ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 2, pp: (27-39), Month: April - June 2019, Available at: www.researchpublish.com

PREVALENCE AND ASSESSMENT OF ESBL-PRODUCING ORGANISMS IN THE SOUTH- SOUTH GEOPOLITICAL ZONES OF NIGERIA

ANDY, I.E., TIKU, D.R., OKPO, E.A., UTSALO, S.J.

Department of Microbiology, Faculty of Biological Sciences, University of Calabar-Nigeria

Abstract: **The study to assess the prevalence of ESBL-producing organisms in the South-South geopolitical zones of Nigeria was investigated. Clinical specimens of urine, stool, sputum, blood, high vaginal swabs, wound, pus and urinogenital swabs were obtained from health resource centers in Akwa Ibom, Cross River State, Delta Edo and Rivers States, all in South-South geopolitical zone of Nigeria. Double disk synergy test (DDST) was used to detect ESBL production. Quick identification was confirmed using Ceftazidime. Inositol Amphotericin B Agar (CIVA). Results from the study revealed that a total of 130 (20.1%) isolates were positive for ESBL production. Delta State had the highest percentage occurrence of 30.5% and Rivers State was least with 9.4%. Urinogenital swab had the highest production for ESBL with 31(27.2%). Results of fitting logistic regression in univeriate and multivariable analysis revealed significant association of ESBL production with Isolate type (p<0.001) and States (p<0.0001). No association was observed for Source (p-value 0.60) and Patient location (p-value = 0.50). Isolate of *E.coli* were 1.83(95%, (11.20-2.79) times likely to be ESBL positive than those of *K. pneumonia.* Compared to Akwa Ibom, Edo Delta, Rivers and Cross River States were 2.02 (95%, CI 1.05-3.89), 3.20(95%, CI1.69-6.06) and 3.14(95%, CI 1.61-6.14) respectively, were likely to have ESBL positive result. Rivers State was not significant with p-value of 0.47. The study has revealed the presence of ESBL- producing organisms in the study area. It is therefore recommended that concerted efforts must be made to control bacterial resistance to third generation cephalosporin caused by extended spectrum beta lactamases.**

Keywords: **ESBL-producing organisms, Clinical specimens, South-South geopolitical zone.**

1. INTRODUCTION

β -lactamases are the most common mechanism of resistance to beta- lactam antibiotics including the third- generation cephalosporins to which extended spectrum β-lactamase plays a huge role among Enterobacteia ceae (Chidinma *et al.*, 2018). Though there are different diverse β-Lactamases, the etended spectrum β-lactamases (ESBL's) have been known to be of very clinical importance (Raji *et al.*,2013). ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillin, first, second and third generation cephalosporins and aztreonam by hydrolysis excluding the cephamycins and carbapenems (Lee *et al.*,2011). ESBLs have been reported worldwide in many different generation of Enterobacteriaceae and *Pseudomonas aeruginosa* (Raji *et al.*, 2013). Their prevalence among clinical isolates varies from country to country and from institution to institution (Paterson and Bonomo, 2005; Jemima & Verghase, 2008) . In the United States, the occurrence of ESBL production in Enterobacteriacae ranges from 0 to 25% depending on the institution with national coverage around 3%. Vast majority of reports in the first decade after the discovery of ESBLS were from France (Agrawal *et al.*, 2008). The first large outbreak in France were reported to have occurrence in 1986 in which fifty-four patients in three intensive care units were infected and the infection spread to four other wards. This led subsequently to a dramatic proliferation of ESBLS in France. By the early 1990's, 25 to 35% of posocomially acquired *K*lebsiella *pneumoniae* isolates in France were ESBL producing (Marty and Jalier 1998; Friedmann *et al.*, 2009), whereas in

Netherlands a survey of eleven Hospital laboratories showed that less than one percent of *E. coli* and *K. pneumonia* strains possessed on ESBL. In recent years, infection control interventions has been accompanied by a decrease in incidence of ESBL producing *Klebsiella pneumonia* (Albertii *et al.*, 2002). It has become very important to study the prevalence of ESBL- producing organisms because of their increasing antimicrobial resistance and the decreasing number of new drugs available against such organisms (Chidinma *et al.*, 2018). Though the first detection of ESBLs in Nigeria is still not known and a national study for the actual prevalence of ESBL- producing bacteria in Nigeria is lacking, some reports have shown the increasing prevalence of ESBL- producing bacteria in some parts of the country (Iroha *et al.*, 2017). The implications of the prevalence of ESBL producing organisms in Nigerian communities cannot be overlooked. The wide and irrational use of antibiotics, especially the broad spectrum β-lactams, in our local communities allow for the emergence and spread of resistance strains of bacteria that render available drugs ineffective for treatment (Ejikeugwu *et al.*, 2013). Keeping in view the economic and clinical importance of ESBL- producing bacteria, this study was conducted to investigate the prevalence of ESBL producing organisms in the South-South Geopolitical zones of Nigeria.

