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Laboratory survey of extended spectrum beta-lactamase producing Enterobacteriaceae from selected tertiary hospitals in south-eastern Nigeria

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Abstract:

Background: Extended spectrum beta-lactamases are produced by Gram-negative bacteria and most strains producing them belong to the family Enterobacteriaceae. The greatest challenge with ESBL-producing Enterobacteriaceae is their propensity to acquire multidrug resistance traits. This study aimed at determining the prevalence of ESBL-producing Enterobacteriaceae among selected tertiary hospitals in south-eastern Nigeria.
Methods: A total of 400 Enterobacteriaceae isolates were obtained from patients attending five selected tertiary hospitals and were identified to species level by Gram staining and conventional biochemical tests. Screening for ESBL production was determined by the Kirby-Bauer disk diffusion method using 30µg disk of ceftriaxone, cefuroxime, cefpodoxime, ceftazidime, and aztreonam while confirmatory test was done using combination disk test based on the 2016 CLSI guidelines.
Results: The prevalence of ESBL production among Enterobacteriaceae isolates from selected hospitals in southeast Nigeria is 61.5% (246 of 400). Among the isolates obtained, the highest prevalence was observed in *Klebsiella oxytoca* (100%) while the least prevalence was seen in *Morganella morganii* (50.0%). *Escherichia coli* and *Klebsiella pneumoniae* had rates of 61.8% and 62.3% respectively. Among the States of the south-east Nigeria, selected hospital in Ebonyi had a prevalence of 83.5%, Abia 63.6%, Anambra 61.5%, Enugu 51.7% and Imo 36.5%. The prevalence of ESBL-producing Enterobacteriaceae differ significantly between the States (p=0.000).
Conclusion: ESBL-producing Enterobacteriaceae strains have been isolated from different participants, from the selected tertiary hospitals in south-eastern Nigeria. Therefore, we report a high prevalence of ESBL-producing Enterobacteriaceae in south-eastern Nigeria.

Keywords: ESBL, Enterobacteriaceae, resistant strains, southeast Nigeria

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Enquête en laboratoire sur les entérobactéries productrices de bêta-lactamases à spectre étendu de certains hôpitaux tertiaires du sud-est du Nigéria

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Abstrait:

Contexte: Les bêta-lactamases à spectre étendu sont produites par des bactéries à Gram négatif et la plupart des souches qui les produisent appartiennent à la famille des entérobactéries. Le plus grand défi des entérobactéries

productrices de BLSE est leur propension à acquérir des traits de résistance multidrogue. Cette étude visait à déterminer la prévalence des entérobactéries productrices de BLSE parmi certains hôpitaux tertiaires du sud-est du Nigéria.
Méthodes: Un total de 400 isolats d'Enterobacteriaceae ont été obtenus de patients fréquentant cinq hôpitaux tertiaires sélectionnés et ont été identifiés au niveau de l'espèce par coloration de Gram et tests biochimiques conventionnels. Le dépistage de la production de BLSE a été déterminé par les méthodes de diffusion sur disque de Kirby-Bauer en utilisant un disque de 30 µg de ceftriaxone, cefuroxime, cefpodoxime, ceftazidime et aztreonam tandis que le test de confirmation a été effectué en utilisant un test de disque combiné basé sur les lignes directrices de 2017 du CLSI.
Résultats: la prévalence de la production de BLSE parmi les isolats d'Enterobacteriaceae provenant d'hôpitaux sélectionnés dans le sud-est du Nigeria est de 61,5% (246 sur 400). Parmi les isolats obtenus, la prévalence la plus élevée a été observée chez *Klebsiella oxytoca* (100%) tandis que la prévalence la plus faible a été observée chez *Morganella morganii* (50,0%). *Escherichia coli* et *Klebsiella pneumoniae* avaient des taux de 61,8% et 62,3% respectivement. Parmi les États du sud-est du Nigéria, certains hôpitaux d'Ebonyi avaient une prévalence de 83,5%, Abia 63,6%, Anambra 61,5%, Enugu 51,7% et Imo 36,5%. La prévalence des entérobactéries productrices de BLSE diffère considérablement d'un État à l'autre (p=0,000).
Conclusion: des souches d'entérobactéries productrices de BLSE ont été isolées de différents participants, dans les hôpitaux tertiaires sélectionnés du sud-est du Nigéria. Par conséquent, nous signalons une prévalence élevée d'entérobactéries productrices de BLSE dans le sud-est du Nigéria.

