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ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF CLASS D OXA ß-LACTAMASES PRODUCING BACTERIA IN KANO STATE, NIGERIA

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ABSTRACT
This study was carried out to determine the antibiotic susceptibility profile of Gram negative bacteria obtained from three different hospitals for class D Oxa ß-lactamases in Kano metropolis. The clinical isolates include: Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus sp and Salmonella spp. A total of 500 clinical isolates were screened phenotyphically using double discs synergy test. Confirmatory tests were performed among the suspeced isolates according to the Clinical and Laboratory Standard Institutes guidelines (CLSI). A total of 13 antibiotic discs were used for sensitivity test including: Amoxicillin/clavulanic acid, ceffazidime, cefepime, cepatozime, cefuruxime, imipenem, meropenem, ciprofloxacin, genetamicin, levoflaxacin, nitrofuranton, tigercyline and ofloxacin. Nine antibiotic discs were used on lactose fermenters and seven antibiotics were used on non-lactose fermenters. The result of the prevalence of class D oxa ß-lactamases producing bacteria among the clinical isolates revealed that, 26.40% of the clinical isolates were confirmed positive for class D oxa Beta lactamases production with the highest prevalence in E. coli (37.88%) and absent in K. oxytoca. The isolates producing this enzyme were sourced mainly from urine (55.17%) or wound swabs (26.44%). The antibiotic susceptibility profile using class D blaOxa showed that E. coli has overall resistance to clavulanic acid and third generation cephalosporins, with high susceptibility profile to tigercycline. (52.0%) and nitrofuranton (49.00%). K. pneumoniae has the highest susceptibility with tigercyclin and Nitrofuranton (56.7% and 56.4% respectively). The most active agents against non-lactose fermenters were meropenem, tigercycline and levofloxacin. The data highlighted the widespread of antibiotic resistance associated with bla OXA among the Gram -negative bacterial isolates in hospitals from Kano metropolis. The attention of the authorities and healthcare sectors is needed urgently towards the rising spate and widespread resistance due to class D oxa ßlactamases so as to device a method to curb this threatening trend.
Key words: Antibiotic susceptibility, resistance, lactose fermenters, non-lactose fermenters.

INTRODUCTION

Antimicrobial resistance (AMR) is not a recent phenomenon, but it is a critical health issue today. Over several decades, to varying degrees, bacteria causing common infections have developed resistance to each new antibiotic and AMR has evolved to become a worldwide health threat. With a dearth of new antibiotics coming to market, the need for action to avert a developing global crisis in health care is increasingly urgent (WHO, 2002). The rising prevalence of multidrug-resistant bacteria has emerged as one of the greatest challenges to quality healthcare delivery with a greater burden on developing nations, accounting for a large proportion of hospital-acquired infections (Conen *et al*., 2015; Okoche *et al.*, 2015). One of the mechanisms developed by bacterial species to confer resistance to antibiotics is their ability to produce class D oxa ß-lactamases enzymes. Beta-lactamases are enzymes produced mostly by Gram-negative bacteria. They are often responsible for resistance to ß-lactam antibiotics by organisms possessing them (Bush *et al.*, 1995). They are among the penicillinases that hydrolyze oxacillin and cloxacillin and are poorly inhibited by clavulamic acid (Poirel *et al.*, 2004).

Since their discovery in Turkey in 2004 (Carrer *et al.*, 2010), OXA ß-lactamases have spread to Europe, the Middle East and Africa (Egypt and Senegal). They are responsible for 5-20% of outbreak of nosocomial infections in intensive care unit, burn, oncology and neonatal units (Kotra *et al.*, 2002).

Poor hygienic practices, indiscriminate use of antibiotics as well as lack of monitoring of microbial drug resistance has created suitable condition for the emergence and controllable spread of these enzymes in Nigeria (Arzai and Adamu, 2008). There is therefore an urgent need to reduce cost of point of-care diagnostics (Carrer *et al.,* 2010).
In view of the considerable medical significance of this wide spread class of enzymes, this research seeks to detect the presence of OXA class D ß-lactamases among Gram-negative clinical bacterial isolates in Kano metropolis and to determine their antibiotic susceptibility profile.

MATERIALS AND METHODS

Sample Collection

Ethical approval to collect samples for the study was obtained from Ethical Committee of the Aminu Kano Teaching Hospital (AKTH), Kano. A total of 500 clinical Gram-negative bacterial isolates were collected from the Aminu Kano Teaching Hospital (AKTH), Kano, Murtala Muhammad Specialists Hospital (MMSH) and Muhammad Abdullahi Wase Specialists Hospital (MAWSH) Kano. The clinic number, type of specimen, sex and age of the patients were documented. The isolates include; *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Proteus vulgaris, Pseudomanas aeruginosa, Salmonella typhi* and *.Salmonella paratyphi*. The isolates were obtained from different clinical samples (wound, ear, vaginal swabs, blood, cerebro-spinal fluid, urine, sputum and stool). They were identified by using Gram staining, MacConkey and Chocolate agar, swarming activity and confirmed with Microbact 12E Identification kit (Oxoid Ltd. Basingstoke, Hants, UK).

