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Phenotypic Detection of Carbapenemases in Members of Enterobacteriacea in Kano, Nigeria

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ABSTRACT

Background and Objectives: A study was carried out to determine the occurrence and prevalence of carbapenemase production among members of Enterobacteriacea in Aminu Kano Teaching Hospital (AKTH) Kano-Nigeria. **Materials and Method**: A total of 135 isolates of *Escherichia coli, Klebsiella pneumoniae, Proteus spp.,* and *Serratia spp* were screened for susceptibility to Meropenem and Imipenem (10µg, Oxoid, England) by disk diffusion assay in order to determine suspected carbapenemase producing isolates. Isolates with zones of inhibition to Meropenem measuring < 21mm were subjected to confirmatory test using Modified Hodge's Test. **Results**: Results show that only 8.9% (16/135) of the isolates were susceptible to Meropenem while 92.6% (125/135) were susceptible to Imipenem. The prevalence of carbapenemases among *Enterobacteriaceae* was 14.0% (19/135) and the highest prevalence was found among *K. pneumoniae* (16.7%; 2/12), followed by *Proteus spp.* (16.0%; 4/25), and *E. coli* (12.5%; 13/96). No carbapenemases were detected in *Serratia spp*. All the carbapenemase producing bacteria were resistant to Meropenem and sensitive to Imipenem. **Conclusion**: This study therefore showed that carbapenemases occurs in some members of Enterobacteriacea in Kano, Nigeria. The enzymes occur at an alarming rate in Kano, raising fear of multiple drug resistance by Gram negative bacterial pathogens.

Keywords: *Detection, Prevalence, Carbapenemases, Enterobacteriacea, Meropenem, Imipenem, Kano*.

I. INTRODUCTION

A number of resistance mechanisms can circumvent the efficacy of carbapenems. Predominant among these is the production of carbapenem-hydrolyzing β-lactamases (carbapenemases). Infection with carbapenem-resistant *Enterobacteriaceae* or carbapenemase-producing *Enterobacteriaceae* is emerging as an important challenge in health-care settings [1]. Currently, carbapenem-resistant *Klebsiella pneumoniae* is the species most commonly encountered in the United States. They have the ability to hydrolise pencillins, cephalosporins, monobactams and carbapenems [2]. Bacteria producing these β-lactamases may cause serious infections in which the carbapenemase activity renders many β-lactams ineffective and these have been associated with high rates of morbidity and mortality, particularly among persons with prolonged hospitalization and those who are critically ill and exposed to invasive devices [3].

Although known as "Carbapenemases", many of these enzymes recognize almost all hydrolysable β-lactams, and are resilient against inhibition by all commercially viable β-lactamase inhibitors [4,5].

A uniform and standardized phenotypic tool for the detection of carbapenemases is still lacking. Recently, the CLSI issued recommendations for the phenotypic screening of carbapenemase producers among species of *Enterobacteriaceae*: MICs of ertapenem (ETP), meropenem (MEM), and imipenem (IPM) of 2, 2 to 4, and 2 to 4 µg/ml, respectively (or a zone of inhibition by ETP or MEM of 21 mm in diameter in the disk diffusion [DD] assay), may indicate isolates with carbapenemase production, and this phenotype should be confirmed by the Hodge method [1].

Early recognition of producers of carbapenemases has become mandatory and crucial for controlling the spread of carbapenemase-producing bacteria as treatment of infections caused by pathogens producing carbapenemases, poses a serious challenge because these infections are resistant to all commonly used antibiotics. Carbapenems are the only reliably active antibiotics against many multiresistant gram-negative pathogens producing extended-spectrum beta-lactamases (ESBLs) and *AmpC* enzymes. In most cases, an array of pathogens will be negative for both AmpC and ESBL enzymes, but still resisting almost (if not all) third and fourth generation cephalosporins. Hence this study was carried out in order to evaluate the occurrence and prevalence of carbapenemases among members of Enterobacteriaceae in Kano, Nigeria.