2. MATERIAL AND METHODS

Collection of isolates

Six hundred and forty- six isolates were obtained from various clinical specimens including urine, wound swab, stool, blood, high vaginal swab and sputum from two hospital in five out of the six states of the South-South Geopolitical Zones namely; Akwa Ibom (University of Uyo Teaching Hospital and St. Luke's Hospital Anua in Uyo), Cross River State (University of Calabar Teaching Hospital and General Hospital Calabar), Delta (Federal Central Hospital Asaba and NNPC clinic), Edo (University of Benin), Rivers (University of Port Harcourt and Braith Waite memorial Hospital, Port Harcourt). The isolates were collected on nutrient agar slants and immediately transported to the laboratory in cool boxes.

Control strains

The negative control strains *E. coli* ATCC 25922 was obtained from medical research laboratory, Benin, Edo- State, while the positive control of *Klebsiella pneumoniae* was obtained from in house positive strain. They were preserved on agar slants at - 20^0C until use.

Identification of isolates

The bacterial isolates were identified using Standard Biochemical Methods and also API-20E method of identification. The API-20E identification system is a standardized miniaturized version of conventional biochemical procedures used to identify enterobacteriaceae and non-fastidious gram negative rods. An inoculum of the test isolate was transferred into a test tube containing 5ml normal saline. The enlongated flap of water was added to the bottom to provide humid atmosphere during incubation. The API plastic strip was then placed in the incubation tray. The 5ml bacterial suspension was introduced into the API strip pouches using a Pasteur pipette in drops. Each pounch/tube contains reagent for a specific biochemical test. The tubes for ONPG, TDA, IND, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY and ARA were filled up.

The tubes for ADH, LDC, ODC, H_2S and URE were slightly under filled. These tubes were later filled up with mineral oil after inoculation to create anaerobic conditions. The tubes with (CIT), (VP) and (GEL) which are in brackets were completely filled up. The lid was placed on the incubation tray and at 35^0C for 18 to 24 hours after which they were removed and read accordingly.

To the tubes for TDA, I drop of 10% ferric chloride was added. Positive result is indicated by a change to reddish brown. For indole test, 1 drop of Kovac's reagent was added and a pink colour gave a positive result for the VP test, one drop of 40% potassium hydroxide and one drop of 6% α-napthol were added and observation of a pink colour after about 10 minutes gave a positive result. All positives were assigned corresponding numbers and codes. The codes were then used to read off the particular organism from the analytical profile index. Where the organism numerical profile was not found in the index, software for API-20E system was used.

Detection of ESBL producing *E.coli* and *K. Pneumoniae*

Extended spectrum beta lactamase producing *E. coli* and *K. pneumoniae* were detected in a two step procedure; preliminary screening and phenotypic confirmatory tests as recommended by HPA(2006).

Preparation of antibiotic disc

A cork borer was used to perforate Whatman No. 3 filter paper discs of 6mm diameter. One hundred each was counted into four McCartney bottles and sterilized in hot air oven at 160^0C for 1 hour. The antibiotics were first reconstituted water (SDW) to get the required concentration. One millimeter (1ml) of each antibiotic were pipette into their appropriately labeled McCartney bottles containing 100 sterile discs, each disc soaked contains 30mcg of antibiotic.

Disc diffusion method (preliminary or initial screen test) inoculum preparation

At least 3-5 pure colonies form 24 hour culture of all *E. coli* and *K. pneumoniae* including control strains were inoculated into 5ml of normal saline. The suspension was adjusted to a turbidity of 0.5 McFarland standard (1-2 cfu/ml).