Antibiotic susceptibility test

All the clinical isolates collected were tested for potential class D blaOxa production as presumptive tests using the Cefpodoxime (10µg) and the Ceftriaxone (30µg) antibiotic discs. All the isolates suspected of class D oxa ß-lactamases production in the presumptive test were furthered confirmed using the double discs synergy according to Cheesbrough (2010). The antimicrobial discs used are oxacillin (10µg), cloxacillin (10µ), the amoxicillin-clavulanic acid (30µg), ceftazidine (30µg), and cefepime (30µg) (Oxoid England). Antimicrobial susceptibility testing was performed on the isolates that produced class D blaoxa enzymes using Clinical and Laboratory Standard Institute (CLSI, 2012) protocol. A total of 13 different antibiotics were used, these are: Amoxicillin/clavulanic acid (30µg), ceffazidime (30µg), cefepime (30µg), cepatozime (30µg), cefuruxime (30 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), genetamicin (10 µg), levoflaxacin (5µg), nitrofuranton (300 µg), tigercyline (15 µg) and ofloxacin (5µg). Amoxicillin/clavulanic acid, ceftazidime, cefatoxime, cefuroxime, ciprofloxacin, gentamicin, nitrofuraton, ofloxacin, and tigercyline were tested on lactose-fermentative bacteria while imipenem, meropenem, levofloxacin, cefepime, ceftazime, tigercyline, and Amoxicillin/clavulanic acid were tested on non-lactose fermentative bacteria. The control and test plates were examined to ensure confluent growth (Cheessbrough, 2010). The plates were incubated over night at 35°C. The inhibition zone diameters around the disks were measured and interpreted according to the CLSI (2012) guideline. The data obtained were analyzed in frequency and percentage tables.

RESULTS

The result obtained for the severity of class D Oxa ß-lactamases among the clinical isolates obtained in Kano is presented in Table 1. The result showed that 58.40% of the isolates obtained from the three hospitals from Kano produce class D oxa ß-lactamases. However, *E. coli* has the highest prevalence of species producing class D Oxa ß-lactamases (34.25%) while *P. vulgaris* has the least prevalence of Beta-lactamases producers (0.34%).
Similarly, the result from the CSLI test (Table 2) indicated that 26.40% of the clinical isolates obtained from the three hospitals in Kano were confirmed to produce class D oxa Beta lactamases. More so, the highest prevalence was found in *E. coli* (37.88%) while the enzyme production was found to be absent in *K. oxytoca*.
Furthermore, most of the ß-lactamases producing bacteria (Table 3) were sourced from either urine (55.17%) or wound swabs (26.44%). However, non of the isolates collected from cerebro-spinal fluids and stool were found to be ß-lactamases producers.
More so, the highest prevalence of Beta lactamases producing bacteria (Table 4) were obtained from the samples obtained from AKTH (62.50%) while the least was found in samples from MAWSH (5.56%)
However, the antibiotic susceptibility profile of Class D OXA ß-lactamases among lactose fermenters was shown in Table 5.
The result showed that, *E. coli* showed overall resistance to amoxycillin-clavulanic acid, agumentin and ceptazidime. It was also resistant to cefuroxime and ciprofloxacin, gentamacin and with high susceptibility profile with tigercyclin (52.0%) and nitrofuranton (49.00%).

K. pneumoniae has the highest susceptibility with tigercyclin and Nitrofuranton (56.7% and 56.4% respectively).

Similarly, the result for the antibiotic susceptibility profile of Class D OXA β-lactamases among non-lactose fermenters is presented in Table 6. The result showed that, Levofloxacin has the highest susceptibility in *Proteus spp* (20.6%) followed by meropenem with (18.1%) and leftazidine has the least susceptibility profile. In *Psudomonas aeruginosa* Tigenocline *and* meropenem have the highest susceptibility of 7.1% each. Furthermore, *P. aeruginosa* was resistance to Amoxyclavulanic acid. However, *Salmonella spp* have the highest susceptibility rates with impenum, meropenem and tigercycline; with no MIC values with cepfime, feftazidime and amoxiclav.

