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(RESEARCH ARTICLE)

Phenotypic detection of Carbapenamase among *Klebsiella pneumoniae* isolated from clinical samples using modified Hodged test

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Abstract

Carbapenemases are microbial enzymes that confer resistance to virtually all available beta-lactam antibiotics and the most frequent carbapenemases are the *Klebsiella pneumoniae* Carbapenamase (KPC). Detection of carbapenemases is a significant infection control strategy as the enzymes are often associated with extensive antimicrobial resistance, therapeutic failures and mortality associated with infectious diseases. A total of 400 clinical samples were collected from different groups of patients in Abubakar Tafawa Balewa University Teaching Hospital, Bauchi, Nigeria and 118 K. pneumoniae were isolated using standard microbiological techniques. The isolates were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method, then screened for Carbapenamase production using modified Hodge test. The results indicated that the isolates were resistant to Ampicillin (61.9%), Ceftriaxone (50.8%) and Ceftazidime (50.8%), then Ciprofloxacin (54.2%), but predominantly sensitive to Imipenem (66.9%), Eterpenem (60.2%) and Meropenem (65.3%). It was found that 38 (32.2%) of the isolates phenotypically shows the presence of Carbapenamase, with highest frequency of (40.7%) among patients, mainly adult females with cases of Urinary Tract Infections (UTIs) and the least from wound (11.8%).This study revealed that the isolates produced other beta-lactamases than KPC or variants of Carbapenamase that cannot be detected by modified Hodge test, thus shows low resistance to carbapenems. Therefore further studies is needed to genotypically confirm the presence of KPC in these isolates.

Keywords: *Klebsiella pneumoniae*; Carbapenem; Modified hodged test; Carbapenamase

1. Introduction

Klebsiella is a Gram-negative bacterium that can cause different types of infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. *Klebsiella* bacteria are normally found in the human intestines, as commensals. *Klebsiella i*nfections commonly occur among sick patients who are receiving treatment for other conditions, whose care requires devices like ventilators or intravenous catheters, and patients who are on prolonged antimicrobial therapy [1]

Klebsiella pneumoniae have increasingly developed antimicrobial resistance to some antibiotics like carbapenems due to production of an enzyme known as Carbapenamase, thus referred to as KPC-producing bacteria. Infections caused by KPC-producing bacteria can be difficult to treat because fewer antibiotics are effective against them [2]. In such cases, a

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microbiology laboratory must run tests to determine which antibiotics will treat the infection. *Klebsiella pneumoniae* carbapenemases (KPCs) producing bacteria have emerged as a cause of multidrug-resistant nosocomial infections worldwide [2, 3]. KPCs are plasmid-encoded enzymes capable of hydrolysing a broad spectrum of beta-lactams, including carbapenems and monobactams [3].

Carbapenamase is an enzymes that hydrolyse a group of antibiotic called carbapenems which was a drug of choice for the treatment of multidrug resistance bacteria especially Extended spectrum beta lactamase producers by binding to the penicillin binding proteins (PBAs), inhibiting the synthesis of bacterial cell wall [4]. Among the beta-lactamases, the carbapenemases are the most crucial, because of their ability to hydrolyze all beta-lactam antibiotics, including the carbapenems. The major health concern is with transmissible carbapenemases, which can be acquired unpredictably by important nosocomial pathogens such as *Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* [5].

Carbapenemases belong to molecular classes A, B, and D. The class A enzymes (Bush group 2f) are inhibited to various degrees by Clavulanate and usually hydrolyze penicillins or cephalosporins more efficiently than carbapenems [6]. For this reason, some, such as KPC enzymes, lack potent Carbapenamase activity and may be considered ESBLs that also hydrolyze carbapenems [7]. There are three classes of Carbapenamase usually identified, class A of the KPC type, class B of the New Delhi Metallo-βeta-lactamase (NDM-1), IMP and Verona Integron-encoded metallo-βeta-lactamase (VIM type), and class D of the OXA-type[5, 6].