Inoculation of test plates

Within 15 minutes after adjusting the turbidity of the inoculums, a sterile cotton swab was dipped into the suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This was done to remove excess suspension of inoculums from the swab. The swab was the used to inoculate a dried surface of Mueller-Hinton agar plate by streaking over the entire sterile ager three times. These plates were rotated to ensure appropriate and even distribution of inoculums. At the final step, the rim of the agar plate was swabbed.

Application of antibiotic discs to inoculated plate

The three antibiotic discs of Cefotaxime (30mcg), Ceftazidime (30mcg) and Ceftriazone (30mcg) were place on each plate. Each disc was pressed down to ensure complete contact with the agar surface. Within 15 minutes after the discs were applied, the plates were placed inverted in 37^0C incubator. The plates were later examined after 18 to 24 hours incubation. Upon examination, the zone sizes were measured by use of millimeter rule.

Confirmatory test using double disk synergy

Sterile forceps was used to place discs of augmentin (amoxiclav) at the centre of the plate. Disc containing the three antibiotics were placed adjacent to the disc containing the amoxiclav, 30mm centre to centre. Care was taken to ensure that the discs had contact with the incubator. Enhancement of the zone of inhibition by 5mm is taken as positive for ESBL production (HPA, 2006).

Reading of phenotypic confirmatory test plates

After 16-18 hours of incubation, each plate was examined and the diameters of inhibition were measured. An organism was said to be an ESBL producer if there was an increase and enhancement of zone of inhibition and formation of ellipsoidal zones (HPA, 2006).

Storage of isolates

To prepare an isolate for freezing, each isolate was grown overnight (18-20hours) on blood or MacConkey agar. A heavy suspension of growth was made in the stock medium (glycerol broth 20% v/v glycerol) by using a small volume of stock medium approximately 1ml. all ESBL producing *E.coli* and *K. pneumoniae* were kept frozen at -70^0C in stock medium until needed.

Recovery of isolates

Isolates were recovered from storage when needed by sub-culturing from frozen stock medium (glycerol stock) onto blood or MacConkey agar and incubated over night (18-20hours) at 37^0C in the incubator. Detection of ESBL producers using Ceftazidime Inositol Vancomycin Amphotericin β-agar (CIVA) medium.

This medium rapidly shows the production of ESBLs. It comprises peptone of casein, beef extract, inositol, sodium chloride, bromothymol blue agar. All ingredients were dissolved in sterile distilled water and thoroughly mixed. The mixture was sterilized by autoclaving at 121^0C, 151bs P.S. for 15 minutes. The base was cooked and antibiotics added. Components of CIVA medium were ; Agar (17.0g), Inositol (12.0g), Rentone(11.0g), Sodium chloride (8.5g); Beef extract (3.0g), Bromothymol blue (0.03g), Vancomycin (4.5mg/L), Ceftazidime (2mg/l).

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 2, pp: (27-39), Month: April - June 2019, Available at: www.researchpublish.com

3. RESULTS

Guide for ESBL identification

The guide for ESBL identification is presented in table 1. It showed that for cephalosporin such as cefotaxime (30mcg), ceftazidime (30mcg) and ceftriazone (30mcg), the diameter of inhibition zone are ≤ 27mm, and ≤ 25mm respectively.

TABLE 1: Guide for ESBL identification

Cephalosporin	Diameter of Inhibition Zone
Cefotaxime (30mcg)	≤ 27mm
Ceftazidime (30mcg)	≤ 22mm
Ceftriazone (30mcg)	≤ 25mm

Distribution of ESBL producing isolates

The distribution of ESBL producing *E.coli* and *K. pneumonia* as obtained from the study area is presented in Table 2. The highest number of isolates came from Rivers State, *E. coli* (79) and *K. pneumonia* (70), totaling 149 isolates. The least was Cross River State, *E.coli* (52) and *K. pneumoniae* (50), with a total of 102.

Percentage distribution of ESBL producing isolates is presented in Fig. 1. It showed that River State had the highest percentage distribution of ESBL producing isolates (23.1) as compared to other states with, Akwa Ibom (21.1), Edo (20.2), Delta (19.8) and Cross River (15.8). Sources of *E.coli* and *K. pneumoniae* based on sample types.

From each study state where different specimen types were obtained, Akwa Ibom and Edo have six specimen types namely; Pus, sputum, stool, urinogenital swab, urine and wound swab. Delta state had five sample namely; sputum, stool, usw, urine and wound swab. Cross River State and Rivers had only four sample types; blood, hvs USW and urine. This is presented in Table 3.

