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PREVALENCE OF EXTENDED SPECTRUM β-LACTAMASES (ESBLs) AMONG ENTEROBACTERIACEAE IN MURTALA MOHAMMED SPECIALIST HOSPITAL, KANO, NIGERIA

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ABSTRACT
Confirmed variants of enterobacteriaceae isolated from 143 patients that attended Murtala Mohammed Specialist Hospital Kano, were screened for extended spectrum β-lactamases (ESBLs) production using Clinical Laboratory Standards Institute (CLSI) breakpoint. Suspected ESBLs producers were subjected to confirmation using Disc Replacement Method (DRM). Standard discs of augmentin {AMC 30µg (Oxoid, England)}, ceftriaxone {AUF 30µg (Oxoid, England)} and ceftazidime {RP 30µg (Oxoid, England)} were used in the screening. Of the 143 isolates screened, 114 (79.72%) were Gram negative isolates belonging to the family enterobacteriaceae. Among the enterobacteriaceae isolates screened, the results of CLSI breakpoint test showed that 76 (66.7%) were ESBLs producers viz: Citrobacter spp. (3), Enterobacter spp. (2), E. coli (28), Klebsiella spp. (18), Morganella morganii (7), Proteus spp. (13), Salmonella spp. (1), Serratia spp. (1), Shigella spp. (2) and Yersinia spp (1). On subjecting the CLSI positive isolates to DRM, only 47 (41.2%) were confirmed ESBLs producers. These include; Citrobacter spp. (1), E. coli (20), Klebsiella spp. (12), Morganella morganii (4), Proteus spp. (8), Salmonella spp. (1) and Shigella spp. (1). ESBLs occur at an alarming rate among enterobacteriaceae isolates in Kano which calls for government intervention in the healthcare setting.

Keywords: Prevalence, ESBLs, Isolates, Augmentin, Ceftazidime, Ceftriaxone

INTRODUCTION

Beta-lactam antimicrobial agents are the most common drugs for the treatment of bacterial infections and account for over 50% of global antibiotic consumption (Kotra, *et al*, 2002). Bacterial resistance to β-lactam antibiotics has significantly increased in recent years and has been attributed to the spread of plasmid mediated β-lactamases (Sanders, 1992). Some of these organisms have produced new forms of the older enzymes such as the extended–spectrum β-lactamases (ESBLs) that can hydrolyze newer cephalosporins and aztreonam (Paterson and Bonomo, 2005).

ESBLs are enzymes that mediate resistance to extended–spectrum (third generation) cephalosporins such as ceftazidime, cefotaxime and ceftriaxone as well as monobactams such as aztreonam (NCCLS, 1999). These ESBLs have been found worldwide in many different genera of enterobacteriaceae (Bradford, 2001).

More than 200 different natural ESBLs variants are known at present which account for resistance in an increasing variety of Gram-negative species (Bradford, 2001) with their distribution being far from uniform (Marchandin *et al*., 1999).

With β-lactams being the most frequently prescribed antimicrobials, the emergence of ESBL-producing organisms in clinical infections can result in treatment failure which constitutes a serious threat to current β-lactam therapy. A preliminary study was conducted to detect ESBLs and describe ESBL type in Clinical isolates of *E. coli* from Granada, Spain. A total of 62 isolates were screened using the VITEK 2 system, disk diffusion method and epsilon test. Fourteen randomly selected isolates were subjected to genetic analysis in order to detect the ESBL type. Out of the 62 isolates with ESBL detected by VITEK 2 system, 61 (98.4%) were confirmed to contain ESBLs by disk diffusion and Epsilon test. The prevalent ESBL type in *E. coli* was CTX-M-9 (Solórzano *et al.,* 2006).

In Nigeria, seven hundred and Forty Seven isolates obtained from Muhammad Abdullahi Wase Specialist Hospital, Kano were screened for ESBLs production. The result showed that 37 (9.25%) isolates were ESBL producers based on DDST while 20 (5.00%) were positive using NCCLs break points. Four species identified as ESBLs producers include *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli* and *Salmonella* spp. (Yusha'u *et al.,* 2007).

In a study at Ebonyi State University, *Klebsiella pneumoniae* isolated from urine, blood and stool samples was investigated. A total 105 clinical isolates of *Klebsiella pneumoniae* were isolated from urine (43), stool (38) and blood (24). Sensitivity studies were carried out using disc diffusion method by Kirby-Bauer and phenotype characterization of ESBL was carried out using double disc synergy test (DDST).

