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Original article

Molecular detection of carbapenem resistant *Klebsiella pneumoniae isolated* from clinical specimens in Jos, Nigeria

Jude Yunzoom Nkup*[*1], Sarah Anzahi Joseph [1], Yusuf Amuda Agabi [1], Veronica David [1], Zakari Hashimu [1], Caroline Chiamaka Madubulum [1], Amanyi David [2], Ujah Otobo [3,4], Gloria Albert Goyil [1], Nanya Amos Cirfat [1], Joseph Aje Anejo-Okopi [1], Samirah Awak David [1]

1- *Department of Microbiology, Faculty of Natural Sciences, University of Jos, Jos, Nigeria.*
2- *Department of Family Medicine, Faculty of Medical Sciences, University of Jos, Jos, Nigeria.*
3- *Department of Obstetrics and Gynecology, Faculty of Medical Sciences, Jos University Teaching Hospital, Jos, Nigeria.*
4- *College of Public Health, The University of South Florida, Tampa, USA.*

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ABSTRACT

Background: *Klebsiella pneumoniae* is among the most frequent opportunistic pathogens causing wide range of infections and the emergence and spread of carbapenem resistant *Klebsiella pneumoniae* are problems currently threatening global health. The present study aimed to determine the prevalence of carbapenem resistant *Klebsiella pneumoniae* and to detect carbapenem resistance genes in Jos, Nigeria. **Methods:** A total of 19 clinical specimens were collected from Jos University Teaching Hospital (JUTH) and National Veterinary Research Institute Vom, Jos, Nigeria. *Klebsiella pneumoniae* was identified by cultural and biochemical methods. Antibiotic susceptibility test was performed using modified Kirby-Bauer disc diffusion technique. Carbapenem resistant *Klebsiella pneumoniae* isolates were tested for carbapenemase production and blaKPC, blaVIM,and blaNDM genes using Modified Hodges Test and multiplex PCR respectively. **Results:** Of the 19 clinical specimens examined, 8(42.10%) were positive for *Klebsiella pneumonia* and most of the isolates were recovered from urine 5(62.50%), followed by sputum 2(25.00%) and isolates from wounds swab recorded the lowest occurrence, 1(12.50%). Antibiotic susceptibility test showed that isolates were highly resistant to gentamicin (62.50%), ceftriaxone (50.00%), amoxicillin/clavulanic acid (62.50%), ciprofloxacin (37.50%), cefepime (62.50%), clarithromycin (50.00%) and meropenem 3(37.50%). Polymerase chain reaction screening for blaKPC, blaVIM,and blaNDM genes which code for carbapenemases among the 3 meropenem resistant *Klebsiella pneumoniae* isolates were not detected. **Conclusion:** The expression of high phenotypic antibiotic resistance recorded in this study could be suggestive of other resistance genes which this study was limited for. Therefore, monitoring of carbapenem resistant *Klebsiella pneumoniae* with the aim of screening other antibiotic resistance genes is strongly recommended.

Introduction

Antimicrobials have been considered to be on the losing side in the therapeutic battle against pathogenic microorganisms; however, carbapenems which are regarded as the drug of last resort are the preference for severe infections caused by antimicrobial resistant strains [1]. The genus *Klebsiella* belongs to the *Enterobacteriaceae* family and comprises of gram negative opportunistic non

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* *Corresponding author:* Jude Yunzoom Nkup
E-mail address: jnkup@yahoo.com

motile pathogens with a mucoid aspect. The gastrointestinal tract serves as a reservoir and often the latent source of infections [2]. It is one of the leading causes of hospital and community acquired infections manifesting as pneumonia, urinary tract infections and abscesses [3].

Different antibiotic resistance mechanisms especially those resistant to carbapenems have been identified in *Klebsiella pneumoniae.* The production of beta lactamase enzymes is one of the most important mechanisms [4]. According to the ambler classifications, beta lactamase enzymes types A,B,C and D have been identified based on the molecular structure and homology, and are divided into several sub-classes. The most clinically important carbapenems enzymes gene is carbapenem resistant *Klebsiella pneumoniae* (CRKP) belonging to the ambler class A (KPC type) [5].