TABLE 2: Distribution of *E. coli* and *K. pneumoniae* isolates from the study states

States	*E. coli*	*K. pneumoniae*	Total
Akwa Ibom	83	53	136
Cross River	52	50	102
Delta	72	56	128
Edo	88	43	131
Rivers	79	70	149
Total	374	272	646

TABLE 3: Sources of *E. coli* and *K. pneumoniae* from the states based on sample types

Samples	AK			CR			DE			ED			RI		
	Tot	Ec	Kp	Tot	Ec	Kp	Tot	Ec	Kp	Tot	Ec	Kp	Tot	Ec	Kp
Blood	0	0	0	10	0	10	0	0	0	0	0	0	27	0	27
Hvs	0	0	0	18	1	17	0	0	0	0	0	0	0	0	0
Pus	11	8	3	0	0	0	0	0	0	13	4	9	12	5	7
Sputum	22	16	6	0	0	0	13	3	10	13	2	11	0	0	0
Stool	40	24	16	0	0	0	25	11	14	30	25	5	0	0	0
Usw	27	14	13	21	10	11	24	18	6	22	16	6	20	11	9
Urine	24	18	6	54	41	12	38	31	7	37	34	3	102	68	34
Ws	12	3	9	0	0	0	15	5	10	17	6	11	0	0	0
Total	136	83	53	102	52	50	128	72	56	131	88	43	149	79	70

LEGEND

Ak-Akwa Ibom, CR-Cross River, DE-Delta, ED-Edo, RI-Rivers, HVS-High Vaginal Swab, USW-Urinogenital Swab, EC-*Escherichia coli*, Kp-*Klebsiella pneumoniae*

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 2, pp: (27-39), Month: April - June 2019, Available at: www.researchpublish.com

Comparison of ESBL producers among sample types

The proportion of ESBL producers compared to non-producers on the basis of sample types is presented in Fig. 2. The highest sample obtained in this study was urine (224), out of which 56(22.0%) were positive for ESBL production. Urinogenital, the second highest with 14 gave 31(27.2%) positive. The least sample was high vaginal swab in which out of 18, 4(22.2%) was positive. The 37 blood samples obtained in the study did not yield any ESBL producer. The difference was not statistically significant (p-value = 0.60) comparison of ESBL producers among isolates.

A comparison was made between the isolate type and ESBL production. In this study, *E. coli* had the highest ESBL of 89(23.8%) out of 285 isolates obtained while *K. pneumonia* gave 41(15.1%) out of 231 isolates. However, a significant association among isolates types (p-value = 0.01) was observed. This result is presented in Fig. 3.

Comparison of ESBL producers between patients service areas

Figure 4 presents the relationship of ESBL production with patient service areas. It was observed that in patients were 311,s out of which 66(21.2%) were positive and out patients were 335 with 64(91.1%) as positives. The difference in ESBL production among patient service areas was not statistically significant (p-value = 0.50).

