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PREVALENCE OF CARBAPENEM RESISTANCE IN CLINICAL BACTERIAL SPECIES ISOLATED FROM KANO, NORTH WEST NIGERIA

Umar, M. K., Mohammed, Y. and Dabo, N. T.
1. Microbiology Department Federal University Dutsin-Ma, Katsina Nigeria.
2.Medical Microbiology Department Bayero University Kano Nigeria.
3 Microbiology Department Bayero University Kano Nigeria.
Correspondence author's email maryamkabir0663@gmail.com; Tel: 08027820841

ABSTRACT
Carbapenems are antimicrobials of last resort used in the treatment of highly drug resistant bacterial pathogens including cephalosporin resistant strains. However, Carbapenem-resistant strains are emerging worldwide, and resistance rates have reached 50-60% in some countries. Increasing rates of bacterial resistance to beta lactam antibiotics such as Cephalosporins and Carbapenems are of great concern, especially in low income countries where treatments with such antibiotics (Carbapenems) are not frequent. In Nigeria, data regarding Carbapenemase and other beta lactamase mediated resistance are scarce, this poses a great danger to general health care delivery system. This study was carried out to determine the susceptibility pattern of bacterial strains (from patients attending Aminu Kano Teaching Hospital) to Carbapenems. The antimicrobial susceptibility pattern of the clinical bacterial isolates (obtained from Microbiology Department of Aminu Kano Teaching Hospital) to 3rd generation cephalosporins (cefotaxime and ceftriaxone) was determined by Kirby Bauer disc diffusion method, according to CLSI guidelines, resistant isolates were further subjected to Carbapenem (Imipinem and Meropenem) susceptibility testing. Out of 157 Cephalosporin resistant isolates analyzed, 52 (14.9%) were Carbapenem resistant, Escherichia coli had the highest frequency of Carbapenem resistant strains of 20(31.3%) while Pseudomonas aeruginosa had the least frequency of 3(18.8 %). The findings of the study showed that Carbapenem resistance occurred in Kano with a prevalence rate of 14.9%, indicating a danger of limited treatment options in the near future. This emphasizes the need for effective infection prevention and control measures to avoid the spread of Carbapenem resistant Enterobacteriaceae in hospital setting.
Keywords: Carbapenem, Cephalosporins Enterobacteriaceae, Kano, Resistance

INTRODUCTION

Carbapenems namely imipenem, meropenem, doripenem, and ertapenem, are bactericidal β-lactam antimicrobials with proven efficacy in severe infections caused by extended spectrum β-lactamase (ESBL) producing bacteria (Francis and Eric, 2018). They are among the antibiotics of last resort against infections caused by antibiotic resistant bacteria, currently in use worldwide as a result of the rising resistance to cephalosporin antimicrobials in the Enterobacteriaceae group, however, resistance to this important class of antibiotic is increasing due to various resistance mechanisms developed by the bacteria. Most notably among these mechanisms is the production of carbapenemase**s**, which are group of broad spectrum β-lactamase enzymes with hydrolytic activities against all cephalosporins, and Carbapenems, leaving narrow therapeutic options (Eshetie *et al.,* 2015). Carbapenems are structurally related to Penicillins, they exert their effect by inhibiting cell wall synthesis, which leads to cell death. They are active against gram-negative, gram-positive, and anaerobic organisms (Francis and Eric, 2018). Carbapenem Resistant Enterobacteriaceae (CRE) are able to survive on dry surfaces for weeks to months, which is long enough to be potentially involved in transmission (Havil *et al.,* 2014). The spread of CRE has emerged as a major public health concern, consequently, Updates on the epidemiology of CRE and early detection of carriers is essential to stop any spread.

There is a need for continuous monitoring of antibiotic susceptibility pattern, since a poor treatment occurs when an infection is treated with an antibiotic to which the infectious agent is resistant, this lead to increase in morbidity and mortality rate. This study is therefore, aimed to assess the prevalence and associated risk factors of CRE in Kano.

MATERIALS AND METHODS

Collection of Isolates

A total of 348 previously identified clinical bacterial isolates comprising of *Escherichia coli* (151), *Klebsiella pneumonieue,* (101), *Proteus mirabilis* (56), *Proteus vulgaris* (21), *and* P*seudomonas aeruginosa* (19) from clinical specimens of urine, blood, wounds, sputum, semen and high vaginal swab, endo cervical swabs, and the control strain *E. coli ATCC 25922* were collected from the Microbiology Department of Aminu Kano Teaching Hospital (AKTH), Kano over a period of six months from June to December, 2014, and their identifications were re-confirmed based on their cultural characteristics, Grams reaction, and biochemical characteristics, as described by Cheesbrough, (2006).