The result of the study revealed that 38 (36.2%) isolates were positive for ESBL production 15 (34.9%) isolates were from urine, 10 (26.3%) from stool and 13 (54.2%) from blood respectively (Iroha *et al.*, 2008). The study was aimed at determining the rate occurrence of ESBLs among enterobacteriaceae isolates in the study site via isolation of enterobacteriaceae from clinical specimens and detection of ESBLs among the isolates.

MATERIALS AND METHODS

a) Source of Samples

The site for sample collection was Murtala Mohammed Specialist Hospital Kano, a referral Hospital patronized by people having different nutritional status from within and outside Kano state. This informed the choice of the laboratory as the study site as nutrition is among the predisposing factor for infection with ESBLs producing organisms (Paterson and Bonomo, 2005).

b) Gram's Stain reactions of bacterial isolates

Bacterial colonies formed after culturing samples from patients were Gram stained as described by Brooks *et al.* (1989).

c) Biochemical characterization of the isolates

Gram negative isolates were further subjected to indole, motility, citrate utilization, urease production, hydrogen sulfide production as well as lactose fermentation according to standard procedures described by Cheesbrough (2004). KIA reactions are based on the fermentation of lactose and glucose and the production of hydrogen sulphide. A yellow butt (acid production) and red-pink slope indicate the fermentation of glucose only. The slope is pink-red due to a reversion of the acid reaction under aerobic conditions. Cracks and bubbles indicate gas production from glucose fermentation. A yellow slope and a yellow butt indicate the fermentation of lactose and possibly glucose. A red pink slope and butt indicate no fermentation of glucose or lactose. Blackening along the stab line or throughout the medium indicates hydrogen sulphide (H_2S) production. For Simmon's Citrate agar a bright blue colour indicates a positive citrate test and no change in colour of the medium indicates a negative citrate test. The isolates produce pink colour in Urea agar when positive for urease and no pink colour when urease negative. When the strain is urease producing, the enzymes will break down the urea to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red. Lactose peptone broth brings about a change in colour of the medium from purple to yellow, brown or pale purple.

d) Inoculum Standardization

The isolates were cultured on prepared Brain Heart Infusion (BHI) agar (Biotech, England) plates and incubated for at 37^{o}C for 24 hours so as to obtain confluent growth for sensitivity test. A loopful of isolates from BHI plates were dispensed in sterile normal saline to match the 0.5 McFarland standards for sensitivity tests as described by NCCLS (1999).

e) CLSI Breakpoint Test For ESBLs Screening

The sensitivity of standard inocula of isolates to ceftriaxone (AUF 30µg) and ceftazidime (RP 30µg) discs were determined on Mueller Hinton Agar (Biotech, India) using Kirby Baueur method (NCCLS, 1999).

f) Disc Replacement Method (DRM) For ESBLs Confirmation

This was described by Casals and Pringler (1990). Two amoxy-clav (AMC 30) discs were placed on Mueller Hinton agar (Biotech, India) inoculated with the bacterial isolates. After one hour at room temperature, the discs were removed and replaced with Ceftazidime (RP 30) and Cefriaxone (AUF 30). Each cephalosporin disc was also placed independent of the initial augmentin discs and the plates were incubated at 37^{o}C for 18-24hours and read for evidence of ESBLs production.

RESULTS

Of the 143 bacterial isolates subjected to Gram's staining technique, the isolates were separated into Gram-positive (29) and Gram-negative (114). Biochemical characterization of the 114 isolates confirmed tem as members of enterobacteriaceae. Of the 114 enterobacterial isolates subjected to ESBL detection using CLSI breakpoint, 76(66.7%) were found to be positive (Table 2) which included; *Citrobacter* spp. (4)*, Enterobacter* spp. (3)*, E. coli* (28)*, Klebsiella* spp. (18)*, Morganella morganii* (7)*, Proteus* spp. (13)*, Salmonella* spp. (1)*, Serratia* spp. (1)*, Shigella* spp. (2*)* and *Yersinia* spp. (1). However, only 47(41.2%) were confirmed to be ESBL producers based on DRM (Table 3) which included; *Citrobacter* spp. (1)*, E. coli* (20)*, Klebsiella* spp. (12)*, Morganella morganii* (4)*, Proteus* spp. (8)*, Salmonella* spp. (1) *and Shigella* spp. (1)*.* Inhibition zones of ≤22mm against ceftazidime disc and ≤25mm against ceftriaxone disc were suspicious for ESBLs production in CLSI method. Positive DRM result was indicated by an increase in inhibition zone of 5mm and above between the inhibition zones formed by the augmentin-replaced cephalosporin discs and those placed independently.