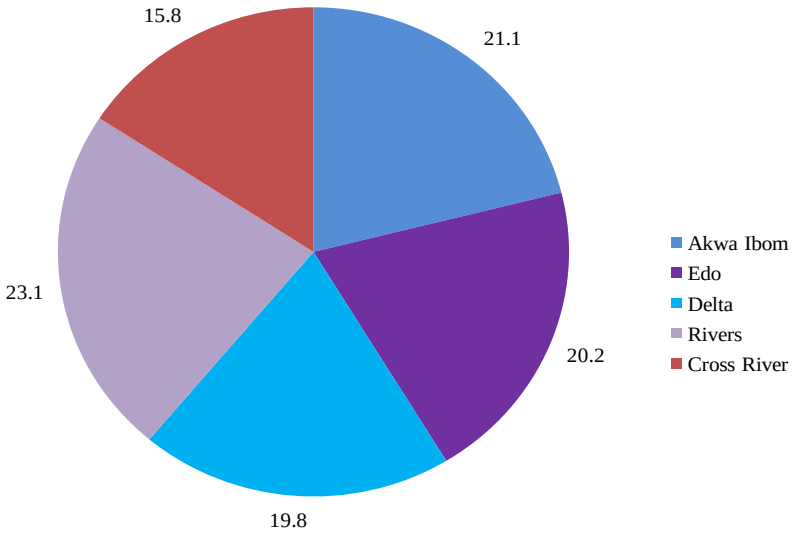

Fig. 1: Distribution of ESBL producers in the study states

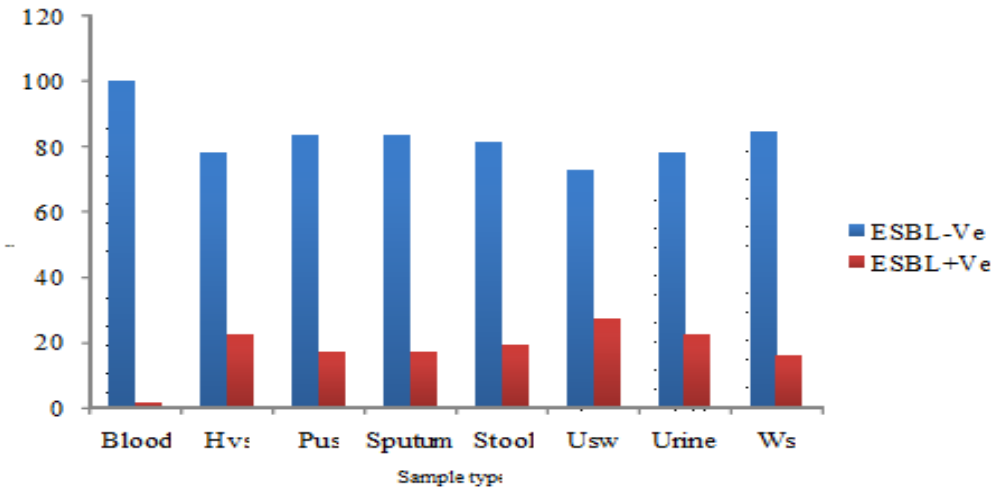

Fig. 2: Comparison of ESBL producers among sample type

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 2, pp: (27-39), Month: April - June 2019, Available at: www.researchpublish.com

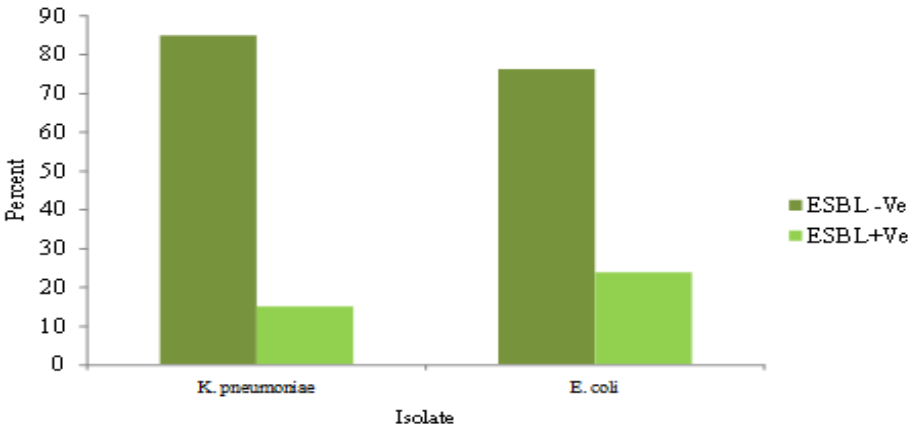

Fig. 3: Comparison of ESBL producers among isolates

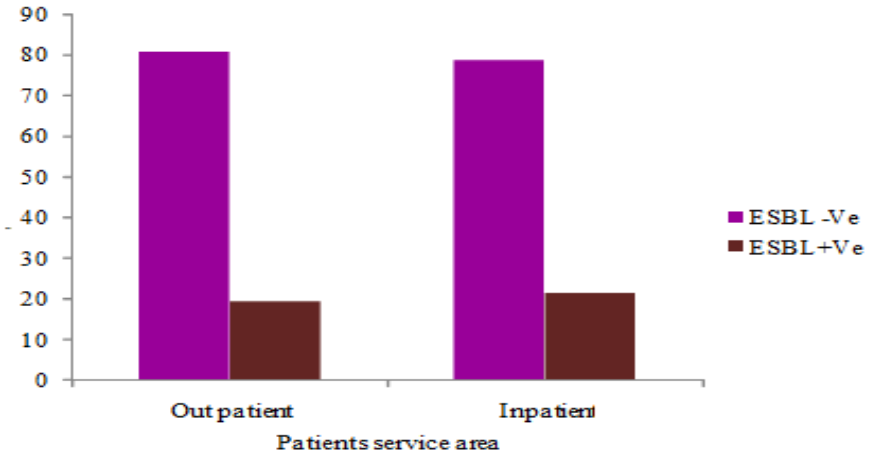

Fig. 4: Comparison of ESBL producers between patients service area

ESBL production in the study side

The proportion of ESBL production in relations to study states as illustrated in a map showing the positives in the study states is presented in Fig. 5. It showed that Delta State had the highest 39(30.5%) while Rivers state had the least 14(9.4%). The difference among states was statistically significant (p-value <0.0001)

