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DETECTION OF EXTENDED-SPECTRUM β-LACTAMASES AMONG GRAM NEGATIVE ISOLATES FROM GOMBE SPECIALIST HOSPITAL USING DISC REPLACEMENT METHOD

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ABSTRACT
A total of 500 clinical bacterial isolates from various sources including stool, urine, sputum and swabs obtained from Gombe State Specialist Hospital between July, 2011 and January, 2012 were used in this study. Gram's stain reaction of the isolates separated them into Gram-positives (200) and Gram-negatives (300). Biochemical tests confirmed the identity of the Gram-negative isolates to be members of the enterobactericeae, which included Klebsiella pneumoniae (60), Escherichia coli (98), Providencia Spp. (32), Morganella moganii (32), Shigella Spp. (14), Citrobacter freundii (14), Serratia marcescens (10), Salmonella paratyphi A (10), Yersinia enterocolitica (8), Proteus vulgaris (4), Salmonella typhi (2) and Pseudomonas aeruginosa (16). Of the 300 Gram-negative isolates subjected to screening using Cefpodoxime (CPX 10µg, oxoid England) and Cefotaxime (CTX 30µg, Oxoid England) for ESβL- production based on Clinical Laboratory Standard Institute (CLSI) breakpoint, 250 (83.33%) were found to be positive which included K. pneumoniae (40), E. coli (92), Providencia Spp. (30), M. morganii (20), P. aeruginosa (14), Shigella Spp. (14), C. freundii (12), S. marcescens (6), and Y. enterocolitica (6), S. paratyphi A (10), P. vulgaris (4) and S. typhi (2). However, one hundred and sixty four, 164 (65.6%) were confirmed ESβL- producers based on DRM using Amoxicillin-clavulanate (AMC 30µg, Oxoid England) which included; K. pneumoniae 32(19.50%), E. coli 52(31.71%), Providencia Spp 20(12.20%), M. morganii 16(9.76%), P. aeruginosa 8(4.88%), Shigella Spp. 12(7.32%), C. freundii 6(3.66%), S. marcescens 4(2.44%), S. paratyphi A 8(4.88%), Y. enterocolitica 6(3.66%), P. vulgaris (0.0%), and S. typhi (0.0%).
Keywords: Detection, ESBLs, Clinical isolates, Disc Replacement Method, Gombe

INTRODUCTION

Betalactam antimicrobial agents are the most common drugs for the treatment of bacterial infections and account for over 50% of global antibiotic usage (Kotra *et al.,* 2002). Beta lactamase production by Gram negatives is the important single mechanism of resistant to beta lactam antimicrobial agents. The beta lactamases are enzymes that hydrolyzed the beta lactam ring of beta lactam antibiotic, thereby rendering them inactive.

Extended- spectrum β-lactamases (ESβLs) are enzymes that mediate resistance to extended-spectrum (third-generation) Cephalosporins such as Ceftazidime, Cefotaxime, and Ceftriaxone as well as Monobactams sush as Aztreonam (NCCLS, 1999). Organisms that produce ESβLs remain important factor to consider as responsible for therapy failure with Cephalosporins and have serious consequences for infection control (Paterson and Bonomo, 2005).

The extended spectrum β-lactams became widely used in the treatment of serious infections due to Gram- negative bacteria in 1980's (Bradford, 2001). Resistance to these newer β-lactams emerge quickly and the first report of plasmid-encoded β-lactamases capable of hydrolyzing the extended- spectrum cephalosporins was published in 1983 (Bradford, 2001; Samaha-kfoury and Araj, 2003).

ESβLs occur predominantly in the family Enterobacteriaceae leading to outbreaks of nosocomial infections in intensive care units, burn, oncology and neonatal units (Kohler *et al.*, 1999).

A study was conducted to detect ESβLs and determine the ESβL type in clinical isolates of *E. coli* from Granada, Spain. A total of 62 isolates were screened using the Vitek 2 system, Disc diffusion method epsilon test. Fourteen isolates were randomly selected and subjected to genetic analysis in order to detect the ESβL type. Out of the 62 isolates with ESBL detected by Vitek 2 system, 61 (98.4%) were confirmed to contain ESβLs by disc diffusion and Epsilon test. CTX-M-9 ESβL was typed in E. coli (Solorzano *et al*., 2006).

A research was conducted at Murtala Mohammed Specialist Hospital, Kano, Nigeria to determine the prevalence of ESβLs among members of Enterobacteriaceae. Of the 114 Enterobacterial isolates subjected to ESβL detection using CLSI breakpoint, 76 (66.7%) were found to be positive which included *Citrobacter* spp.(4), *Enterobacter* spp. (3), *Escherichia 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, 47(41.2%) were confirmed to be ESBL producers based on DRM which included; *Citrobacter* spp. (1), *E. coli* (20), *Klebsiella* spp. (12), *M. morganii* (4), *Proteus* spp.(8), *Salmonella* spp. (1) and *Shigella* spp. (1) (Yusha'u *et al*., 2010).

