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Prevalence of extended-spectrum β-lactamases (ESBLS) producing enterobacteriaceae in Kano, Nigeria

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ABSTRACT : One thousand one hundred and fifty one (1151) bacterial isolates, four hundred and four (404) obtained from Aminu Kano Teaching Hospital (AKTH) and seven hundred and forty seven (747) obtained from Muhammad Abdullahi Wase specialist Hospital (Nassarawa), Kano, Nigeria, were subjected to Gram staining reactions. Gram negative isolates were subjected to biochemical characterization using standard procedures. All confirmed enterobacteriaceae isolates were subjected to screening for extended spectrum β-lactamases (ESBLs) production using Clinical and Laboratory Standards Institute (CLSI) breakpoint. Suspected ESBLs producers were subjected to Double Disc Synergy Test (DDST) using standard discs of Augmentin {(AMC 30µg (Oxoid, England)}, Cephotaxime (Ce 30 µg) and Ceftazidime (Ca 30µg) {Hi-Media, India. Of the 815 Gram negative isolates, 795 (77.55%) were members of enterobacteriaceae family. These include; *Citrobacter species 47 (5.77%), Enterobacter species 32 (3.93%), E. coli 272 (33.37%), Klebsiella species 118 (14.48%), Proteus species 139 (17.05%), Providencia species 8 (0.98%), Salmonella species 175 (21.47%), Shigella species 4 (0.49%).* Among the enterobacteriaceae isolates screened, the results of CLSI breakpoint test showed that 82 (10.31%) were ESBLs producers of which only 44(5.53%) were confirmed ESBLs producers using DDST. These include; *Citrobacter species* 2 (4.23%), *Enterobacter species* 1 (3.13%), *Escherichia coli 13* (4.78%), *Klebsiella species 9* (7.63%), *Proteus species* 6 (4.32%) and *Salmonella species* 13 (7.43%).

Key Words: Enterobacteriaceae; β-Lactamase; Prevalence study.

Introduction

Extended spectrum β-lactamases (ESBLs) are enzymes that confer variable level of resistance to oxyiminocephalosporins such as cephotaxime, ceftazidime and monobactams. They occur predorminantly in the family enterobacteriaceae with *Klebsiella pneumoniae* been the most commonly reported worldwide and it is responsible for 5-20% of outbreaks of nosocomial infections in intensive care units, burn, oncology and neonatal units (Kotra, *et al*, 2002).
At present there exist more than 200 different natural variants worldwide which constitute serious threat to current β-lactam therapies and represent major therapeutic challenges for clinicians (Lin et al, 2005).

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This research is important at the present situation because of the collapse of primary healthcare system and the unavailability of drugs in the hospitals in this country which resulted in most of the people resorting to purchase of drugs over the counters and in some cases from roadside hawkers which led to the use and overuse of drugs that expose them to the danger of acquiring ESBL-producing organisms. 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. the objectives of this research are:

1. To detect the presence or otherwise of ESBL-producing enterobacteriaceae among clinical isolates.
2. To determine the rate of occurrence of ESBLs among members of the family enterobacteriaceae.

Materials and Methods

ANTIBIOTICS

The antibiotics used in this research were; Augmentin (AMC 30µg) (Oxoid, England), Cephotaxime (Ce 30µg) (Hi-Media, India) and Ceftazidime (Ca 30µg) (Hi-Media, India).

ISOLATES

One thousand one hundred and fifty one (1151) bacterial isolates were collected from the Microbiology laboratories of Aminu Kano Teaching Hospital (AKTH) and Muhammad Abdullahi Wase Specialist Hospital (Nassarawa) Kano.

GRAM'S STAINING

This was carried out as described by Brooks *et al.* (1989).

BIOCHEMICAL TESTS

Gram negative isolates were further subjected to indole, motility, citrate utilization, urease production, hydrogen sulfide production as well as acid and gas production according to standard procedures described by Cheesebrough (2004).

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. Few colonies 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).