II. MATERIALS AND METHODS

Sample collection

(i) Bacterial Isolates

A total of 135 clinical bacterial isolates of *Escherichia coli, Klebsiella pneumoniae, Proteus spp,* and *Serratia spp* were collected from the Pathology Department of Aminu Kano teaching Hospital (AKTH), Kano over a period of three months.

(ii) Control Strain

The control strain *E.coli ATCC 25922*, was also collected from the Aminu Kano Teaching Hospital (AKTH), Kano-Nigeria.

Screening of Pathogens for Carbapenemases

The bacterial isolates were screened for carbapenemases according to CLSI guidelines [1].In this method carbapenem antibiotic Meropenem and Imipenem discs (10µg, Oxoid, England) were used. The antibiotic discs were placed on the surface of inoculated Mueller Hinton Agar (MHA) (Hi-media, India) plates using a sterile forceps. The discs were placed about 30mm apart and the plates were incubated for 24 hours at 37^{o}C after which zones of inhibitions were read.

Isolates that showed a zone of inhibition ≤ 21mm in diameter for Meropenem or ≤23mm in diameter for Imipenem were considered as suspected carbapenemase producers and were subjected to confirmatory test by the Modified Hodges Test (MHT).

Phenotypic confirmation of carbapenemases (Modified Hodges Test)

In this method, a Mcfarland suspension of *E.coli* ATCC 25922 was evenly inoculated with a sterile cotton swab on surface of MHA plates. Two discs each of Meropenem and Imipenem (10µg, Oxoid, England) were placed on the surface of MHA 30mm apart lying diagonally opposite to each other. In a straight line, by means of a sterilized wire loop, the test organisms were streaked from the edge of one disc Meropenem (MEM) to edge of the other Imipenem (IPM). Up to four organisms were tested on the same MHA plate. The plates were incubated at 37 ^{o}C for 24 hours.

They were examined for a clover leaf type indentation or flattening at the intersection of the test organism and *E. coli ATCC 25922* within the zone of inhibition of the carbapenem susceptibility disc as described by Anderson [6].

III. RESULTS AND DISCUSSION

Resistance to carbapenems has been reported in places worldwide, such as Israel [7], China [8], America [9] and France [10] with varying prevalences. In this study, the susceptibility of the isolates to MEM and IPM was 8.9% and 92.6%, respectively (Table 1)

Table 1: Susceptibility of some members of Enterobacteriacea to Meropenem and Imipenem according to CLSI breakpoints

S/N	Bacterial isolates	No screened	No susceptible to meropenem (zone >21mm in diameter) (%)	No susceptible to Imipenem (zone >23mm in diameter) (%)
1	*E. coli*	96	12(12.5)	89(93)
2	*K. pneumoniae*	12	1(8.3)	11(92)
3	*Proteus spp*	25	3(12)	24(96)
4	*Serratia spp*	2	0(0)	1(50)
	Total	**135**	**16 (8.9)**	**125(92.6)**

Breakpoint zone diameters of 20-22mm (for intermediate sensitivity) were employed for both MEM and IPM.
Figures in parenthesis are percentages

The control strain was found to be 100% susceptible to the two carbapenems as a quality control. This is in accordance with CLSI [1] guidelines which require a zone size of 28-34mm for 10-µg/mL meropenem disk by the Disk Diffusion Method. The high sensitivity of the isolates to Imipenem is in agreement with the findings of Kiffer [11] and Turner [12] who reported that overall, worldwide susceptibility to carbapenems is 98% among enterobacteriaceae, whereas Imipenem susceptibility ranges from 60% to 83% for *Ps. aeruginosa* and *Acinetobacter baumannii* (2004 to 2005) surveys. Similarly, this study showed that carbapenemases producing isolates were 75-100% sensitive to IPM (Table 3)

Table 3: Susceptibility of Carbapenemase Producing Isolates to Meropenem and Imipenem

S/N	Bacterial isolates	No Positive for carbapenemases	No suscebtible to Meropenem	% susceptibility to Meropenem	No susceptible to Imipenem	% susceptibility to Imipenem
1	*E. coli*	13	0	0	9	75
2	*K. pneumoniae*	2	0	0	2	100
3	*Proteus spp*	4	0	0	3	75
	Total	**19**	**0**		**14**	