Mots-clés: BLSE, Enterobacteriaceae, souches résistantes, sud-est du Nigeria

Introduction:

Some bacteria produce beta-lactamase enzymes which renders β-lactam antimicrobial agents inactive by hydrolyzing the beta-lactam rings thereby conferring resistance against β-lactam antibiotics. However, bacteria which produce beta-lactamases usually express resistance to other antimicrobial agents and as such frustrate therapeutic interventions (1). Extended spectrum beta-lactamases (ESBLs) are a group of enzymes that possess the ability to deactivate the beta-lactam rings of penicillins, first, second and third generation cephalosporins, and aztreonam, but they are inhibited by clavulanic acid (2). There are several molecular variants of ESBLs designated as TEM-1, TEM-2, SHV, OXA, CTX-M and PER amongst others but the most prevalent types are TEM and SHV enzymes (3).

ESBLs are chromosomally or plasmid-mediated. When plasmid-mediated, they are encoded on large plasmids that also carry genes which mediate resistance to other antimicrobial agents such as tetracyclines, aminoglycosides, chloramphenicol, trimethoprim and sulphonamides. Therefore, organisms which produce ESBLs usually manifest resistance to multiple antibiotic classes (4), thereby posing very serious therapeutic challenges from limited treatment options, with severe, and in some cases, fatal clinical outcomes (2). Being plasmid-encoded, ESBLs can easily be transferred from one bacterium to another via horizontal gene transfer. Although this can occur within the community, it is most often observed in healthcare facilities, and is a major challenge in nosocomial infections. A number of factors create suitable conditions for their spread within the hospital setting, and these include; poor hygienic practices in hospitals especially those in developing countries, indiscriminate antibiotics use, empirical antibiotic prescription and therapy not supported by the laboratory, absence of antimicrobial resistance surveillance programs and inadequate infection control practices (1,5).

ESBLs are produced by Gram-negative bacteria and most strains producing them belong to the family Enterobacteriaceae. The most common mechanisms of resistance to third generation cephalosporins by Enterobacteriaceae are through the production of ESBLs. These organisms are not only present within the hospital settings but have become prevalent also in the community. Furthermore, ESBLs have been isolated from commensals in human, animals and sewage. Therefore, environment may serve as reservoirs of organisms producing ESBLs (6), which have been reported to be responsible for the worldwide spread of ESBLs (7).

The emergence of ESBLs in Enterobacteriaceae compromises the efficacy of antibiotics, and infections due to these organisms are associated with high morbidity, mortality and treatment costs (3,8). By far, the greatest challenge associated with ESBL-producing Enterobacteriaceae is their propensity to acquire multidrug resistance traits. Other challenges are gross limitation of treatment options, prolonged hospital stay and ability to spread from persons both within the hospital and the community with epidemic potential, and also to become established as endemic pathogen within community (9,10).

The drugs of choice for treatment of infections caused by ESBL-producing Enterobacteriaceae are the carbapenems. However,

with these strains becoming endemic, there have been increasing dependence and widespread use of the carbapenems and at present, studies have reported the presence of resistance to carbapenems (11). Furthermore, failure of antibiotic therapy has resulted in high mortality rates in patients infected with these bacteria (12). Globally, resistance to anti-microbial agents has been identified as a menace to public health (13), and developing countries are major regions for these multidrug resistant bacteria (14).

There have been studies conducted at different times in individual State of the south-eastern region of Nigeria but none had been conducted across all the States at once. Therefore, this study aimed to determine the prevalence of ESBL-producing Enterobacteria-ceae isolates obtained from selected tertiary hospitals in each of the five States that make up the south-eastern region of Nigeria.