Detection of Carbapenamase-producing bacteria can be difficult, as their presence does not always produce a resistant phenotype on conventional disc diffusion or automated susceptibility testing methods. These enzymes are often associated with laboratory reports of false susceptibility to carbapenems which can be potentially fatal [8]. However, most clinical laboratories do not attempt to detect carbapenemases on routine basis, due to the lack of availability of guidelines and procedures or lack of knowledge and expertise. The routine susceptibility tests may be unreliable and special tests are therefore required to detect the resistance mechanisms involved [8]. Considering the infection control implications and the high morbidity and mortality associated with infections caused by *K. pneumoniae*, it is essential that susceptibility methods identify carbapenem resistance rapidly and reliably [9, 10].

Modified hodged test is very crucial for the detection of Carbapenamase producing bacteria, as automated method such as VITEK-2 is not reliable because Carbapenamase-producers have MIC below the clinical carbapenems break point [11, 12]. The test has been widely used in clinical setting and it's been recommended as phenotypic techniques for identification of Carbapenamase activity by CLSI guideline [5, 13]. Carbapenamase-producing *Klebsiella pneumoniae* isolates are important causative agents of nosocomial infections associated with significant mortality rates [14, 15]. Rapid detection and typing of these bacteria is critical especially for surveillance purposes, to prevent outbreaks and optimize antibiotic therapy. This study therefore aimed to determine the effectiveness of modified Hodged test as one of the most sensitive phenotypic test for detection of KPC producers among *Klebsiella pneumoniae*.

2. Materials and methods

2.1. Study Design

This is a hospital based, descriptive and cross-sectional study involving both in and out-patients attended Abubakar Tafawa Balewa University Teaching Hospital Bauchi from June to December, 2016.

2.2. Sample collection

Total of four hundred (400) clinical samples were collected from Abubakar Tafawa Balewa University Teaching Hospital, the samples includes urine, sputum, wound swab and High vaginal swab.

2.3. Culture and Identification of *K. pneumoniae*

The samples were inoculated on CLED, MacConkey and Blood agar media (Oxoid, UK). The plates were incubated at 37^0C for 24 hours. Preliminary identification of *K. pneumoniae* was carried out based on morphological features, Gram reaction, microscopy and biochemical tests according to Microbiological standards [16, 17].

2.4. Antimicrobial susceptibility testing

Susceptibility of all isolates to the groups antimicrobial agents such as fluoroquinolones, third generation cephalosporins and carbapenems were determined by the disk diffusion method on Mueller-Hinton agar using

antibiotic disks from Oxoid Ltd. (Basingstoke, UK) according to the recommendations and interpretation of CLSI (2017). The antibiotics used were Ampicillin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Eterpenem (10µg), Imipenem (10µg), and Meropenem (10µg). Strains of *K. pneumoniae* ATCC 1705 and ATCC 1706 were used as positive and negative control respectively**)**. Isolates that shows susceptible or intermediate to disk diffusion (i.e. 16mm-21mm) were selected for Carbapenamase detection.

2.5. Modified Hodge test

A loopful of 1:10 dilution of *Escherichia coli* (ATCC 25922) was used as carbapenems susceptible organism recommended by CLSI (2014). The dilution was streaked on Mueller Hinton agar plate and a 10µg of Meropenem antibiotic disk was placed at the centre of the plate, the test organism (*K. pneumoniae*) and control organisms (*K. pneumoniae* ATCC 1705 and 1706) were streaked in straight line from the edged of the disk to the edge of the plate. The plates were then incubated at 35^0C for 16-24hours [17]. The results were interpreted as positive modified hodged test by clover leaf–like indentation of the *E. coli* 25922 susceptible strain growing along the test organism.

2.6. Statistical analysis

The data obtained were analyzed with SPSS version 20.0 using Chi-square at 95% to test degree of association.

3. Results

In this study, a total of 118 *K. pneumoniae* isolates were recovered from 400 clinical, specimens out of which 38 (32.2%) were Carbapenamase-producers (Table 1). Most of the isolates were from female patients (55.1%) within 41 to 50 years age (22.9%), but highest Carbapenamase-producers (34.2%) was found in 31 to 40 age group.

It was found that the isolates (Table 2) were resistant to some of the Beta-lactam antibiotics such as Ampicillin (61.9%), Ceftriaxone (50.8%) and Ceftazidime (50.8%), then Ciprofloxacin (54.2%), but predominantly sensitive to some carbapenems notably, Imipenem (66.9%), Eterpenem (60.2%) and (65.3%). Highest frequency of KPC producers (40.7%) were found in the urine samples (Table 3) and the least were from wound swab (11.8%).