The prevalence of carbapenemases among the members of Enterobacteriaceae in AKTH is 14.0% (19/135) (Table 2)

Table 2: Prevalence of carbapenemases among members of Enterobacteriacea in AKTH

S/No	Bacterial species	No screened	No resistant to Meropenem (zone <21mm in diameter)	No positive for carbapenemaes	% prevalence
1	*E. coli*	96	87	13	13.5
2	*K. pneumoniae*	12	12	2	16.7
3	*Proteus spp*	25	22	4	16.0
4	*Serratia spp*	2	0	0	0.0
	Total	**135**	**121**	**19**	**14.0**

This finding is also in accordance with findings of Yusuf and Arzai [13], who reported a prevalence of 13.3% among gram negative bacterial isolates in Murtala Muhammad Specialist Hospitals (MMSH) in Kano, Nigeria. A higher prevalence was observed by Queenam and Bush [14] who reported KPC (a type of carbapenemases) prevalence of 22% and 59% for Brooklyn and NewYork, respectively. The lower prevalence in Kano may be attributed to the fact that carbapenem were not commonly sold in Nigeria because of their high cost, but the higher prevalence as compared with MMSH can be as a result of the fact that majority of those that attend AKTH are of higher socio-economic class which can afford to buy the antibiotics despite their their high cost. The origin or source of these enzymes is unknown, but probably they were imported from abroad or they emerged locally and spread by gene tranfer. Perhabs the patients may have a history of travel to endemic areas for treatment such as India, Pakistan or United State.

Equally important is that substantial amount patients of AKTH are usually being referred from other hospitals such as MMSH, this will facilitate their wide spread in the community.

The highest prevalence of carbapenemases producers was in *K pneumoniae* (16.7%). This was followed by *Proteus spp* (16%), and *E. coli* (13.5%). The highest prevalence of carbapenemases in *K. pneumoniae* agreed with the finding of Landman [15] who reported that over one-third of *K. pneumoniae* collected in 2006 in New York, USA carried the carbapenemase KPC. KPC Carbapenemases are the most prevalent found mostly on plasmids in *Klebsiella pneumoniae*. A much lower prevalence of carbapenemases (0.15%) was reported by Jones [16] in Tel Aviv, Israel. The multiple resistance capability of these bacteria in Kano, Nigeria may be due to multiple factors including uncontrolled antibiotic usage, inappropriate dosing regimens, wide spread of counterfeit and substandard antibiotics and local hospital practices concerning isolation of patients with multi-resistant pathogens which is poorly managed in Nigeria. Majority clinical laboratories in Kano are unaware of their occurrence while few have problems detecting beta lactam hydrolyzing enzymes such as extended-spectrum beta-lactamases (ESBLs), plasmid-mediated AmpC beta-lactamases and carbapenemases. Confusion exists about the reasons why treatment failure still persists. Clinicians blamed the laboratory scientist of reporting wrong sensitivity pattern, while the fact is that these laboratories lack adequate funding, equipment, and expertise to provide a rapid and clinically relevant antibiotic

testing service. These problems have contributed to their uncontrolled spread and sometimes to therapeutic failures.

If the spread of carbapenemases in hospitals is not controlled, the reliance on carbapenems in treatment of infection caused by multiple drug resistance bacteria (ESBL, AMPC producers) may be jeopardized.

Limitation of the Study

One limitation of our study is that the isolates were not screened for additional beta-lactamases e.g. ESBL other than *carbapenemases*, which often give a false positive result with MHT. Also the sample size was relatively small and a single-center design was used. The gram-negative bacteria were not tested for bla_{KPC} due to lack of equipment.

IV. CONCLUSION

From the findings of the study the following conclusions were drawn:

- Carbapenemases was detected in almost all the members of Enterobacteriaceae tested.
- Carbapenemase production among certain bacterial pathogens in Kano occurs at an alarming rate, raising fears of resistance to a multitude of antibiotics in the treatment of clinical infections.
- Most of the Carbapenemase-Producing pathogens are highly resistant to Meropenem but sensitive to Imipenem.

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