Table 1: Occurrence of class D OXA β-lactamases based on severity Kano

Bacterial Species	No. of Screened Isolates	No. of Isolates Positive	Prevalence(%)
E. coli	187	100 (53.48%)	34.25
K.pneumoniae	130	78 (60.00%)	26.71
K. oxytoca	3	2 (66.67%)	0.69
P. mirabilis	87	66 (75.86%)	22.60
P. vulgaris	29	1 (3.45%)	0.34
P. aeruginosa	56	38 (67.86%)	13.01
S. paratyphi	2	2 (100.00%)	0.69
S. typhi	6	5 (83.33%)	1.71
TOTAL	**500**	**292 (58.40%)**	**100**

Standard Error; 14.18, Standard Deviation: 40.10 Mean: 36.5

Table 2; Prevalence of Class D β-lactamases Producing Bacteria based on CLSI

Bacterial Species	No of Isolates Screened	No of Isolates Confirmed Positive	Prevalence (%)
E. coli	187	50 (26.74%)	37.88
K. pneumonia	130	34 (26.15%)	25.76
K. oxytoca	3	0 (0.00%)	0.00
P. mirabilis	87	20 (22.99%)	15.16
P. vulgaris	29	7 (24.14%)	5.30
P. aeruginosa	56	14 (25.00%)	10.60
S. paratyphi	2	2 (100.00%)	1.52
S. typhi	6	5 (83.33%)	3.78
TOTAL	**500**	**132 (26.40%)**	**100**

Standard Error : 6.19, Standard Deviation: 17.53, Mean: 16.5

Table 3: Distribution of Class D Oxa β-Lactamase producing bacteria from Sources

S/N	Sources of Bacterial Species	No of Isolates Producing Oxa D	Prevalence (%)
1	Urine	96	55.17
2	Catherter tip	10	5.75
3	Wound swab	46	26.44
4	Ear swab	00	0.00
5	High virginal swab	3	1.72
6	Blood	18	10.35
7	Cerebro spinal fluid	00	0.00
8	Stool	0	0.00
9	Sputum	1	0.57
	TOTAL	**174**	**100**

Standard Error: 10.80, Standard deviation: 32.41 Mean: 19.33

Table 4: Prevalence of Class D OXA β-lactamase isolates producers Among Hospitals

Hospitals	No of Isolates Screened	No of Isolate Positive	% Prevalence (%)
AKTH	311	180 (57.88%)	62.50
MMSH	169	92 (54.44%)	31.94
MAWSH	20	16 (80.00%)	5.56
TOTAL	**500**	**288 (57.60%)**	**100**

Standard Error: 47.35 Standard deviation: 82.07 Mean: 96.00

Table 5; Antimicrobial Susceptibility Profile of Lactose Fermenters

Antibiotics	*E. coli*				*Klebsiella spp*			
	Sen	**Inter**	**Res**	**Res%**	**Sen**	**Inter**	**Res**	**Res %**
AUG	0.00	0.00	100	0.00	0.00	0.00	78	0.00
CAZ	5	12	83	5.0	1	10	69	1.3
CPR	7	30	63	7.0	4	26	50	5.1
CRX	1	3	96	1.0	4	8	68	5.1
CXM	0.00	0.00	100	0.00	1	0.00	79	1.3
Gen	45	5	50	45.0	30	2	48	38.5
NIT	49	24	27	49.0	44	2	34	56.4
OFL	30	8	62	30.0	29	7	44	37.2
TGC	52	26	22	52.0	45	21	14	57.7
Total	189	108	203	189	158	76		202.6

Key: Sen = Sensitive Inter = Intermediate Res = Resistance Res % = Resistance Percentage

Table 6: Antimicrobial Susceptibility Profile of non-Lactose Fermentors

Antibiotics	*Proteus spp*				*Pseudomonas aeruginosa*				*Salmonella spp*			
	Sen	**Inter**	**Res**	**Res%**	**Sen**	**Inter**	**Res**	**Res %**	**Sen**	**Inter**	**Res**	**Res %**
AUG	0.00	0.00	116	0.00	0.00	0.00	56	0.00	0.00	0.00	7	0.00
CAZ	2	10	104	1.7	2	2	52	3.6	0.00	1	6	0.00
FEP	13	9	94	11.2	3	1	52	5.4	0.00	---	7	0.00
IPM	7	13	96	6.0	1	6	49	1.8	4	1	2	57.1
LEV	24	10	82	20.6	3	1	52	5.4	3	---	4	42.8
MEM	21	18	72	18.1	4	3	49	7.1	5	1	1	71.4
TCG	39	13	64	33.6	4	5	47	7.1	`5	2	---	71.4
Total	**106**	**73**	**512**	**91.2**	**17**	**18**	**301**	**30.4**	**17**	**5**	**20**	**242.7**

Key: Sen = Sensitive Inter = Intermediate Res = Resistance Res % = Resistance Percentage