Susceptibility Testing of the Isolates

The antimicrobial susceptibility pattern of the isolates to Cefotaxime and Ceftriaxone, Meropenem and Imipinem was determined using Kirby-Bauer disc diffusion method as described by Clinical Laboratory Standard Institute (CLSI, 2013) guidelines. The Mueller Hinton agar was prepared and sterilized as instructed by the manufacturer. It was then Poured into 90 mm diameter sterile petri dishes to a depth of 4 mm (about 25 ml per plate). Using a sterile wire loop, three pure isolates of the test organisms were touched and emulsified in a tube of 4 ml sterile physiological saline. In a good light the turbidity of the suspension was matched to the turbidity standard (0.5 Mac Farland) by viewing against a sheet of white paper. A sterile swab was dipped into the tube, and removed immediately, excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension, this was then used to evenly streak the plate of Mueller Hinton agar in three directions, rotating the plate approximately 60 degrees to ensure even distribution. With the petri dish lid in place, the surface of the agar was allowed to dry for 3 minutes. Using sterile forceps, the appropriate antimicrobial discs were placed and evenly distributed on the inoculated plate. Within 30 minutes of applying the discs, the plates were inverted and incubated aerobically at 37°C for 18 h. after the incubation, a ruler was placed on the underside of the plate across the center of the disc to measure the diameter of the zone of inhibition surrounding the disc in millimeter, the diameters of the zones of inhibition were then compared with the standards for interpretation as described in (CLSI, 2013).

RESULTS AND DISCUSSION

Susceptibility of the isolates to Cefotaxime is presented in Table 1, it shows that out of 348 isolates analyzed a total of 173 (49.7%) isolates were sensitive, 144 (41.4%) isolates were resistant. While only 31 (8.9%) isolates were intermediately sensitive. *Proteus vulgaris* was the most susceptible species to cefotaxime with a total number of 14(66.6%) susceptible isolates out of 21 isolates, while *Pseudomonas aeruginosa* was the least susceptible species with only 4(21.0%) susceptible isolates out of 19 isolates.

Susceptibility of the bacterial isolates to Ceftriaxone is presented in Table 2 it shows that out of the 348 isolates analyzed, a total of 211(60.6 %) were sensitive, while 115(33.0%) isolates were resistant, and only 22(6.4%) isolates are intermediately sensitive. *Proteus mirabilis* was the most susceptible species with a total number of 44(78.6%) susceptible isolates out of 56 isolates, while *P. aeruginosa* is the least susceptible species with only 4(21.0%) susceptible isolates out of 19 isolates.

Table 1: Susceptibility of the Isolates to Cefotaxime

S/No.	Bacterial isolates	No.Screened	No Susceptible (%)	No. Resistant (%)	No. Intermediate (%)
1	*Escherichia coli*	151	75(49.7)	59(39.0)	17(11.3)
2	*Klebsiella pneumoniae*	*101*	46(45.5)	44(43.6)	11(10.9)
3	*Proteus mirabilis*	56	34(60.7)	20(35.7)	2(3.6)
4	*Proteus vulgaris*	21	14(66.6)	6(28.6)	1(4.8)
5	*Pseudomonas aeruginosa*	19	4(21.0)	15(79.0)	0(0.0)
	Total	348	173(49.7)	144(41.4)	31(8.9)

Table 2: Susceptibility of the isolates to Ceftriaxone.

S/No.	Bacterial isolates	No. Screened	No. Susceptible (%)	No. Resistant (%)	No. Intermediate (%)
1	*Escherichia coli*	151	91(60.3)	52(34.4)	8(5.3)
2	*Klebsiella pneumoniae*	101	57(56.4)	34(33.7)	10(9.90)
3	*Proteus mirabilis*	56	44(78.6)	10(17.9)	2(3.6)
4	*Proteus vulgaris*	21	15(71.4)	6(28.6)	0(0.0)
5	*Pseudomonas aeruginosa*	19	4(21.0)	13(68.4)	2(10.5)
	Total	348	211(60.6)	115(33.0)	22(6.4)

The prevalence of cephalosporin resistance in our study (41.4% cefotaxime and 33.0% ceftriaxone) is relatively lower than what is reported in a recent study by Aminu *et al.,* (2021) in which 129 (44.2%) of the bacterial isolates analyzed were resistant to cephalosporins, this may be due to the fact that they analyzed isolates obtained only from intensive care units of the hospital where a higher prevalence of resistant species is expected.