Materials and methods:

Study area

The southeastern region of Nigeria lies within the coordinates 5°25'N and 8°05'E, and is made up of five States; Abia, Anambra, Ebonyi, Enugu and Imo. It has an area of approximately 76,000 square kilometers. According to the 2006 census, the population of the region was approximately 16,395,555. The region has three types of vegetation; mangrove swamps and tidal waterways dominate the coastal areas; tropical rainforests dominate the regions further north of the swamps while guinea savannah dominates the northernmost parts of the region.

Study design and sampling technique

This was a cross-sectional study designed and carried out in five selected tertiary hospitals in south-eastern Nigeria. Among eleven tertiary hospitals located within the region, five were selected by simple random sampling technique.

Study population

The participants were patients who presented with clinical manifestations that suggested the presence of infection(s) caused by any member of the Enterobacteriaceae, whose appropriate clinical specimens were sent to the microbiology laboratory of each hospital for microscopy, culture and sensitivity.

Ethical consideration

Ethical approval was obtained from the State Ministries of Health of Abia, Ebonyi, Enugu and Imo States, with approval numbers; AB/MH/AD/904 /T.167, EBSMOH/ERC/061/19, MH/MSD/REC18/045, and CON/MH/MD/195/1 respectively. Informed consent was obtained from the participants or from their parents/ guardians (for those below 18 years). The participants were assured that their identities will not be linked to any data. The study was conducted following strict adherence to international, national and institutional ethical guidelines and in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Sample size

The sample size was calculated using the formula; $N=Z^2PQ/d^2$ where; N=minimum sample size, Z=1.96 (Standard deviation of a normal distribution taken at 95% confidence interval which corresponds to 1.96), d=0.05 (degree of accuracy set at 0.05 for 95% confidence interval, and P=50% (expected prevalence from available literature, however, no previous epidemiological study of this nature has been conducted within the study area hence a prevalence of 50% was assumed), and Q=1-P. Therefore, $N=1.96^2x\ 0.5(1-0.5)/0.05^2$ gives an estimated sample size of 384, which was increased to 400 to compensate for 10% attrition.

To determine the number of samples that would be collected from each centre, the probability proportion by size was calculated. To determine this, the average number of patients received in the medical microbiology laboratory from each centre per month was obtained (Table 1). The formula used to calculate the sample size to be collected from each centre was given as "a/b x n", where a=average total patients received by each centre per month, b=total number of patients, and n=sample size.

Inclusion and exclusion criteria:

Patients who gave informed consents and in whom Enterobacteriaceae were isolated from their specimens were included in the study. Patients who gave consent but in whom Enterobacteriaceae was not isolated from their specimens were excluded from the study. Also, based on the prescription on the patients' case folder and on verbal interview, patients on combined antibiotic therapy were excluded from the study.

Specimen collection and bacteria identification:

Specimens were collected from the participants based on the requests on their laboratory request forms. Enterobacteriaceae were isolated from various specimens such as urine, sputum, cerebrospinal fluids, stool,

blood, semen, wound, high vaginal, ear, throat, urethral and eye swabs.

The identification of the isolates was performed using standard microbiological methods described by Cheesbrough (15) and Forbes et al., (16), which includes Gram reaction and conventional biochemical tests such as indole, methyl red, Voges-Proskauer, citrate utilization, oxidase, urease, triple sugar iron, and sugar fermen- tation reactions.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done using Kirby-Bauer disk diffusion test. Mueller-Hinton agar plates were prepared following the Manufacturer's instructions. Using sterile swab sticks for each isolate, the plates were seeded with 0.5 McFarland turbidity equivalent of the bacterial suspension made on sterile normal saline, they were then allowed to stand for 20 minutes. Thereafter standard antimicrobial disks were placed on the surface of the media, each disk being well spaced from the other. The plates were subsequently incubated in ambient air at 37^0C for 18 hours. Results were read and interpreted using guidelines of the Clinical and Laboratory Standards Institute (17).

