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Laboratory Evaluation of Clinical Bacterial Isolates for Detection of Carbapenemases-Producing *Enterobacteriaceae* in Kano, North West, Nigeria

Y. Mohammed[1*], N. T. Dabo[2] and M. K. Umar[2]

[1]Department of Medical Microbiology and Parasitology, Bayero University Kano, Nigeria.
[2]Department of Microbiology, Bayero University Kano, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors YM and NTD did the study design and wrote the protocol. Authors YM and MKU did the statistical analysis and literature searches while analyses of study was by author NTD. All authors read and approved the final manuscript.

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ABSTRACT

The spread of carbapenemase-producing *Enterobacteriaceae* is a global problem; however, no exact data on the epidemiology of carbapenemase in Kano, Northern Nigeria is available. The study was aimed to detect the occurrence and prevalence of carbapenemase production among clinical bacterial isolates in Aminu Kano Teaching Hospital, Kano, Nigeria. From March to August 2014, a total of 94 clinical bacterial isolates comprising of *E. coli* (44), *Klebsiella pneumoniae* (27), *Proteus mirabilis* (19) and *Proteus vulgaris* (4) were screened for susceptibility to 3rd generation Cephalosporins using Kirby-Bauer disc diffusion method and for carbapenemase production using Modified Hodges Test. Result obtained showed that 7(7.4%) of the isolates were found to produce carbapenemase. Highest prevalence of carbapenemase production was found in *E. coli* (9.09%) followed by *Klebsiella pneumoniae* (7.40%) *Proteus mirabilis* (5.26%) and none were found in *Proteus vulgaris* (0%). Urine samples were found to be with the highest prevalence of 57.1% when

**Corresponding author: Email: drymohd@yahoo.com;*

compared with sputum (42.9%). This showed that carbapenemase-mediated resistance occurred in Kano state and uncontrolled spread may lead to treatment failure.

Keywords: Antimicrobials; detection; carbapenemases; bacterial isolates; Kano.

1. INTRODUCTION

Antibiotic resistance remains a major global public health problem that leads to increasing healthcare costs, extra length of hospital stay, and treatment failures [1]. An emerging problem is the spread and increasing prevalence of carbapenemase-producing gram-negative bacteria. For example, outbreaks of *Klebsiella pneumoniae* carbapenemase (KPC)-positive *K. pneumonia* occurred in the USA in 2001 and subsequently spread throughout the world; New Delhi metallo-*β*-lactamase (NDM-1)-positive *K. pneumoniae* was imported from India and spread to the United Kingdom in 2010 [2]. *Enterobacteriaceae* are inhabitants of the intestinal flora and are among the most common human pathogens, causing infections such as cystitis and pyelonephritis with fever, septicemia, pneumonia, peritonitis, meningitis, and device-associated infections. *Enterobacteriaceae* are the source of community- and hospital-acquired infections. They have the propensity to spread easily between humans (hand carriage, contaminated food and water) and to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons [3].

The Carbapenems namely imipenem, meropenem, ertapenem, and doripenem are the antimicrobials of last resort used in treating infections due to highly drug resistant bacteria [4]. These antimicrobial agents became crucial in the management of life-threatening healthcare-associated and community acquired infections [5]. Carbapenems are no longer fully effective in the CRE (Carbapenems Resistance *Enterobacteriaceae*) epidemic. The paucity of novel antimicrobials in development escalates the antimicrobial resistance problem, severely reducing the available therapeutic choices [6].

The mechanism of carbapenemase production is the most prominent mechanism of carbapenem resistance and is distinct from other antimicrobial resistance such as impaired permeability due to porin mutations, although the susceptibility patterns for carbapenemase producing isolates and those with porin mutations can be identical [7]. CRE infections pose a serious threat to public health due to high mortality rates, resistance to commonly used antibiotics, limited treatment options, and the potential for widespread dissemination , mortality rates of up to 40% to 50% have been reported [8]. 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 [9].

Their rapid dissemination is worrisome and necessitates not just surveillance study but also studying occurrence and prevalence in a hospital setting to formulate a policy of therapy. Hence this study was carried out in order to detect the occurrence and prevalence of carbapenemase production among clinical bacterial isolates in Aminu Kano Teaching Hospital, Kano, Nigeria.