Susceptibility of the bacterial isolates to Imipinem is presented in Table 3 it shows that out of the 157 isolates analyzed, a total of 101(64.3 %) were sensitive, while 34(21.7%) isolates were resistant, and only 22(14.0%) isolates are intermediately sensitive. *Proteus vulgaris and Pseudomonas aeruginosa* were the most susceptible species with a total number of 6(75%) and 12 (75%) susceptible isolates out of 8 and 16 isolates respectively, while *Klebsiella pneumoniae* is the least susceptible species with 30(61.20%) susceptible isolates out of 49 isolates.

Table 3: Susceptibility of the isolates to Imipinem

S/No.	Bacterial isolates	No.Screened	No Susceptible (%)	No. Resistant (%)	No. Intermediate (%)
1	*Escherichia coli*	64	40(61.5)	19(29.7)	5(7.8)
2	*Klebsiella pneumoniae*	49	30(61.2)	9(18.4)	10(20.4)
3	*Proteus mirabilis*	20	13(65.0)	4(20.0)	3(15.0)
4	*Proteus vulgaris*	8	6(75.0)	1(12.5)	1(12.5)
5	*Pseudomonas aeruginosa*	16	12(75.0)	1(6.3)	3(18.7)
	Total	**157**	**101(64.3)**	**34(21.7)**	**22(14.0)**

Susceptibility of the bacterial isolates to Meropenem is presented in Table 4 it shows that out of the 157 isolates analyzed, a total of 107(68.1 %) were sensitive, while 37(23.6%) isolates were resistant, and 13(8.3%) isolates were intermediately sensitive. *Escherichia coli* was the most susceptible specie with a total number of 48(75.0%) susceptible isolates out of 64 isolates, while *Proteus mirabilis* is the least susceptible specie with only 9(45.0%) susceptible isolates out of 20 isolates

Table 4: Susceptibility of the isolates to Meropenem

S/No.	Bacterial isolates	No.Screened	No Susceptible (%)	No. Resistant (%)	No. Intermediate (%)
1	*Escherichia coli*	64	48(75.0)	12(18.7)	4(6.3)
2	*Klebsiella pneumoniae*	49	36(73.0)	10(20.4)	3(6.1)
3	*Proteus mirabilis*	20	9(45.0)	11(55.0)	0(0.0)
4	*Proteus vulgaris*	8	4(50.0)	2(25.0)	2(25.0)
5	*Pseudomonas aeruginosa*	19	10(63.0)	2(25.0)	4(25.0)
	Total	**157**	**107(68.1)**	**37(23.6)**	**13(8.3)**

Our findings (in 2014) of 21.7 % and 23.6% resistance to Imipinem and Meropenem respectively, is relatively lower than that of Yusuf *et al.,* (2017) three years later in the same state who found about 36.6% and 40.3% resistance to Imipinem and Meropenem respectively, this may be due to differences in study populations, and period of sample collections, and it indicates increasing prevalence of resistant species in the state over the years.

From our findings the susceptibilities of *Ps. aeruginosa* to both Imipinem and Meropenem is 75% and 63% respectively, this is relatively lower than what was reported in Lagos by Aibinu *et al*., (2007) who reported 95.9% susceptibility of *Ps. aeruginosa* to both Imipinem and Meropenem. In 2008, *E. coli* and *K. pneumoniae* were 100% susceptible to imipinem in the same state (Enwuru *et al*., 2011). While in 2011, resistance of *E. coli*, *K. pneumoniae* and *Ps. aeruginosa* to Imipinem ranged only from 6-9% in Kano State (Yusuf *et al*., 2012). Many factors have contributed to increasing resistance to antibiotics in Nigeria over the years, common ones have been attributed to uncontrolled sales, lack of proper and sustainable infection prevention and control (IPC) measures, inappropriate and personal prescriptions of antibiotics, and widespread of substandard antibiotics.