Screening for ESBL-production

All Enterobacteriaceae isolates were first screened for ESBL production using Kirby-Bauer single disk diffusion with ceftriaxone (30µg), cefuroxime (30µg), cefpodoxime (30 µg), ceftazidime (30µg) and aztreonam (30µg) (Oxoid, UK). Isolates with inhibition zones < 27 mm (ceftriaxone), < 25mm (cefuroxime), < 22 mm (cefpodoxime and ceftazidime) and < 25 mm (aztreonam) were preliminary identified as ESBL producers and subjected to phenotypic confirmatory test.

Confirmatory test for ESBL production

Confirmatory test for ESBL production was done with combination disk test described by CLSI (17) with minor modifications, using ceftazidime disk in combination with clavulanic acid. 10 µL of a 1000 µg/10ml stock solution of clavulanic acid was added to ceftazidime disks each day of testing. A ≥5mm increase in zone diameter of the combined disk over the zone diameter of single ceftazidime disk confirmed an isolate as ESBL-producer.

Data analysis

Data were analysed with the Statistical Package for the Social Sciences (SPSS) version 20.0. Bar and pie charts were used for the presentation of some variables; descriptive analysis, frequency tables and percentages were used for univariate analysis while Chi square test was used for bivariate analysis. P value < 0.05 was considered significant for all analyses.

Table 1: Sample size calculation from the probability proportion by size

S/N	Centre	(a)	Sample size (n)
1	Federal Medical Centre, Umuahia	900	77
2	Alex Ekwueme Federal University Teaching Hospital, Abakaliki	1200	103
3	Enugu State University Teaching Hospital, Parklane	1350	116
4	Imo State University Teaching Hospital	600	52
5	Chukwuemeka Odumegwu Ojukwu University Teaching Hospital	600	52
	Total (b)	**4650**	**400**

Results:

A total of 400 patient participants were enrolled in the study, among these, 192 (48%) were males while 208 (52%) were females (male: female ratio was approximately 1:1). The age group of the participants and the total number obtained from each of the State is presented in Table 2. The specimen types collected from the participants are presented in Table 3. The most frequently collected specimens were urine from 137 (34.3%), stool from 50 (12.5%), and wound swab from 50 (12.5%) while eye swab was the least frequently collected specimens from 1 (0.3%) participant.

The Enterobacteriaceae isolates obtained from the specimens and the frequency distribution is presented in Table 4. Among the isolates, *Escherichia coli* had the highest overall frequency (30.8%), followed by *Klebsiella pneumoniae* (19.3%) while *Klebsiella oxytoca* had the least frequency of 0.3%. Other organisms isolated include; *Citrobacter freundii, Yersinia enterocolitica, Salmonella enterica, Enterobacter aerogenes, Providencia stuartii, Shigella sonnei, Proteus mirabilis, Morganella morganii* and *Serratia marcescens.*

The prevalence of confirmed ESBL-producing Enterobacteriaceae is presented in Table 5. Among the 400 isolates, 246 (61.5%) were ESBL producers while 154 (38.5%) were non-producers. Among the ESBL producers, *E. coli* had a frequency of 61.8% (76/123) while *K. pneumoniae* had a frequency of 62.3% (48/77). However, the highest frequency was observed among *K. oxytoca* (100%) followed by *S. sonnei* (77.8%), *E. aerogenes* (77.3%) and *Y. enterocolitica* (64.3%) while the least frequency was observed in *M. morganii* (50.0%). When subjected to statistical analysis, the prevalence of ESBL-producers among the isolates tested was non-significant (p=0.721).

When the prevalence of ESBL-producing isolates was compared within the age group of the participants, there was no significant relationship between age group distribution and the prevalence of ESBL-producing Enterobacteriaceae (p=0.834). However, the highest ESBL-producing organisms were isolated from participants within the age group of 70 years and above (70.0%), followed by age group 60-69 (63.6%) whereas age group 40-49 years had the least rate of ESBL-producing Enterobacteriaceae (51.4%).

When the prevalence rates of ESBL-producing Enterobacteriaceae were compared among the five States of the southeast region, Ebonyi had the highest prevalence rate (83.5%) while Imo had the least (36.5%). The prevalence rates for other States were 51.7% for Enugu, 61.5% for Anambra and 63.6% for Abia (Table 6). The prevalence rates of ESBL-producing Enterobacteriaceae differed significantly between the States (p=0.000).