CLSI BREAKPOINT TEST FOR ESBLS SCREENING

The sensitivity of standard inocula of isolates to Cephotaxime (Ce 30µg), Ceftadime (Ca 30µg) and Ceftazidime/Clavulanic acid (Cac 30µg) (Hi-Media, India) disks was determined on Mueller Hinton Agar (Biotech, India) using Kirby Bueur method (NCCLS, 2002).

DOUBLE DISK SYNERGY TEST (DDST) FOR ESBLS CONFIRMATION

The improved procedure of Jarlier *et al*, 1988 was employed in the screening of isolates for ESBL production on Mueller Hinton Agar (Biotech, India).

Results

Of the 1151 isolates collected from the two sampling sites [i.e. Aminu Kano Teaching Hospital and Muhammad Abdullahi Wase Specialist Hospital (Nassarawa GRA), Kano] and screened using Gram's staining technique, 336(29.19%) were Gram positive while 815(70.81%) were Gram negative (Table 1). Out of the Gram's negatives, 795 were identified as members of the enterobacteriaceae family and the remaining 20 were *Pseudomonas* species (Table 2).

Of the 795 enterobacterial isolates subjected to ESBL detection using DDST, 82(10.31%) were found to be positive (Table 3; Plates 1 and 2) which include; *Citrobacter* species *(4), Enterobacter*

species *(4), E. coli (21), Klebsiella* species *(14), Proteus* species *(15) and Salmonella* species *(24)* with no detection in *Providencia* and *Shigella* species. However, only 44(5.53%) were positively confirmed to be ESBL producers based on NCCLS breakpoint Test (Table 4) which include; *Citrobacter* species *(2), Enterobacter* species *(1), E. coli (13), Klebsiella* species *(9), Proteus* species *(6) and Salmonella* species *(13).*