Table 1 Distribution of *Klebsiella pneumoniae* isolates according to patients' demographic characteristics

Patients characteristics	**No. of Samples collected (n=400)**	**No. (%) of *K. pneumoniae* isolates (n=118)**	**No.(%) of Carbapenamase-producers (n = 38)**
Age group (years)			
0 – 10	65	11 (9.3)	00 (0.0)
11 – 20	45	19 (16.1)	04 (10.5)
21 – 30	52	17 (14.4)	08 (21.0)
31 – 40	74	19 (16.1)	13 (34.2)
41 – 50	86	27 (22.9)	11 (28.9)
51 – 60	38	14 (11.9)	00 (0.0)
61 – 70	27	08 (6.8)	02 (5.3)
Above 70	13	03 (2.5)	00 (0.0)
Gender			
Male	132	53 (44.9)	15 (39.5)
Female	268	65 (55.1)	23 (60.5)

Table 2 Antimicrobial susceptibility pattern of *Klebsiella pneumoniae* isolates from this Study

Antimicrobial Agents	**No. (%) of *K. pneumoniae* isolates (118) and Susceptibility pattern**			
	Disc potency (µg)	**Sensitive**	**Resistant**	**Intermediate**
Ampicillin	30	45 (38.1)	73 (61.9)	00 (00)
Amikacin	30	65 (55.1)	50 (42.4)	03 (2.5)
Ceftriaxone	30	54 (45.8)	60 (50.8)	04 (3.4)
Cefotaxime	30	60 (50.8)	58 (49.2)	00 (00)
Ceftazidime	30	58 (49.2)	60 (50.8)	00 (00)
Ciprofloxacin	5	54 (45.8)	64 (54.2)	00 (00)
Imipenem	10	79 (66.9)	36 (30.5)	03 (2.5)
Eterpenem	10	71 (60.2)	45 (38.1)	02 (1.7)
Meropenem	10	77 (65.3)	38 (32.2)	03 (2.5)

Table 3 Carbapenamase production in *K. pneumoniae* isolates according to Modified Hodge test (MHT) in this study

Clinical samples	**No. (%) of sample collected**	**No. (%) of *K. pneumoniae* isolates**	**No. (%) of MHT Positive isolates**
Urine	142 (35.5)	48 (40.8)	23 (60.5)
High vaginal swab	82 (20.5)	18 (15.3)	10 (26.3)
Sputum	103 (25.8)	38 (32.2)	03 (7.9)
Wound swab	73 (18.3)	14 (11.9)	02 (5.3)
Total	400 (100)	118 (29.5)	38 (32.2)

4. Discussion

Carbapenems resistance can be mediated by production of carbapenemases or by the combination of outer membrane porin expression disruption and production of various β-lactamases [18]. Carbapenemases may confer resistance to virtually all available beta-lactam antibiotics. The most frequent carbapenemases are the *Klebsiella pneumoniae* Carbapenamase (KPC) enzymes. KPC-producing bacteria are found in many countries [19, 20].While the KPC enzyme is still found most commonly in association with *K. pneumoniae*, the blaKPC gene has now been identified in numerous other members of the Enterobacteriaceae [10].

In this study, there are only 32.2% Carbapenamase-producing *Klebsiella pneumoniae* isolated from various clinical specimens, which implies that the enzyme could be present in other enterobacteriaceae, as reported by previous studies [21]. The KPC enzyme was originally described in 2001 in a *Klebsiella pneumoniae* isolate from North Carolina [19]. Since then, KPC has disseminated worldwide and, although harboured predominantly by *Klebsiella pneumoniae*, it has also been identified in numerous other genera of the *Enterobacteriaceae.* The KPC isolates in this study were mainly in urine specimens from female patients in the middle age group. It was reported that [22, 23]. *Klebsiella* is one the common pathogen associated with UTI. Our result is slightly lower than 32.4 % recorded in 2018 in Maiduguri by Mohammed *et al.* [24], and both are higher than12.4% obtained in 2015 by Oduyebo *et al*. [25], which shows that carbapenems resistance is increasing in this area and it could pose serious treatment challenges. In Athens, Daikos *et al.* [1] identified 163 (79.5%) KPC isolates from 205 patients with cases of septicaemia, in an observational study conducted between 2010 and 2011.