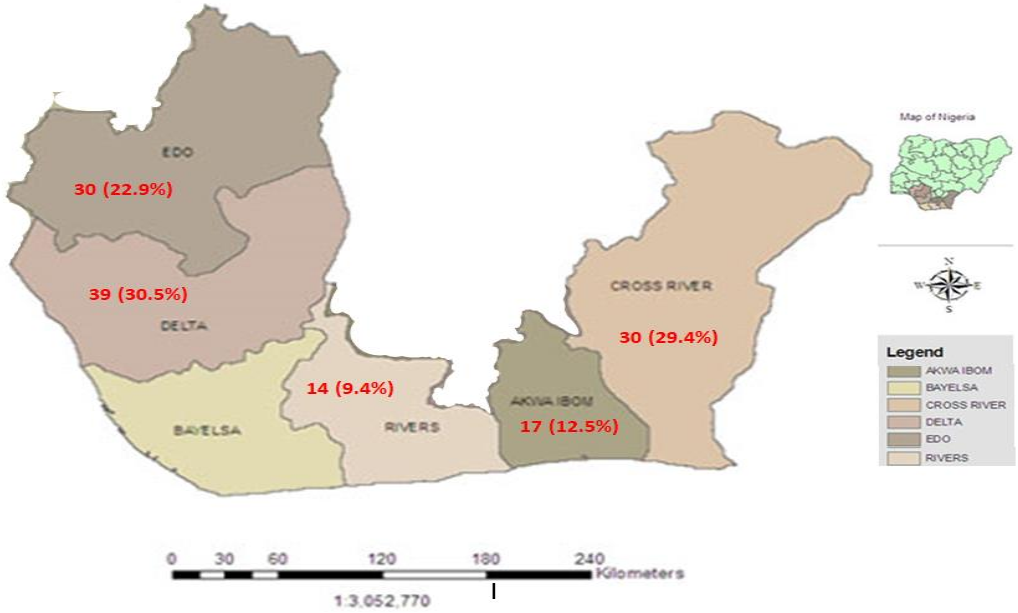

Fig. 5: Map of study area showing ESBL positives

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 2, pp: (27-39), Month: April - June 2019, Available at: www.researchpublish.com

Recovery of potential ESBL producer (phenotypes) and producers from clinical specimens from Akwa Ibom State

From the urine specimen, 7 out of 24 were putative producers while 2 were the phenotypes of the 11 pus samples obtained, 1 was putative producer. A total of 10 phenotypes were obtained while the putative producers were 17. The result is as presented in Table 4.

Recovery potential ESBL producers from clinical samples from Cross River State

A total of 19 phenotypes were obtained. The highest was obtained from urine (10) and the least (3) was from urinogenital swab. The putative producers were 31. This results is presented in Table 5.

Recovery of ESBL phenotypes and producers from clinical samples from Delta State

A total of 17 phenotypes were obtained from Delta State. The highest from urine was 4, stool, USW and pus each had 3, while the least were obtained from sputum and wound swab, 2 in each case. The putative producers were 39. This result is presented in Table 6. Plate 1 shows the detection of ESBL production using DDST method.

Recovery of ESBL phenotypes and producers from clinical samples from Edo state

A total of 22 phenotypes were obtained from Edo State. Of these, USW gave 5 and was the highest while the least was sputum with 2 phenotypes. Putative producers were 30. This result is presented in Table 7.

Recovery of ESBL phenotypes and producers from clinical samples from River State

A total of 7 phenotypes were obtained. The highest from urine was 5 and the least from USW was 2. The putative producers were 14. This result is presented in Table 8

Detection of ESBL producers using CIVA medium

This result is presented in Table 9. It shows the growth characteristics of the isolates on the medium *E. coli* produced large flat colourless colonies while *K. pneumonia*e produced yellow and extremely muciod colonies.