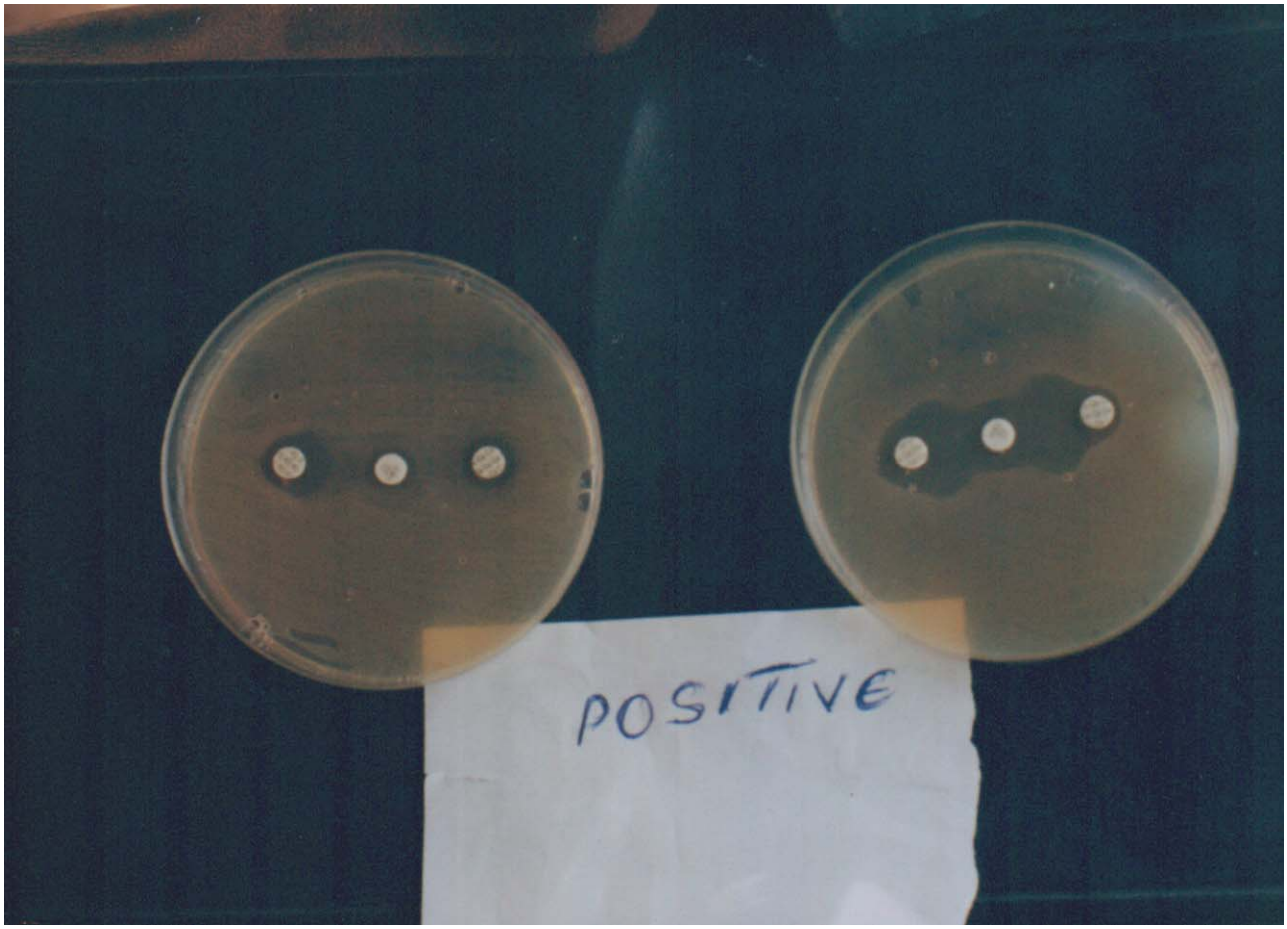

Plate 1: ESBL positive plates based on DDST

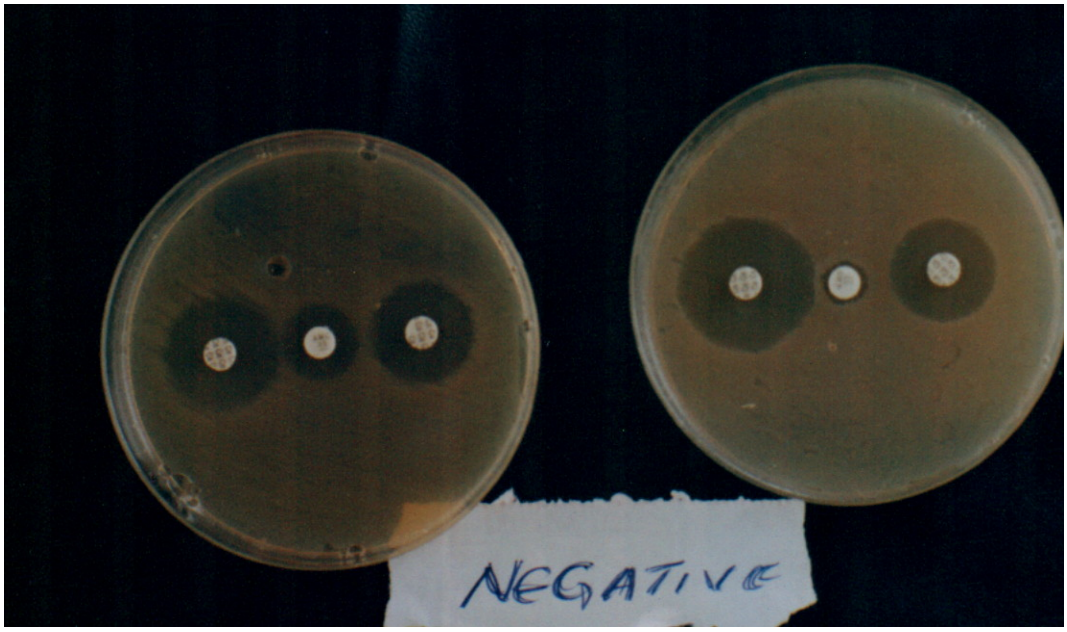

Plate2: ESBLs negative plates based on DDST

Table 1: Gram's Staining Reactions of bacterial isolates.