In Iran, Bina *et al*. [23] reported that 57% of the *Klebsiella* isolates were from women and highest frequency of susceptibility among *K. pneumoniae* was associated to Imipenem (86.1%) and Meropenem (85.5%), which is consistent with the findings of our study. They also found 80.5% (33 of 41) isolates as Carbapenamase-positive by MHT, but all the isolates were negative for PCR amplification of the bla-KPC gene, as also recently reported by Agbanya *et al*. [26] in Nigeria, with 73.3% sensitivity to Imipenem. However, In Portugal, Aires-de-Sousa *et al*. [20] showed that out of the 46

Carbapenamase producers in their study in Portugal,13(28%) were susceptible at lower frequency to imipenem and 10 (22%) to Meropenem.

The high antimicrobial sensitivity of *K. pneumoniae* to carbapenems in our study is an indication that the isolates might be harbouring other beta-lactamases than KPC. It can be another variants of Carbapenamase present, which cannot be detected by our study, hence the reason for low MHT positive. The low resistance to carbapenems in this study could be due to the fact that, our hospitals does not have a regular prescription pattern for carbapenems probably due to their high costs and are not usually the first line drugs used in the treatment of infectious disease in this area [24]. Other factors which contribute to carbapenem resistance is that the enzymes have social and microbiological epidemiology which embraces patient to patient transmission of KPC [27], which is low in our study area.

The sensitivity and specificity of phenotypic methods KPC detection like MHT varies, depending on prevalence of Carbapenamase resistance and the study area. Our results showed low specificity of MHT for KPC as compared to very low tests 1.5% and 7%in Nigeria and Taiwan respectively [26, 28]. Another study in Argentina revealed 57% MHT positive [29]. In addition, CLSI [12], reported more than 90% sensitivity and specificity in USA. Nordmann *et al*. [30] showed high MHT sensitivity for KPC identification. Azimi *et al.* [31], suggested the use of MHT as a primary laboratory screening test for *K. pneumoniae* Carbapenamase especially in patients whom Meropenem and Imipenem is used as the first line of antimicrobial treatment.

Modified Hodges test was used in this study to phenotypically detect Carbapenamase from the Klebsiella isolates, which has its shortcomings [16, 32]. It was reported that KPC confirmatory test options are limited to the modified Hodge test (MHT), which is subjective and suffers from false-positive results, and PCR-based methods, which may not be available in all laboratories [33]. The issue of confirmation has led others to evaluate Boronic acid inhibition for KPC confirmation. However, these methods also inhibit AmpC activity and are, therefore, not specific to KPC [30]. The performance of automated systems for the detection of KPC-producing *K. pneumoniae* has been also evaluated [14].Matrix-assisted laser desorption-ionization time-of-flight-mass spectrometry (MALDI-TOP MS) is a promising, rapid and economical method for the detection of Carbapenamase-producing *K. pneumoniae* strains that could be successfully introduced into the routine diagnostic microbiology laboratories [14, 15].

Due to the difficult elucidation and false positive results, MHT cannot be used as a confirmatory test for identification of the *K. pneumoniae* Carbapenemases (28, 29). False-positive results are a more common in isolates producing AmpC and CTX-M beta-lactamase [34, 35, and 36]. Azimi *et al*. [31] found that 64% (28 of 44) of the *Klebsiella* isolates as resistant to carbapenem with 36% sensitive. But unlike in our studies, their MHT was positive in all of carbapenem-resistant isolates. However, all the isolates were negative for presence of KPC genes thus concluded that MHT is not specific-enough for the detection of KPC. Other studies [23, 37, 38, 39] recommended the MHT as a suitable method for approving Carbapenamase production, but should accompany with PCR method for the bla-KPCgene, which has the additional advantage for confirming the specific KPC presenting the bacterial strains

5. Conclusion

The present study found that MHT might not be an appropriate phenotypic method for confirming Carbapenamase production, considering the low positive test results and high sensitivity of the isolates to common carbapenems drugs. Other standard methods, including molecular assays should therefore be employed to ascertain the presence of Carbapenamase in *Klebsiella pneumoniae*.