2. MATERIALS AND METHODS

2.1 Collection of Isolates

A total of 94 bacterial isolates of *Escherichia coli, Klebsiella pneumoniae, Proteus* spp*,* and *Serratia* spp from clinical specimens of urine, wounds, sputum, stool and high vaginal and endo cervical swabs, *and* the control strain *E. coli ATCC 25922* were collected from the Pathology Department of Aminu Kano Teaching Hospital (AKTH), Kano over a period of six months from June to December, 2014.

2.2 Susceptibility Testing

The antimicrobial susceptibility pattern of the isolates to Cefotaxime and Ceftriaxone was determined using Kirby-Bauer disc diffusion method [10].

2.3 Screening of Pathogens for Carbapenemases

The bacterial clinical isolates that were resistant to any or both of the above 3rd generation cephalosporin were screened for carbapenemases [11]. In this method carbapenem antibiotic, meropenem and imipenem discs (10 µg, Oxoid, England) were used. The antibiotic discs were placed on the

surface of inoculated Mueller Hinton Agar (MHA) plates using a sterile forceps. The discs were placed about 30 mm apart and the plates were incubated for 24 hours at 37℃ after which zones of inhibitions were read. Isolates that showed a zone of inhibition ≤ 19 mm in diameter for meropenem or ≤19 mm in diameter for imipenem were considered as suspected carbapenemase producers and were subjected to confirmatory test by the Modified Hodges Test [10].

2.4 Confirmation of Carbapenemases (Modified Hodges Test)

In this method, a 0.5 McFarland suspension of *E. coli* ATCC 25922 was prepared in 5 ml of saline and the suspension was diluted to 1:10 by adding 0.5 Mc Farland to 4.5 ml of saline, this was then used to evenly inoculate the surface of MHA plates using a sterile cotton swab. Meropenem disc (10 µg, Oxoid, England) was placed on the center of MHA using sterile forcep, by means of a sterilized wire loop, the test organism was streaked from the edge of the disc to the edge of the plate [10]. The plates were incubated at 37°C for 24 hours. After incubation MHT positive test showed a clover leaf like indentation of the *Escherichia coli* 25922 growing along the test organism growth streaked within the disk diffusion zone. While MHT Negative test showed no growth of the *E. coli* 25922 along the test organism growth streaked within the disk diffusion zone [11].

3. RESULTS

The 94 clinical bacterial isolates used during this research work analyzed were confirmed, using various biochemical tests to be *E. coli* (48%) as the most frequent spp. followed by *K. pneumoniae* (28.7%), *P. mirabilis* (20.2%), and the least frequent spp. is *P. vulgaris* (4.2%).

On subjecting the above clinical isolates to susceptibility testing using Kirby-Bauer disc diffusion method, a total of 47 (50.0%) isolates were resistant to cefotaxime, 23 (24.5%) were resistant to ceftriaxone. Out of 49 isolates that were resistant to any or both of the tested cephalosporin 12 (24.5%) were resistant to meropenem and 5 (41.67%) were resistant to imipenem.

These carbapenems resistant isolates are the suspected carbapenemase producers and they are subjected to a confirmatory tests using modified hodge test in which 7 out of the 12 were confirmed to be carbapenemase producers which include *E. coli* 4 (9.09%), *K. pneumoniae,* 2 (7.40%), *Proteus mirabilis 1* (5.26%), and *P. vulgaris* 0 (0%).

4. DISCUSSION

The study showed that carbapenemase producing organisms (CPO) exist in some of clinical bacterial isolates in Kano State, with a prevalence of about 7.4% this is in agreement with the findings of [12] which reported carbapenemase production with a prevalence of 14.0% in 2010. Few months later another research published by Yusuf et al. [13] reported a decreased prevalence of 13.32% in Kano Nigeria, this shows that the carbapenemase producers exist among clinical bacterial isolates in Kano State, but with a low prevalence. This may be due to unavailability and cost of the antibiotics in Kano where only few can afford to buy it, and they are prescribed for only life threatening infections.