Klebsiella pneumoniae are ambler class A plasmid encoded enzymes that are capable of hydrolyzing all beta lactam antibiotics including monobactams, extended spectrum cephalosporin and carbapenems. Additionally, microbial resistance against carbapenem has become a global concern and studies on the detection of carbapenemase producing *Klebsiella* specie (CPK) isolates in clinical settings are increasingly being reported [6]. The rise in antimicrobial resistance seen in *Klebsiella pneumoniae* has become a major problem in health care systems and given the substantial impact of CRKP on patients' outcome and the need to identify resistance genes to curtail the spread of infections. Therefore, this study aimed to determine the prevalence of carbapenem resistant *Klebsiella pneumoniae* and to detect carbapenem resistance genes in some clinical specimens in Jos, Nigeria.

Materials and Methods

Study area and design

This work was a prospective and descriptive study conducted on a pool of clinical specimens collected between January and February, 2020 among individuals attending Jos University Teaching Hospital (JUTH) and National Veterinary Research Institute (VOM), in Plateau state, Nigeria. The hospitals are among the major hospitals located within the state providing essential, specialized and referral medical and surgical services to residents of Plateau state and patients from neighboring states.

Ethical clearance was obtained from the Ethical Review Committee of Jos University Teaching Hospital in Plateau state, Nigeria. Informed consent was obtained from each study subject and confidentiality of patient information and samples was maintained at all times. Spent samples were appropriately disposed by autoclaving.

Specimens collection and culturing

Nineteen (19) pools of clinical specimens were collected in sterile well-labeled disposable containers and taken to microbiology laboratory for processing and examination.

The 19 hospital specimens (11 urine, 5 sputum and 3 wound swabs); sputum and swab specimens were cultured on blood agar (Oxoid) and MacConkey agar (Oxoid), while urine specimens were cultured on Cysteine Lactose Electrolytes Deficient (CLED) agar and incubated at 37°C for 24 hours. Thereafter, suspected *Klebsiella pneumonia* isolates were identified by Gram stain and biochemical tests (indole test, catalase test, urease test, citrate test, oxidase test and triple sugar (TSI)) as described [7].

Antibiotic susceptibility testing

Antibiotic susceptibility test was carried out using the modified Kirby–Bauer disc diffusion method as outlined in the current European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [8]. The *Klebsiella pneumonia* isolates were tested for antibiotic susceptibility using the following commercially available discs (Oxoid Ltd, Basingstoke, UK): carbapenems (meropenem (10µg); cephalosporins (ceftriaxone (30µg), cefepime (30µg) and ceptazidime (30µg)); fluoroquinolones (ciprofloxacin (5µg));aminoglycosides (gentamicin (10µg); penicillins (amoxicillin/clavulanic acid (30µg) and macrolide (clarithromycin (15µg)) on Mueller Hinton (Oxoid) agar plates. Incubation was performed at 35°C for 24 hours. After incubation isolates with zonediameters <25 mm to meropenem (10µg) as stated inthe EUCAST guidelines for the detection of resistance were classified as carbapenem resistant and were tested for carbapenemase production.

Phenotypic detection and carbapenemase production

The phenotypic detection of carbapenemase was performed using the Modified Hodges test (MHT) as described [9]. Briefly, a 0.5 McFarland standard suspension of *E. coli* ATCC

25922 in saline was prepared, and a dilution of 1:10 was streaked on Mueller Hinton Agar plate. Meropenem (10µl) disk was placed at the center of the plate. *Klebsiella pneumoniae* was streaked in a straight line out from the edge of the disk to the edge of the plate incubated overnight at 35^0C for 24hours. Interpretation of both negative and positive tests was done according to CLSI 2017 [9].

Polymerase chain reaction (PCR) detection of carbapenemase encoding genes

The detection of carbapenem resistance genes was performed by multiplex PCR. Isolates were screened by PCR amplification using specific primers for the detection of carbapenemases genes (*blaKPC*, *blaVIM*,and *blaNDM*). The specific primers and length of expected PCR products are presented in **table (1)**. Genes were amplified using primers and conditions as described (10), amplified products were electrophoresed on a 1.5% agarose gel and stained with ethidium bromide. The results were observed with a gel imaging system and photographed. A 100bp DNA ladder was used.