Gram's Reaction	No. observed	% occurrence
+ve	336	29.19
-ve	815	70.81
Total	**1151**	**100**

Discussion

The sites of isolation of the test organisms were Aminu Kano Teaching Hospital (AKTH) and Muhammad Abdullahi Wase Specialist Hospital (Nassarawa GRA), Kano. The reason for choosing these hospitals is that the former was a referral hospital and mainly patronized by the elites while the latter was patronized by many people from within and outside Kano.

Of the 1151 bacterial isolates collected from the two hospitals screened using Gram's staining technique, 336(29.19%) were Gram positive while 815(70.81%) were Gram negative (Table 1). High occurrence of Gram negative organisms among the isolates is an indication of possible outbreaks of infection since the organisms are pathogenic once found living outside their natural habitat i.e. gastrointestinal tract.

On subjecting the Gram negative isolates to biochemical tests, 795(97.55%) were identified as members of the enterobacteriaceae family and the remaining 20(2.45%) were *Pseudomonas* species (Table 2).

On comparing the two sampling sites, a variation exists (at P = 0.05) in the occurrence and distribution of enterobacterial isolates with *E. coli, Klebsiella, Salmonella, Proteus, Citrobacter, Enterobacter, Providencia* and *Shigella* species being identified among samples obtained from Muhammad Abdullahi Wase Specialist Hospital (Nassarawa) amongst which *Salmonella* species has the highest prevalence (33.0%).

Table 2: Occurrence of Gram negative species based on Biochemical Reactions.

S/No.	Isolates	Glu.	Lac.	Urea	Cit.	Mot.	Ind.	Slope	Butt	H_2S	Gas	No. identified	% occurrence
1	*Citrobacter spp.*	+	+	+/-	+	+	-	R/Y	Y	+/-	+	47	5.77
2	*Enterobacter spp*	+	+	-	+	+	-	Y	Y	-	+	32	3.93
3	*E. coli*	+	+	-	-	+	+	Y	Y	-	+	272	33.37
4	*Klebsiella spp*	+	+	Slow+	+	-	-	Y	Y	-	+	118	14.48
5	*Proteus spp*	+	-	+	+/-	+	+/-	R	Y	+	+	139	17.05
6	*Providencia spp*	+	-	+/-	+	+	+	R	Y	-	+/-	8	0.98
7	*Pseudomonas spp*	+/-	-	+/-	+	+	-	R	R	-	-	20	2.45
8	*Salmonella spp*	+	-	-	+/-	+	-	R	Y	+	+/-	175	21.47
9	*Shigella spp*	+	-	-	-	-	+/-	R	Y	-	-	4	0.49
	Total											**815**	**99.99**

Key: Glu. = Glucose, Lac. = Lactose, Cit. = Citrate, Mot. = Motility, Ind. = Indole, H_2S = Hydrogen Sulphide production,
R = Alkaline reaction, Y = Acid reaction, ….+ = Late positive reaction, +/- = Few species give negative reactions

Table 3: Screening for ESBLs production among Enterobacterial isolates based on NCCLS Breakpoint Test.

S/No.	Isolates	Number screened	Number positive	% occurrence
1	*Citrobacter spp.*	47	4	8.51
2	*Enterobacter spp*	32	4	12.50
3	*E. coli*	272	21	7.72
4	*Klebsiella spp*	118	14	11.86
5	*Proteus spp*	139	15	10.79
6	*Providencia spp*	8	0	0
7	*Salmonella spp*	175	24	13.71
8	*Shigella spp*	4	0	0
	Total	**795**	**82**	**10.31**

Table 4: Confirmation of ESBLs production among Enterobacterial isolates based on DDST Method.