A higher prevalence was reported elsewhere for example [14] also reported 59% in New York. This high difference in prevalence could be as a result of the aforementioned reasons.

The high prevalence among *E. coli* (9.09%) and *K. peumoniae* (7.40%) agrees with the report of [15] which reported that carbapenem resistance has been cited in up to 4.0% of *Escherichia coli* and 10.8% *Klebseilla pneumoniea* isolates, reported to the national health care safety

Table 1. Susceptibility of the isolates to cefotaxime and ceftriaxone

S/N	Bacterial isolates	No. screened	No. susceptible to cefotaxime (%)	No. susceptible to ceftraxone (%)
1.	*E. coli*	44	24 (55)	28 (64)
2	*K. pneumoniae*	27	8 (30)	15 (56)
3	*Proteus mirabilis*	19	13 (68)	17 (89)
4	*Proteus vulgaris*	4	0 (0)	1 (25)
Total		94	45 (47.9)	61 (64.9)

Table 2. Susceptibility of the isolates to meropenem and imipenem

S/N	Bacterial isolates	No. screened	No. susceptible to meropenem (%)	No. susceptible to imipenem (%)
1.	*E. coli*	21	17 (81)	9 (43)
2	*K. pneumoniae*	16	14 (88)	10 (63)
3	*Proteus mirabilis*	5	5 (100)	3 (60)
4	*Proteus vulgaris*	4	3 (75)	4 (100)
Total		46	39 (84.8%)	26 (56.5%)

Table 3. Prevalence of carbapenemase among the clinical bacterial isolates

S/N	Clinical bacterial isolates	No. of isolates screened	No. of isolates producing carbapenemase	% prevalence
1.	*E. coli*	44	4	9.09
2	*K. pneumoniae*	27	2	7.40
3	*Proteus mirabilis*	19	1	5.26
4	*Proteus vulgaris*	4	0	0
Total		94	7	7.4

network. The high prevalence among *E. coli* (9.09%) also tally with the result of [12] which reported that highest number of carbapenemase producers (MBL type) was found in *E. coli* (89.7%).

Antimicrobial susceptibility pattern of the isolates shows that out of 94 isolates tested 45 (47.9%) were sensitive to cefotaxime, and 61 (64.9%) were sensitive to ceftriaxone, and out of 46 isolates tested for carbapenem susceptibility 39 isolates (84.8%) were sensitive to meropenem, and 26 isolates (56.5%) were sensitive to imipenem.

Based on the analysis of the clinical samples, urine was found to have the highest prevalence (57.1%) of carbapenemase producers, followed by sputum (42.9%) this may be due to the fact that urinary tract infection is on increase worldwide and a multitude of antibiotic is used in their treatment. The reports of urinary pathogen's resistant to wide range of antibiotics in Kano, Nigeria mediated by extended spectrum beta Lactamases, AmpC beta lactamases and carbapenemases have been reported by [16]; [13] and [12] respectively.

5. CONCLUSION

Infections with CRE are an emerging clinical threat and from the findings obtained from this study it is concluded that carbapenemases occurred among clinical bacterial isolates in Kano State raising fears of resistance to a multitude of antibiotics in the treatment of clinical infection.

6. RECOMMENDATIONS

The Carbapenems should not be routinely used as first-line therapy unless the pathogen is multidrug-resistant and is known to be susceptible to these agents. Due to the growing threat of CRE infections in the healthcare setting, it is important for pharmacists and clinicians to be familiar with antimicrobial therapy options, risk factors, and diagnostic indicators. Development of new antimicrobials is needed, in light of limited data with current treatment options and growing resistance. Prevention strategies and good infection control programs should be incorporated at the healthcare system level to limit the transmission of CRE within the hospital. A concerted global commitment to the intelligent use of antimicrobials, better antibiotic stewardship, the implementation of effective infection control strategies, and the development of more effective therapies are desperately

7. LIMITATION OF THE STUDY

Limitation of our study is that the clinical bacterial isolates were not screened for various types of carbapenemases such as MBLs carbapenemases. 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.

CONSENT

It is not applicable.

ETHICAL ISSUES

Ethical approval and administrative clearance for the study were obtained from the ethical

committee of Aminu Kano Teaching Hospital, Kano.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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