MATERIALS AND METHODS

Bacterial isolates

A total of 500 bacterial isolates obtained from Gombe State specialist Hospital were used for this study.

Reaction of the isolates to Gram's staining

The bacterial isolates were subjected to Gram's stain reactions and separated into Gram positives and Gram negatives using the standard procedure described by Cheesebrough, (2000).

Identification of the Gram negative isolates based on biochemical tests

The Gram negative isolates were subjected to biochemical tests which indentified them to be members of the family Enterobacteriaceae and *Pseudomonas aeruginosa* using the standard procedure including Urease test, citrate utilization test, indole test and Kligler Iron Agar test as described by Cheesebrough, (2000).

Inoculum standardization

This was performed according to suggestion made by NCCLS, (1999). The Gram negative isolates were sub cultured onto prepared Brain Heart Infusion (BHI) agar. Following incubation at 37^{o}C for 24hrs, a few loopful of the isolates were dispensed in a sterile normal saline to match the 0.5 McFarland standards for sensitivity.

Clinical Laboratory Standard Institute (CLSI) breakpoint test for ESβLs screening

All the Gram negative isolates were subjected to screening for ESβLs production using Cefpodoxime (CPX 10µg, Oxoid England) and Cefotaxime (CTX 30µg, Oxoid England) discs according to description by NCCLS, (1999).

Disc Replacement Method (DRM)

Suspected ESβLs producers based on CLSI were subjected to confirmatory test using DRM described by Casal and Pringler, (1990). Two Amoxicillin-clavulanate (Augmentin) discs (AMC 30µg, Oxoid England) were placed on prepared Muller-Hinton agar plates inoculated with the Gram negative isolates. After an hour at room temperature, the discs were removed and replaced at the spot of each with Cefotaxime (CTX 30g) and Cefpodoxime (CPX 10g), incubated at 37^{0}C for 24hrs before they were read for the evidence of ESβLs production (increase in zone diameter of ≥5mm from that recorded during screening of confirmed ESβL production).

RESULTS

Biochemical identification of the clinical isolates revealed higher number of Gram-negatives than Gram-positive organisms as shown in Table 1.

Screening of the isolates for the presence of ESBL indicated that the enzymes occur at higher rate among *E. coli* (36.8%) than the other isolates while the least occurrence rate was observed among *Salmonella typhi* (0.8%) (Table 2).

On subjecting the isolates positive for screening test to confirmation using disc replacement method, the highest occurrence rate was observed among *E. coli* (31.7%) than the other isolates while the least occurrence rate was observed among *Salmonella typhi* and *Proteus vulgaris* with 0% each (Table 3).

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

Gram's reaction	Number observed	% occurrence
Positive	200	40
Negative	300	60
Total	**500**	**100**

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

Isolate	Number screened	Number positive	% occurrence
Klebsiella pneumoniae	60	40	16
Escherichia coli	98	92	36.8
Providencia spp	32	30	12
Morganella morganii	32	20	8
Psuedomonas aeruginosa	16	14	5.6
Shigella spp	14	14	5.6
Citrobacter freundii	14	12	4.8
Serratia marcescens	10	6	2.4
Salmonella paratyphi A	10	10	4
Yersinia enterocolitica	8	6	2.4
Proteus vulgaris	4	4	1.6
Salmonella typhi	2	2	0.8
Total	**300**	**250**	**83.33**

Table 3: Confirmed percentage occurrence of ESβLs producers among the isolates based on Disc Replacement Method (DRM)

Isolates	Number screened	Number positive	% occurrence
K. pneumoniae	40	32	19.50
E. coli	92	52	31.71
Providencia spp	30	20	12.20
M. morganii	20	16	9.76
P. aeruginosa	14	8	4.88
Shigella spp	14	12	7.32
C. freundii	12	6	3.66
S. marcescens	6	4	2.44
S. paratyphi A	10	8	4.88
Y. enterocolitica	6	6	3.66
P. vulgaris	4	0	0
S. typhi	2	0	0
Total	**250**	**164**	**65.6**