Table 1. Primer sequences used for detection of carbapenemase encoding genes in *Klebsiella pneumonia* [10].

Gene type	Primer	Primer sequence	Amplicon size(bp)
Carbapenemase genes internal primers	KPC	F-5'-ATTCGCTAAACTCGAACAG-3' R-5'-AAGAAAGCCCTTGAATGAG-3'	1069
	NDM-1	F-5'-ACTTCCTATCTCGACATGC-3' R-5'-TGATCCAGTTGAGGATCTG-3'	1069
	VIM-1	F-5'-GAGCTCTTCTATCCTGGTG-3' R-5'-CTTGACAACTCATGAACGG-3'	1069

Results

Out of the 19 specimens, 8 (42.10%) were positive for *Klebsiella pneumoniae* **(Figure 1)**.

Distribution of the isolates among the clinical specimens shows that most of the isolates were recovered from urine (62.50%), followed by sputum (25.00%) with wound swabs having the lowest occurrence of 12.50% **(Figure 2).**

Eight antibiotics including meropenem (10µg), ceftriaxone (30µg), cefepime (30µg), ceptazidime (30µg), ciprofloxacin (5µg), gentamicin (10µg), amoxicillin/clavulanic acid (30µg) and clarithromycin (15µg) were used to determine the pattern of antimicrobial susceptibility test among the 8 *K. pneumoniae* isolates. Test result was categorized into susceptible (S), resistant (S) and intermediate (I). Of the 8 isolates tested for antibiotic susceptibility, 3(37.50%) showed carbapenem resistance.Isolates also showed high resistance to gentamicin (62.50%), ceftriaxone (50.00%), amoxicillin/clavulanic acid (62.50%), ciprofloxacin (37.50%), cefepime (62.50%) and clarithromycin (50.00%) **(Table 2)**.

The 3 carbapenem resistant *K. pneumoniae* isolates were subjected to detection of *blaKPC*, *blaVIM*,and *blaNDM* resistance genes using PCR, however, the reaction confirmed absence of these genes among the isolates.

Table 2. Antibiotic susceptibility pattern of *Klebsiella pneunomiae* isolates from Jos, Nigeria.

Number of *K. pneumoniae* isolates=8			
Antibiotics	**Susceptible (%)**	**Intermediate (%)**	**Resistance (%)**
Gentamicin (10µg)	2(25.00)	1(12.50)	5(62.50)
Ceftriaxone (10 µg)	4(50.00)	0(0.00)	4(50.00)
Amoxicillin/clavulanic acid (30 µg)	3(37.50)	0(0.00)	5(62.50)
Ciprofloxacin (5 µg)	3(37.50)	2(25.00)	3(37.50)
Cefepime (10µg)	1(12.50)	2(25.00)	5(62.50)
clarithromycin (15 µg)	1(12.50)	3(37.50)	4(50.00)
Meropenem (10 µg)	5(62.50)	0(0.00)	3(37.50)

Figure 1. Prevalence of *Klebsiella pneumoniae* isolated in some clinical specimens in Jos, Nigeria.