The susceptibility of the 157 isolates (Resistant to both or any of the tested Cephalosporins) screened for Carbapenem (Meropenem and Imipinem) susceptibility is presented in Table 5

It shows that a total of 52(14.9%) isolates were Carbapenem resistant. (i.e. they are resistant to either Meropenem, Imipinem or both). These include *P vulgaris* with a total number of 4(50.0%) resistant isolates out of 8 isolates as the most resistant species, and the least resistant species is *P aeruginosa* with only 3(18.8%) resistant isolates out of 16 isolates

Table 5 Prevalence of Carbapenem resistant isolates.

S/No.	Bacterial isolates	No. Screened	Carbapenem resistant isolates (%)
1	*Escherichia coli*	64	20(31.3)
2	*Klebsiella pneumoniae*	49	16(32.7)
3	*Proteus mirabilis*	20	9(45.0)
4	*Proteus vulgaris*	8	4(50.0)
5	*Pseudomonas aeruginosa*	16	3(18.8)
	TOTAL	157	52 (14.9)

The overall prevalence of Carbapenem resistance obtained from our study is 14.9% this is in close agreement with a study in Sokoto by (Olowo *et al.,* 2020) who reported a Carbapenem resistance prevalence of 10.5%, and to the 12.5% reported in Maiduguri by (Mohammed *et al.,*2015), but relatively lower than what was obtained in another research by (Olowo *et al*., 2019) who reported (28.2%) Carbapenem resistance among the Gram-negative bacterial isolates screened.

Our findings also contrasted 36.8% Carbapenem resistance observed among clinical isolates collected from four tertiary hospitals in southwest Nigeria reported by (Enwuru *et al.,* 2011). A higher Carbapenem resistance prevalence of 59%was also reported by Bush (1999), in New York.

The reduced rates of resistance to the Carbapenems in this study was probably because of the high cost of purchase of Carbapenems, which makes it less available for use, in Nigeria, thus resulting in decreased selection pressure for development of resistance. Also, the varying antibiotic resistance rates observed may be attributed to varying levels of regulations regarding antibiotic usage in different localities, and differences in period of the research.

The Distribution of CRE based on source of clinical samples is presented in Table 6, it shows that blood had the highest prevalence of CRE (66.7%) followed by High Vaginal Swab (HVS), sputum and urine in decreasing order, however, none of CRE is found from semen samples.

Table 6 Distribution of CRE Based on Source of Clinical Samples

S/N	Clincal Samples	No.of Isolates Screened	No.of Carbapenem resistant isolates	% Prevalence
1	Urine	79	24	30.4
2	HVS	25	11	44.0
3	ECS	10	2	20.0
4	Sputum	19	6	32.0
5	Wound	11	2	18.1
6	Blood	3	2	66.7
7	Semen	2	0	0.0
8	Pus	4	1	25.0
10	Catheter	4	1	25.0
TOTAL		**157**	**52**	**14.9**

The high prevalence of CRE in blood samples shows that blood can be great risk factor for CRE transmission through various processes like improper use of syringes or needles, inadequate disinfection of skin, or blood transfusion and poor hand washing technique among health practitioners. However, a lower incidence was recorded for wound pathogens (18.1%) and semen (0.0%). This is in close agreement with the findings of Yusuf *et al*., (2015) who also reported a high prevalence of Carbapenemase producers in blood (30.6%) and a relatively lower prevalence in wound swabs (11.1%) and urine (6.3%). Our findings also agree with Abdullahi *et al.,* (2017) who reported a highest prevalence of a novel Carbapenemase, (New Delhi Metallo Beta Lactamase 1(NDM-1)) gene among blood samples in Kano, Northwestern Nigeria

CONCLUSION

The finding of 14.9% Carbapenem resistance prevalence following preliminary screening as observed from this study confirms the existence of Carbapenems resistance in Kano state, hence, the need for enhanced surveillance to improve treatment outcomes. The high prevalence of CRE in blood samples shows that blood can be great risk factor for CRE transmission, this emphasizes the need for proper infection control practices.

RECOMMENDATIONS

The Carbapenems should not be routinely used as first-line therapy unless the pathogen is multidrug-resistant and it should be susceptibility based, it is also important for clinicians to be familiar with antimicrobial therapy options, risk factors, and diagnostic indicators, due to the growing threat of CRE infections in the healthcare setting.

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