Table 2: Demographic variables of patients from whom Enterobacteriaceae were isolated

Demographic variables	Frequency	Percentage
State		
Abia	77	19.3
Ebonyi	103	25.8
Enugu	116	29.0
Imo	52	13.0
Anambra	52	13.0
Age group (year)		
<20	71	17.8
20-29	124	31.0
30-39	46	11.5
40-49	35	8.8
50-59	28	7.0
60-69	66	16.5
70 & above	30	7.5
Gender		
Male	192	48.0
Female	208	52.0

Table 3: Frequency distribution of the specimen types obtained from selected patients

Specimen types	Frequency	Percentage
Urine	137	34.3
Stool	50	12.5
Wound swab	50	12.5
Blood	34	8.5
High vaginal swab	32	8.0
Endocervical swab	29	7.3
Semen	17	4.3
Sputum	14	3.5
Ear swab	11	2.8
Throat swab	10	2.5
Urethral swab	8	2.0
Cerebrospinal fluid	7	1.8
Eye swab	1	0.3

Table 4: Frequency distribution of the Enterobacteriaceae isolates

Isolate	Frequency	Percentage
Escherichia coli	123	30.8
Klebsiella pneumoniae	77	19.3
Citrobacter freundii	41	10.3
Yersinia enterocolitica	28	7.0
Salmonella enterica	27	6.8
Enterobacter aerogenes	22	5.5
Providencia stuartii	19	4.8
Shigella sonnei	18	4.5
Proteus mirabilis	17	4.3
Morganella morganii	14	3.5
Serratia marcescens	13	3.3
Klebsiella oxytoca	1	0.3

Table 5: Distribution of Enterobacteriaceae isolates by ESBL production

Isolate	ESBL production			χ2	*p*-value
	Non-producer (%)	Producer (%)	Total (%)		
E. coli	47 (38.2)	76 (61.8)	123 (30.8)	7.918	0.721
K. pneumoniae	29 (37.7)	48 (62.3)	77 (19.3)		
C. freundii	18 (43.9)	23 (56.1)	41 (10.3)		
Yersinia enterocolitica	10 (35.7)	18 (64.3)	28 (7.0)		
S. enterica	12 (44.4)	15 (55.6)	27 (6.8)		
E. aerogenes	5 (22.7)	17(77.3)	22 (5.5)		
P. stuartii	9 (47.4)	10(52.6)	19 (4.8)		
Shigella sonnei	4 (22.2)	14(77.8)	18 (4.5)		
P. mirabilis	8 (47.1)	9 (52.9)	17 (4.3)		
M. morganii	7 (50.0)	7 (50.0)	14 (3.5)		
S. marcescens	5 (38.5)	8 (61.5)	13 (3.3)		
K. oxytoca	0	1 (100)	1 (0.3)		
Total	**154 (38.5)**	**246 (61.5)**	**400 (100)**		

Table 6: Distribution of Enterobacteriaceae and ESBL production in relation to the States of southeastern Nigeria

State	ESBL production			χ2	*p*-value
	Non-producer (%)	Producer (%)	Total (%)		
Abia	28 (36.4)	49 (63.6)	77 (19.3)	39.560	0.000
Ebonyi	17 (16.5)	86 (83.5)	103 (25.8)		
Enugu	56 (48.3)	60 (51.7)	116 (29.0)		
Imo	33 (63.5)	19 (36.5)	52 (13.0)		
Anambra	20 (38.5)	32 (61.5)	52 (13.0)		
Total	**154 (38.5)**	**246 (61.5)**	**400 (100)**		

Discussion:

This study is essential to raise the awareness about the magnitude of the problem associated with multidrug resistant bacteria with the aim of initiating discussions that can lead to formulation of policies or changes in the existing policies as well as the enforcement of infection control and antibiotic resistance surveillance programs in the southeast region. This is by providing current data on the prevalence of ESBL producing Enterobacteriaceae, taking into cognizance the potential for these resistant strains to spread within the hospital setting and the community, as well as to other regions both within and outside Nigeria. Our study objective is in line with those from other countries (13,14,18,19).