DISCUSSION

Several studies on the prevalence of Class D Oxa Beta lactamases were conducted in different parts of the world among clinical isolates. This study was among the first performed in Nigeria. The study revealed high incidence of this resistance conferring enzyme among the studied clinical isolates with *E. coli* as the most predominant Class D Oxa Beta-lactamases production. This finding is conformity with the findings of Aibinu *et al.* (2003) who reported highest prevalence of Extended Spectrum of Beta Lactamases production of 20.8% in *E. coli* in Lagos Nigeria. Similar result was reported by Yasmin (2012) among some clinical isolates from Nigeria. However, this finding is contrary to that of Yusuf *et al.* (2013) who reported *Shiegella spp* as the most predominant Beta-lactamases producer among clinical isolates from some tertiary health care centers in Kano.

The distribution of ß-lactamases among clinical specimens indicated highest prevalence from the samples isolated from urine. This finding is in agreement with the work of Doughari and Akafa (2009), who reported a higher prevalence rate of 91% ß-lactamase in urine, Iroha *et al.* (2010) who reported high prevalence of 60.3% in urine and Osazuwa and Osazuwa (2011) who also found that ESBLs prevalence was high in urine (61.4%). The high prevalence of ß-lactamses in urine may probably be related to factors like extreme age, female gender, sexual activity, contraception, pregnancy, urinary tract obstruction, neurological dysfunction, antimicrobial use and poor hand washing techniques among health care practitioners, which are some of the factors that can predispose one to urinary tract infection (UTI) development as reported by Eze *et al.* (2015) from Nsuka, Nigeria.

High resistance to ß-lactam was shown in ß-lactamase producers in clinical isolates. This is because ß-lactamases producers have enzymes that relax the active site of the antibiotics. The high resistance to antibiotics reported by this study conforms to the earlier reports by Aibinu *et al.* (2003) who discovered that all ESBLs producing *Enterobacter spp* were resistant to ciprofloxacin. Paterson *et al.* (2000) had reported that globally, 18% of all ESBLs producers were resistant to ciprofloxacin. More so, Eze *et al.* (2015) reported ESBLs producing *E. coli* and *Klebsiella spp* in Nsukka, Enugu State as resistant to ciprofloxacin.

This means that the resistance phenomenon is on the increase. This increasing resistance to several antimicrobial drugs may be due to inappropriate use of antimicrobial drugs (over use, misuse, suboptimal dosage and non-compliance with the treatment duration) which leads to selection pressure. This abuse of antibiotics is reported by Muhammad *et al.* (2010) have impressed a selective pressure that causes discovery of more resistant bacteria. The resistance by ß-lactamase producers was also shown against imipenem. This is in agreement with the work of Asma *et al.* (2014). The resistance can also be attributed to the organisms' ability to encode multiple antibiotic resistance genes as reported by Perez *et al.* (2007). In the present study, ampicillin, amoxyclauve were found to show total resistance. This finding is in conformity with the work of Ullah *et al.* (2009) and Sasirekha *et al.* (2010) who individually reported similar findings. Recent studies by Heritier *et al.* (2005) revealed that both resistant genes exist in carbapenem–resistant clinical isolates of *Acinetobacter spp* from various continents and for even more than 10 years in some countries. In another study by Coelho *et al.* (2006) in Portugal showed that the blaOXA gene exists in the gram–negative enterobacteriaceae and *Pseudomonas spp* in United Kingdom, South Africa and Brazil. This is in conformity with the finding of Alber *et al.* (2004) as blaoxa gene was found in *E. coli, K. pneumoniae, Salmonella spp, Proteus spp* and *Shigella spp.*

In this study, *E. coli* is found to be totally resistance to agumentin, ceptazidime, cefuroxime and ciprofloxacin. This is in line with the work of Adrian *et al.* (2007) in South Africa where *E. coli* isolates were reported to be resistants to third generation cephalosporins, quinolones and ß-lactam antibiotics. The susceptibility test conducted on lactose fermentative bacteria showed that tigercycline was the most active drug against lactose fermentative bacteria. This can probably be due to stearic hindrance from its 9-glyclyamide structure and avoid ribosomal binding, therefore is unaffected by ribosomal alteration and efflux pumps as reported by Adrian *et al.* (2007). The high level of resistance could be due to the ability of the bacteria to harbor more than one multiple antibiotics resistance genes such as gene for production of "efflux pumps" and antibiotic altering enzyme.

CONCLUSION

It was concluded that Class D Oxa ß-lactamases producing bacteria exist among the clinical isolates obtained in Kano with *E. coli* having the highest prevalence. However, the sensitivity test indicated that the organisms were highly resistant to the antibiotics used. This showed that the presence of blaOXA gene is responsible for the high resistance observed by the tested organisms.

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