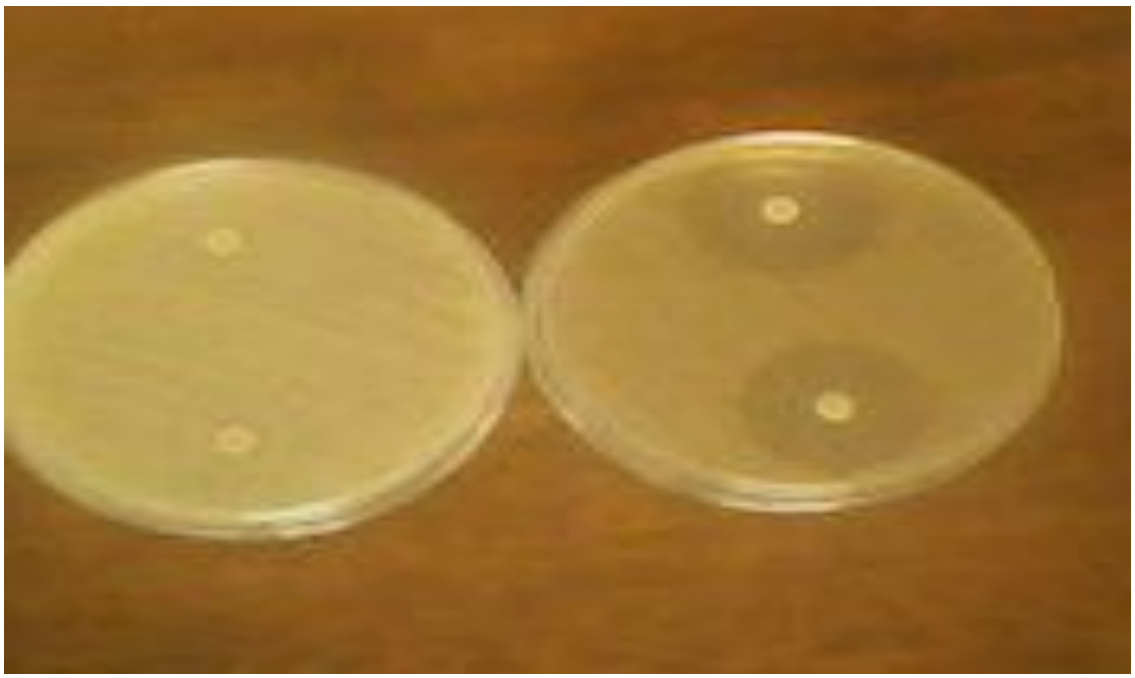

Plate 1: Positive CLSI breakpoint screening test (Left) and confirmed ESβL producer based on DRM (Right).

DISCUSSION

Of the 500 clinical bacterial isolates subjected to Gram's staining reactions, Gram negative isolates were the most predominant in occurrence with 300 (60%) over the Gram positive isolates with only 200 (40%). The high occurrence of Gram negative observed may be related to improper hygiene from the side of the patient which can result in possible outbreak of infection.

On subjecting the Gram negative isolates to screening test for occurrence of ESβLs based on CLSI breakpoint, 250 (83.33%) were found to be positive which included; *K. pneumoniae* (40), *E. coli* (92), *Providencia* spp (30), *M. morganii* (20), *P. aeruginosa* (14), Shigella spp (14), *C. freundii* (12), *S. marcescens* (6), *S. paratyphi A* (10), *Y. enterocolitica* (6), *P. vulgaris* (4) and *S. typhi* (2). However, of the 250 positives in the screening test, one hundred and sixty four, 164(65.6%) were confirmed to be ESβL-producers based on DRM (Table 3). The high occurrence of ESBLs producers among the clinical isolates indicates possible treatment failures using broad spectrum antibiotics directed against common resistant organisms.

Among the ESβLs producers detected, *E. coli* recorded the highest occurrence rate of 52 (31.71%) followed by *K. pneumoniae* with 32(19.50%) which conforms to the findings of Yusha'u *et al.*, (2010) (*E. coli*, 20 and *K. pneumoniae,* 12). Others were *Providencia spp* (20), *M. morganii* (16), *P. aeruginosa* (8), *Shigella spp* (12), *C. freundii* (6), *S. marcescens* (4), *S. paratyphi A* (8), and *Y. enterocolitica* (6). However, there was no evidence of ESβL production observed in the clinical isolates of *P. vulgaris* and *S. typhi* based on the confirmatory test used (DRM). This agrees with the findings of Akujobi *et al.*, (2010) in a study conducted in the sourth-Eastern Nigeria where they detected no evidence of ESβLs among the 6 isolates of *Proteus spp.* tested.

CONCLUSION

From this research, it can be concluded that the percentage occurrence of the ESβLs detected at the study site was high with the calculated value (187.07) being greater than the table value (11.07) at P=0.05 using chi-square statistical analysis which calls for an urgent attention as there may be possibility of treatment failure with cephalosporins.

RECOMMENDATIONS

(a) Efforts should be made to determine the ESβL genotypes using molecular techniques.

(b) Control measures such as proper handling and disinfection of equipments as well as detection of ESβLs producing organisms among the patients and isolation of ESβLs colonized patients should be improved by the Health care settings.

(c) Awareness campaigns on improved hygienic practices should be diversified by both governmental and Non-governmental Organizations so as to reduce the rate of infections and spread of ESβLs among both enterobacterial and other bacterial pathogens.

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