S/No.	Isolates	Number screened	Number positive	% occurrence
1	*Citrobacter spp.*	47	2	4.23
2	*Enterobacter spp*	32	1	3.13
3	*E. coli*	272	13	4.78
4	*Klebsiella spp*	118	9	7.63
5	*Proteus spp*	139	6	4.32
6	*Providencia spp*	8	0	0
7	*Salmonella spp*	175	13	7.43
8	*Shigella spp*	4	0	0
	Total	**795**	**44**	**5.53**

On the other hand, bacterial isolates of *E. coli, Klebsiella, Salmonella* and *Proteus species* were identified from Aminu Kano Teaching Hospital (AKTH) samples with *E. coli* having the highest occurrence rate (44.8%) and the least being in *Salmonella* species (7.17%).

The high occurrence of enterobacteriaceae among the clinical isolates may be due to poor hygienic practices which may result in some of the ESBLs non-producing isolates acquiring plasmids responsible for ESBLs production since plasmids can easily be transferred between organisms living in the same environment and replicate alongside the bacterial chromosome during reproductive processes (Gunseren *et al*, 1999).

Of the 795 enterobacterial isolates subjected to ESBL detection using NCCLS breakpoint, 82(10.31%) were found to be positive (Table 3). These include; *Citrobacter* species *(4), Enterobacter* species *(4), E. coli (21), Klebsiella* species *(14), Proteus* species *(15) and Salmonella* species *(24)* with no detection in *Providencia* and *Shigella* species. However, only 44(5.53%) were positively confirmed to be ESBL producers based on DDST (Table 4). These include; *Citrobacter* species *(2), Enterobacter* species *(1), E. coli (13), Klebsiella* species *(9), Proteus* species *(6) and Salmonella* species *(13).* The variation in ESBLs positive results between the DDST and NCCLS procedures may be due to false positive results caused in organisms with multiple β–lactamases that interfere with the test results which can only be detected using iso-electric focusing and DNA sequencing (NCCLS, 1999).

In general, the percentage prevalence of ESBLs producers among the different species of enterobacterial isolates screened was higher in *Klebsiella* species (7.63%) followed by *Salmonella* species (7.43%), *E. coli* (4.78%), *Proteus* species (4.32%), *Citrobacter* species (4.23%) and the least in *Enterobacter* species (3.13%). There exist significant differences in ESBLs production among the isolates when the results were subjected to chi-square statistical analysis because the calculated value (18.73) was greater than the table value (11.07) at 5% confidence level and 5 degree of freedom (Appendix 5). This conforms to the finding of Hamberger *et al.* (1999) and Moubareck *et al.* (2005). They found that occurrence of ESBLs was high in *Klebsiella* than other species belonging to the family enterobacteriaceae. However, the ocurrence and distribution of ESBLs differs from country to country and from hospital to hospital (Bradford, 2001).

The high occurrence of ESBLs among *Klebsiella* and *Salmonella* species observed in this research is of great concern since infections caused by these organisms (respiratory tract infections and typhoid and paratyphoid fevers) are very common in this part of the country due to the contagious nature and unavailability of qualitative drinking water respectively. The overall prevalence (5.53%) {table 4} observed is high considering poverty level of average Nigerians coupled with the collapse of primary healthcare delivery system which indicates the possibility of treatment failure and/or outbreaks of infections caused by resistant organisms (Ahmad *et al.*, 1999).

On comparing the two sampling sites, a variation exists (at P = 0.05) in the occurrence and distribution of ESBLs with Muhammad Abdullahi Wase Specialist Hospital (Nassarawa) having the highest prevalence of ESBLs (9.85%) in *Salmonella specie* while Aminu Kano Teaching Hospital (AKTH) has the highest prevalence of ESBLs observed in *Klebsiella* species (10.71%). This agrees with the statement of Bradford (2001) that occurrence and distribution of ESBLs differ from country to country and from institution to institution or hospital to hospital.

Recommendations

In view of the worldwide occurrence and quick spread of ESBLs among bacterial pathogens and the problems that may be caused by treatment failure due to infections with ESBLs producing organisms, it could be recommended that;

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

Healthcare settings should improve control measures such as proper handling and disinfection of equipment as well as detection of ESBLs and isolation of patients colonized with ESBLs producing pathogens. Clinicians should reduce the rate at which third and fourth generation cephalosporins are prescribed.

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