Compliance with ethical standards

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Disclosure of conflict of interest

We hereby declare that there is no conflicting/competing interest as authors of this paper.

Disclosure of ethical approval

Ethical approval was obtained from the ATBU teaching hospital research and ethics committee, with verbal informed consent also sought from all patients, prior to specimen and data collection.

References

[1] GL, Tsaousi S, Tzouvelekis LS, Anyfantis I, Psichogiou M, Arygyropoulou A. et al. Carbapenamase-producing *Klebsiella pneumoniae* bloodstream infections: lowering mortality by antibiotic combination schemes and the role of carbapenems. BMC Microbiology. 2014; 58(4): 2322-2328.

[2] Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing enterobacteriaceae. Journal of Antimicrob Chemotherapy. 2013; 68(3): 227-235.

[3] Chen FK, Anderson DJ, Paterson DL. Overview of the epidemiology and the threat of *Klebsiella pneumoniae* carbapenemases (KPC) resistance. Infection and Drug Resistance. 2012; 5: 133-144.

[4] Richter SN, Frasson I, Franchin E, Bergo C, Lavezzo E, Barzon L. KPC-mediated resistance in *Klebsiella pneumoniae* in two hospitals in Padua, Italy: massive spreading of a KPC-3-encoding plasmid and involvement of non-intensive care units. Journal of Antimicrob Chemotherapy. 2012; 4(1): 7-13.

[5] Kabir MH, Meunier D, Hopkins KL, Giske CG, Woodford N. A two-centre evaluation of RAPIDEC® CARBA NP for carbapenemase detection in Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter spp*. Journal of Antimicrob Chemotherapy. 2016; 71(5): 112-119.

[6] Miriagou V, Cornaglia G. Edelstein M. Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clinical Microbiology and Infections. 2010; 16(2): 112–122.

[7] Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clinical Microbiology Reviews. 2007; 20: 440–458.

[8] Asthana S, Mathur P, Tak V. Detection of Carbapenamase-Production in Gram-negative bacteria. Journal of Laboratory Physicians. 2014; 6(2): 69.

[9] Weisenberg SA, DJ Morgan R, Espinal-Witter, DH Larone. Clinical outcomes of patients with *Klebsiella pneumoniae* Carbapenamase-producing *K. pneumoniae* after treatment with imipenem or Meropenem. Diagnostic Microbiology and Infectious Disease. 2009; 64: 233–235.

[10] Doern CD, Michael Dunne Jr. W, Burnham C.A Detection of *Klebsiella pneumoniae* Carbapenamase (KPC) Production in Non *Klebsiella pneumoniae* Enterobacteriaceae Isolates by Use of the Phoenix, Vitek 2, and Disk Diffusion Methods. Journal of Clinical Microbiology. 2011; 49(3):1143-1147.

[11] Carvalhaes CG, Picao RC, Nicoletti AG, Xavier DE, Gales AC. Cloverleaf test (modified Hodge test) for detecting Carbapenamase production in *Klebsiella pneumoniae*: be aware of false positive results. Journal of Antimicrob Chemotherapy. 2010; 65: 249–251.

[12] Clinical Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing: 22nd international supplement. (M100-S22). CLSI, Wayne, PA, USA. 2017.

[13] Nordmann P, Poirel L, Dortet L. Rapid detection of Carbapenamase-producing Enterobacteriaceae. Emerging Infectious Disease. 2012; 18: 1503–1507.

[14] Neonakis IK, Spandidos Detection of Carbapenamase-producers by matrix-assisted laser desorption-ionization time-of-flight-mass spectrometry (MALDI-TOP MS). Journal of Clinical Microbiology and Infectious Disease. 2019; 38(10): 1795-1801.

[15] Sakarikou C, Ciotti I, Dolfa C, Angeletti S, Favalli C. Rapid detection of Carbapenamase-producing *Klebsiella pneumoniae* strains derived from blood cultures by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). BMC Microbiology. 2017; 7(1): 54.

[16] Hrabak J, Chudackova E, Papagianntsis CC. Detection of Carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. Clinical Microbiology and Infections. 2013; 20(9): 839-853.

[17] Cheesbrough M. District Laboratory Practice in Tropical Countries. Part two (2), Cambridge University Press, Cambridge, UK. 2012; 83-89.