDM) showed that none of the CRKP isolates harbors the gene of interest. Since CRKP has been identified in General Hospital Minna, infection control measures such as hand hygiene among healthcare workers, antibiotic stewardship, and judicious use of carbapenems, cephalosporins, fluoroquinolones, and sulphonamides should all be pursued rigorously in order to limit its spread.

ETHICS STATEMENT

Ethical approval with reference number HMB/GHM/136/VOL.III/563 was obtained from the Niger State Hospital Management Board, General Hospital Minna to conduct this study. Participation in the study was entirely voluntary, oral informed consent of each patient was received before inclusion in the study and strict confidentiality of patients' information was maintained.

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APPENDIX

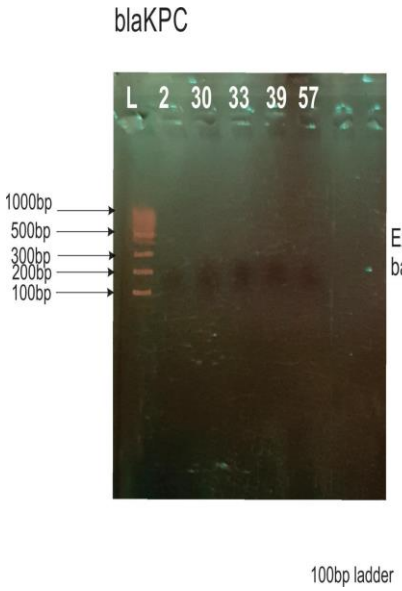

Figure 1a: Gel electrograph of blaKPC

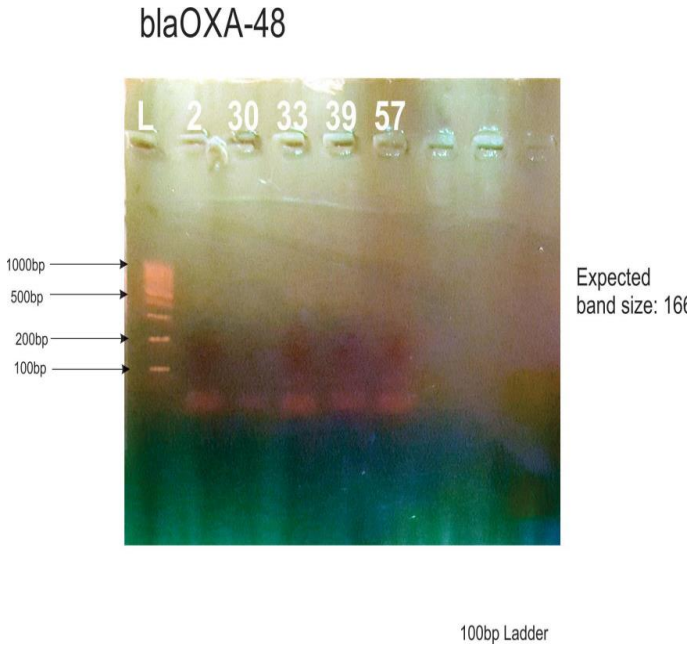

Figure 1b: Gel electrograph of blaOXA48 gene

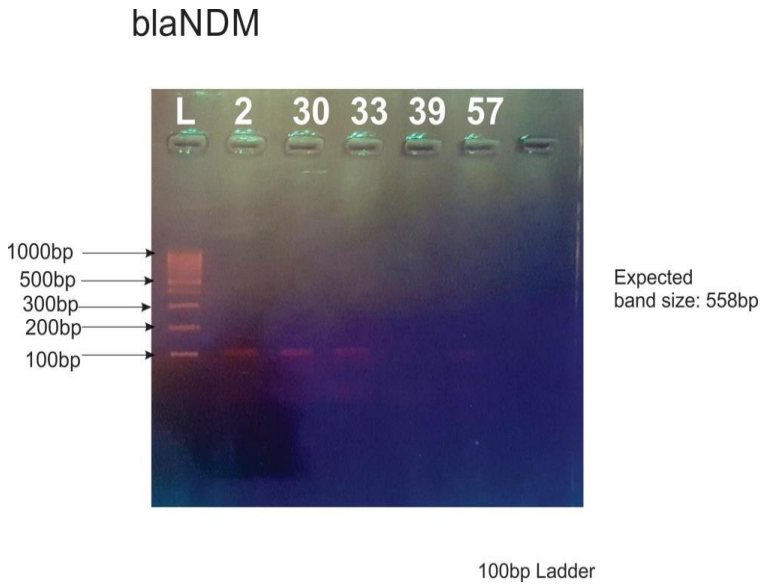

Figure 1c: Gel electrograph of blaNDM gene