Table 1: Gram's staining reactions of the bacterial isolates

Gram's Reaction	No. observed	% occurrence
Positive	29	20.28
Negative	114	79.72
Total	**143**	**100**

Table 2: Percentage occurrence of ESBLs producers among the isolates based on CLSI breakpoint

S/No.	Isolates	Number screened	Number positive	% occurrence
1	*Citrobacter* spp.	4	3	3.9
2.	*Enterobacter* spp.	3	2	2.6
3	*Escherichia coli*	41	28	36.8
4	*Klebsiella* spp.	27	18	23.7
5	*Morganella morganii*	8	7	9.2
6	*Proteus* spp.	21	13	17.1
7.	*Salmonella* spp.	1	1	1.3
8	*Serratia* spp.	3	1	1.3
9.	*Shigella* spp.	4	2	2.6
10	*Yersinia* spp.	2	1	1.3
	Total	**114**	**76**	**66.7**

Table 3: Confirmed percentage occurrence of ESBLs producers among the isolates based on DRM Method

S/No.	Isolates	Number screened	Number positive	% occurrence
1	*Citrobacter* spp.	4	1	2.1
2.	*Enterobacter* spp.	3	0	0.0
3	*Escherichia coli*	41	20	42.6
4	*Klebsiella* spp.	27	12	25.5
5	*Morganella morganii*	8	4	8.5
6	*Proteus* spp.	21	8	17.0
7.	*Salmonella* spp.	1	1	2.1
8	*Serratia* spp.	3	0	0.0
9.	*Shigella* spp.	4	1	2.1
10	*Yersinia* spp.	2	0	0.0
	Total	**114**	**47**	**41.2**

DISCUSSION

Of the 143 bacterial isolates obtained, 29 (20.28%) were Gram positive while 114 (79.72%) were Gram negative (Table 1) which were identified as members of the family enterobacteriaceae. The high occurrence of enterobacteriaceae may be due to poor hygienic practices among the patients which were reported as important factors leading the spread of ESBLs due to non-ESBLs producing strains acquiring plasmids responsible for ESBLs production (Denman and Burton, 1992).

Of the 114 enterobacteriaceae isolates obtained, 76 (66.7%) were positive for ESBLs using CLSI breakpoint (Table 2) which indicates the need for employment of strict control measures inorder to curtail the spread thereby preventing possible outbreak of nosocomial infections. The result also showed that there exist differences in the rate of occurrence of ESBLs among the different bacterial species in each of the procedures employed with highest rate of occurrence observed in *Klebsiella* spp. and least in *Yersinia* spp. having no confirmed case of ESBL production.

There exist a variation in ESBLs positive results between the CLSI breakpoint and DRM procedures that may be due to false positive results displayed in organisms with multiple β–lactamases which interfere with the test results. The use of the two procedures helps in screening out non-ESBLs producing organisms since an organism is considered to be ESBLs producing only when it gives positive reaction using the two procedures (NCCLS, 1999).

The prevalence observed was high which indicates the possibility of treatment failure and/or outbreaks of infections caused by resistant organisms (Ahmad *et al*., 1999). Although the occurrence and distribution of ESBLs differs from country to country and from hospital to hospital (Bradford, 2001), the percentage prevalence of ESBLs among the isolates screened was higher in Klebsiella than other genera isolated in the present study, which conforms to the findings of Hanberger *et al*. (1999) and Moubareck *et al.* (2005). The high occurrence of ESBLs in *Klebsiella* species observed in this research is of great concern since infections caused by this bacterium (particularly respiratory tract infections) are very common in this part of Nigeria due to the contagious nature and resistance of the organism to harsh conditions, which may be due to the presence of capsules that gives some level of protection to the cells (Paterson and Bonomo, 2005).

The occurrence of ESBLs observed among the different clinical isolates calls for the need to improve control measures in healthcare settings so as to curtail the spread ESBLs.

CONCLUSION

ESBLs occur at an alarming rate among enterobacteriaceae isolates from the study site which can result in outbreak of nosocomial infections that may be difficult to treat.

RECOMMENDATIONS

In view of the quick spread of ESBLs among bacterial pathogens and the problems that may be caused by treatment failure due to infections with ESBLs producing organisms coupled with the results obtained in this work, it could be recommended that;

a) Government should strengthen awareness campaigns on improved hygienic practices so as to reduce the rate of infections and spread of ESBLs among both enterobacterial and other bacterial pathogens.

b) Healthcare settings should improve control measures such as proper handling and disinfection of equipment as well as detection of ESBLs producing organisms among the patients and isolation of ESBLs colonized patients.

c) Prescription of third and fourth generation cephalosporins should be regulated.

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