The prevalence of ESBLs is known to vary widely worldwide, even in regions that are closely related. Several studies conducted in different regions of Nigeria on Enterobacteriaceae have reported varying prevalence rates for ESBL-production. A rate of 9.3% was reported for Enterobacteriaceae in Kano (20) while 2.5% for *E. coli* and 5% for *K. pneumoniae* were reported in Ogun State (21). Our study reported prevalence rate of 61.5% for ESBL-production among Enterobacteriaceae isolates in selected hospitals in the south-eastern region of Nigeria. This is higher than the prevalence rates reported from other parts of Nigeria (20,21) and 36.9% rate reported in Central India (22). When compared with previously published data in Ebonyi State, there appeared to be a sustained surge in the prevalence of ESBL-producing Enterobacteriaceae isolates from 16.5% in 2008 (23), 52% among *E. coli* in 2019 (24), to 83.5% in the present study.

The prevalence rates reported from Enugu State have also varied over the years. In 2009, a rate of 11.4% was reported among Enterobacteriaceae isolates (25), and 56.6% for *K. pneumoniae* and 59.4% for *E. coli* in 2010 (26). In 2017, prevalence rates of 59.7% and 40.4% for *Klebsiella* spp and *E. coli* respectively were reported among isolates obtained from orthopaedic wounds (27). Among paediatric population in Enugu metropolis, a prevalence rate of 19.8% for confirmed ESBL-producing Enterobacteriaceae was reported from both healthy and ill participants (28), Another study reported 35% rate among *E. coli* isolates and 75.7% from hospital-acquired infection (29). The presence of ESBL-producing organisms among paediatric population is a cause for concern as it portends an establishment of the strains in Enugu metropolis. Also, the observed increasing prevalence from 2009 to 2019 is an indication of either a non-existent measure to curb the menace of antibiotic resistance or a dilapidation of such control measures if they are available.

In Abia State, a 50.8% prevalence rate of ESBL-producers was reported for urinary isolates in Aba (30), which is slightly lower than the rate obtained from the present study. However, there are no prevalence data on isolates from Umuahia where this current study was conducted. The least prevalence rate (36.5%) reported in our study was among isolates obtained from Owerri, which is comparable to the prevalence of 26.4% for ESBL-producing *E. coli* reported in 2018 (31). However, Duru et al., (32) reported a prevalence of 17% of ESBL-producing *E. coli* and *K. pneumoniae* among asymptomatic persons in Owerri, which represents a group of individuals who may serve as reservoirs of these resistant strains. Worse still, dissemination can continue unnoticed within the community resulting in the establishment as an endemic strain within the locality.

Other studies have detected the presence of ESBL-producing Enterobacteriaceae from animal samples within the south-eastern Nigeria (33). They have also been detected among consumables both within and outside the region. In Abakaliki, a prevalence of 8% was reported from anal swabs of donkeys (34) while 33.3% rate was reported from frozen mackerel fishes sold in the markets (35). In Enugu metropolis, a prevalence of 24.2% was reported among healthy pigs (36), and in Owerri, a prevalence of 22.2% was reported among poultry (37). In other regions, Abubakar and colleagues reported a prevalence of 8.9% and 5.7% for ESBL-producing *E. coli* in chickens and retail eggs in Sokoto metropolis (38).

Conclusion:

To the best of our knowledge, this is the first study to analyse specimens for ESBL-producing Enterobacteriaceae from the entire south-eastern States. Because no study of this nature has been conducted in Umuahia, the results of this present study provide the first information on the prevalence of ESBL-producing Enterobacteriaceae in Umuahia. Generally, our study reports a high prevalence rate of 61.5% for ESBL-producing Enterobacteriaceae in south-eastern Nigeria.

Contributions of authors:

UUI conceived the study, obtained data and interpreted data; UTK designed the study and revised it critically for intellectual content. All authors agreed to the final manuscript.

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