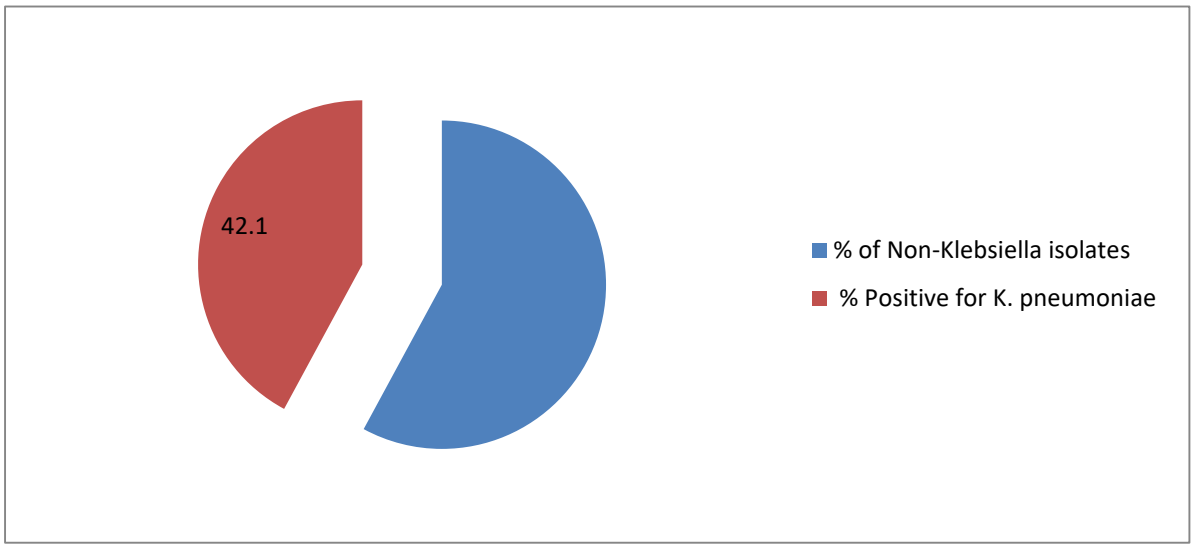
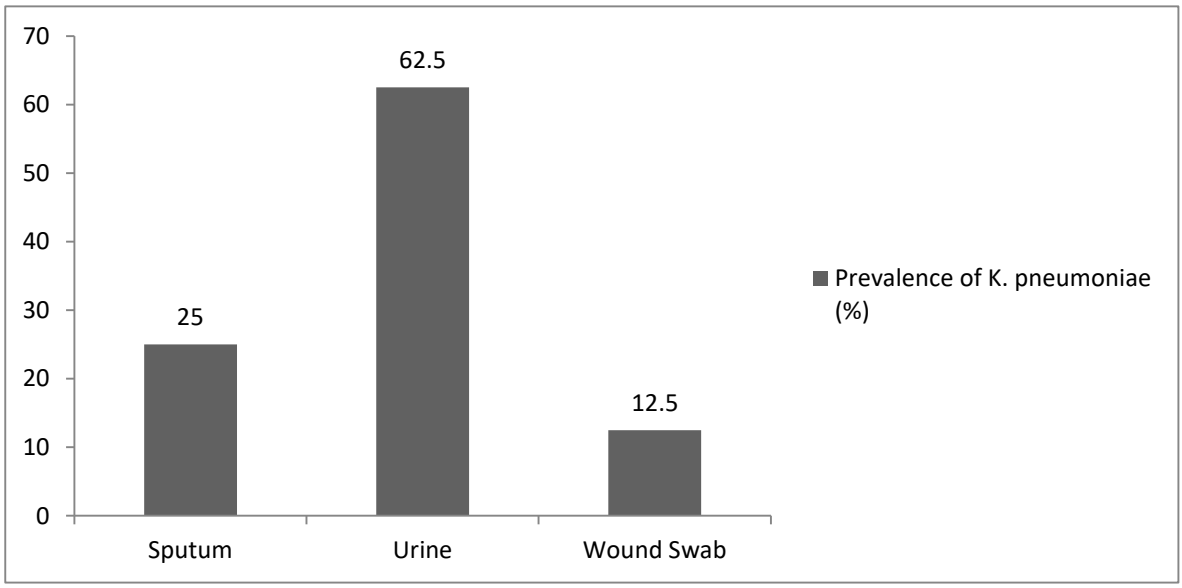


Figure 2. Distribution of *Klebsiella pneumoniae* from different clinical specimens in Jos, Nigeria