TABLE 4: ESBL phenotypes and producers from Akwa Ibom state

Sample type	No. of isolates	Putative producers	Phenotypes
Urine	24	7	4
Stool	40	2	3
Urinogenital swab	27	2	4
Pus	11	1	2
Sputum	22	3	4
Wound swab	12	2	5
Total	136	17	22

TABLE 5: ESBL phenotypes and producers from Cross River state

Sample type	No. of isolates	Putative producers	Phenotypes
Urine	53	15	10
High vaginal swab	18	4	6
Urinogenital swab	21	12	3
Blood	10	Nil	0
Total	102	31	19

TABLE 6: ESBL phenotypes and producers from Delta state

Sample type	No. of isolates	Putative producers	Phenotypes
Urine	38	12	4
Stool	25	9	3
Urinogenital swab	24	5	3
Pus	13	5	3
Sputum	13	3	2
Wound swab	15	5	2
Total	128	39	17

TABLE 7: ESBL phenotypes and producers from Edo state

Sample type	No. of isolates	Putative producers	Phenotypes
Urine	37	11	3
Stool	30	7	3
Urinogenital swab	22	10	5
Pus	12	1	4
Sputum	13	0 (Nil)	2
Wound swab	17	1	3
Total	131	30	20

TABLE 8: ESBL phenotypes and producers from Rivers state

Sample type	No. of isolates	Putative producers	Phenotypes
Urine	102	12	5
Urinogenital swab	20	2	2
Blood	27	0 (Nil)	0
Total	149	14	7

TABLE 9: Growth characteristics of ESBL producers on CIVA medium

Organisms	Characteristics
Klebsiella pneumoniae	Yellow, extremely mucoid colonies
Escherichia coli	Large, flat, colourless colonies

Statistical analysis of data

The objective of the analysis was to study the association of ESBL with isolate type *K. pneumoniae* and *E.coli*, patient service area or status, state from which the sample came and source of the sample considered. Binary logistic regression was used to study both crude or univariate and adjusted multivariable association of ESBL with the proposed predictor. Table 10, 11 and 12 present the result of data analysis. A total of 646 samples from five states were tested for ESBL. It

was observed that 130/646(20.1%) were found to be positive for ESBL. Positive response was higher for *E.coli* isolate (23.8%) samples from source USW (27.8%) and Delta State (30.5%). In-patients showed slightly proportion of positive response (21%) than out- patients. All blood samples in our study did not yield and ESBL producer.

Results of fitting logistic regression on each of the proposed variables indicate significant association of ESBL with *E.coli* (p-value <0.01) and states (p-value 0.0001) based on likelihood ratio test. On the other hand, there was no evidence of such association for source (p-value = 0.60) and in/out-patient status (p-value=0.50). In multivariable analysis as in the univariate analysis, significant association was observed in isolate types (p-value <0.01) and state (p-value<0.0001) with ESBL production in the final multivariate logistic regression model.

Isolates of *E.coli* were 1.83(95 CI 1.20-2.79) times as likely to be ESBL positive as *K.pneumoniae* samples. Compared to Akwa Ibom, Edo, Delta, Rivers and Cross River States were 2.02(95% CI 1.05-3.89), 3.2(95% CI 1.69-606) and 3.14(95% CI 1.61-6.14) times a likely to have ESBL positive result. None of the pair wise interactions between in/out-patient status, source, isolate types and state were found to be significant. For genotype analysis, an attempt to associate each of the genotype with in/out-patient status and isolate using fishers exact test did not show any material relationship. Tables showing crude associations of ESBL with proposed explanatory variables and association of ESBL production with isolates and state.