Discussion

The increased prevalence of *Klebsiella* spp among some clinical specimens has been a public health concern worldwide, as it has been one of the organisms showing higher resistance to available antibiotics. In this study, an overall *K. pneumoniae* prevalence of 42.10% was observed among the clinical specimens collected from Jos University Teaching Hospital (JUTH) and National Veterinary Research Institute (NVRI) Vom, in Jos. The high prevalence of *K. pneumonia*e observed in this study is comparable with the prevalence of 52.2% obtained from a similar study carried out in Mulago, [11] and the 50.0% prevalence rate reported in Mbarara, both in Uganda [12]. Lower rates of *K. pneumoniae* were reported in related studies conducted in Enugu and Calabar Nigeria, by **Nwafia et al**. [13] and **Jombo et al.** [14]. They documented 25.0% and 14.8% prevalence of *K. pneumoniae* from patients with urinary tract infections respectively. Lower rates of *K. pneumonia* were also reported elsewhere. **Turugurwa et al.** [15] documented 28.0% prevalence in Mbarara, Uganda. Also, [16] and [17] respectively reported 20.26% and 12.70% prevalence of *K. pneumoniae* in Uganda. *Klebsiella pneumoniae* infection varies with geographical location [18]. Factors such as unprotected sexual intercourse, peer group influence, low socio-economic status among Nigerian men and women [19] as well as the time and season of specimen collection could be responsible for the high prevalence observed in this study.

Most of the *K. pneumonia* isolates were recovered from urine specimens 5(62.5%), followed by sputum samples 2(25.00%) and the lowest occurrence was in wounds swab 1(12.50%). This finding agrees with previous study by **Olowo-okere et al.** [20] from Sokoto, Nigeria, where they isolated 292 *Enterobacteriaceae* including *K. pneumoniae* from clinical specimens and most were recovered from urine specimens [49.0%). **Turugurwa et al.** [15] also reported highest (50.85%) *Klebsiella* recovery from urine samples irrespective of age and gender in Uganda. Similar findings from other investigators from Uganda reported that urine is the principal source of *Klebsiella* [21, 22]. In another study by **Okoche et al.** [11] whose report is against the present study, reported that *K. pneumoniae* were mostly from pus swabs. Urine sample has the highest source of *K. pneumoniae* in this study is suggestive of urinary tract infections and accounts for almost 40% of nosocomial infections [23]. More so, environmental pressure [15] has been noted as a factor that could be responsible for the variations observed in the recovery of *K. pneumoniae* from clinical specimens. *Klebsiella* is the most heat tolerant among all enteric pathogens [24], having maximal specific growth rate at 37˚C and are reported to survive at higher humidity [25]. Therefore, characteristics seasonalvariation influences recovery of *Klebsiella*, as during the warmest months there are increased reports of *Klebsiella* isolation from urine [15].

Antibiotic susceptibility pattern showed carbapenem resistance in 3 (37.50%)of the tested isolates.Isolates also showed high resistance to gentamicin (62.50%), ceftriaxone (50.00%), amoxicillin/clavulanic acid (62.50%), ciprofloxacin (37.50%), cefepime (62.50%) and clarithromycin (50.00%). The 3 carbapenem resistant *K. pneumoniae* isolates were subjected to PCR for the detection of *blaKPC*, *blaVIM*,and *blaNDM* resistance genes which are responsible for carbapenemases production, however, PCR confirmed absence of these genes among the isolates. **Mohammed et al.** [26] reported *blaKPC* as the predominant carbapenemase gene in a similar study from Maiduguri, northeast Nigeria. Most studies in Nigeria have reported different carbapenem resistance genes from other *Enterobacteriaceae*. [20, 27, 28] reported *blaNDM*-5, *blaOXA*-48 and *blaOXA*-181 as the commonest carbapenemase genes in Nigeria. Our finding may however suggest that the resistance to meropenem could be due to other mechanisms such as efflux pump, decreased outer membrane permeability, porin loss or presence of β-lactams genes, as this was supported by previous studies where *bla*KPC was not detected in *K. pneumoniae* isolates [29, 30].

Conclusion

Emergence and spread of carbapenemase resistant *K. pneumoniae* are among the major problems currently threatening globalpublic health. The expression of high phenotypic antibiotic resistance recorded in this study could be suggestive of other resistance genes which this study was limited for. Therefore, continuous monitoring of carbapenem resistant *K. pneumoniae* with the aim of screening other antibiotic resistance genes is strongly recommended.

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Conflict of interest

All authors have approved the final article and declared no conflict of interest.

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