TABLE 10: Crude association of ESBL with proposed explanatory variables

Variable	Category/unit	Odds ratio	95% Cl	P-value
Isolate	K. pneumoniae	Reference		
	E. coli	1.76	(1.17,2.65)	0.007
In patient	No	Reference		
	Yes	1.14	(0.78, 1.68)	0.50
State	Akwa Ibom	Reference		
	Edo	2.08	(1.08, 3.99)	0.028
	Delta	3.07	(1.63, .77)	0.001
	Rivers	0.73	(0.34, 1.54)	0.402
	Cross River	2.92	(1.50, 5.66)	0.002
Source *	Blood	-		
	HVS	Reference		
	Pus	0.70	(0.17, 2.88)	0.62
	Sputum	0.70	(0.18, 2.69)	0.60
	Stool	0.82	(0. 24, 2.78)	0.75
	USW	1.31	(0.40, 4.28)	0.66
	Urine	0.99	(0.31, 3.13)	0.99
	WS	0.66	(0.17, 2.62)	0.56

*Blood samples not included

TABLE 11: Association of ESBL production with isolates and state

Variable	Category/unit	Odds ratio	95% Cl	P-value
Isolate	K. pneumoniae	Reference		
	E. coli	1.83	(1.20, 2.79)	0.005
State	Akwa Ibom	Reference		
	Edo	2.02	(1.05, 3.89)	0.035
	Delta	3.20	(1.69, 6.06)	0.001
	Rivers	0.76	(0.36, 1.61)	0.47
	Cross River	3.14	(1.61, 6.14)	0.001

Annex-Tables

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 2, pp: (27-39), Month: April - June 2019, Available at: www.researchpublish.com

Tab State Isolate

	Isolate		
State	K. pneumonia	E. coli	Total
Akwa Ibom	53	83	136
Cross River	50	52	102
Delta	56	72	128
Edo	43	88	131
Rivers	70	79	149
Total	272	374	646

Tab source state

	State					
Source	Akwa Cross River		Delta	Edo	Rivers	Total
Blood	0	10	0	0	27	37
HVS	0	18	0	0	0	18
Pus	11	0	13	12	0	36
Sputum	22	0	13	13	0	48
Stool	40	0	25	30	0	95
USW	27	21	24	22	20	114
Urine	24	53	38	37	102	254
WS	12	0	15	17	0	44
Total	136	102	128	131	149	646

Tab source

Source	Freq.	Percent	Cum.
Blood	37	5.73	5.73
HVS	18	2.79	8.51
Pus	36	5.57	14.09
Sputum	48	7.43	21.52
Stool	95	14.71	36.22
USW	114	17.65	53.87
Urine	254	39.32	93.19
WS	44	6.81	100.00
Total	646	100.00	

Tab state inpatient

State	Outpatient	Inpatient	Total
Akwa Ibom	57	79	136
Cross River	54	48	102
Delta	68	60	128
Edo	63	68	131
Rivers	93	56	149
Total	335	311	646

ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 7, Issue 2, pp: (27-39), Month: April - June 2019, Available at: www.researchpublish.com

TABLE 12: Table entries are cell frequencies missing categories ignored

	esbl and inpatient			
	0		1	
State	Outpatient	Inpatient	Outpatient	Inpatient
Akwa Ibom	48	71	9	8
Cross River	38	34	16	14
Delta	51	38	17	22
Edo	50	51	13	17
Rivers	84	51	9	5

4. DISCUSSION

In this study, specimens were obtained from body fluids such as blood, hvs, pus, sputum, stool and urinogential swab, urine and wound swab. This is similar to the studies carried out by other workers in this area of study. The highest number of ESBL producers was obtained from urine, 56(254) followed by urinogential swab. This was closely followed by stool samples in which 18(95) were positive, sputum 8(48), pus 6(36) and HVS 6(36). The blood samples used in this study, however, did not yield any ESBL producers. The reason may be connected with the sterile nature of blood.

Results from other workers showed that urine yields high numbers of ESBL producers. This is believed to be due to the nature of the urine source. The work of Fang *et al.*, (2004) and Kiratisin *et al.*, (2008) also confirmed that the highest numbers were obtained from urine. Some studies have revealed that ESBL production ability may be influenced by several factors; which may include n nature and type of infection, patient status (i.e in-patient and out-patient) and duration of hospitalization. As observed in this study, more-out-patient samples were obtained than in other studies where more in patient samples were obtained. The reason is not very clear as to why we had more out-patient samples; however, it may be connected with the fact that people in this part of the world are not favorably disposed to prolonged hospital study. Even the most serious cases are taken home and the care givers prefer to bring the patients back to the hospital for periodical check-up often times against medical advice. Of all the 646 specimens analyzed in this study for ESBL production, 130 (20.1%) was positive for ESBL production. This is high compared to other studies. In the work of Paterson and Bonomo (2005), it was discovered that out of 10.733 *K.pneumoniae* isolates, 5.7% were positive for ESBL production. In out-patient areas, 1.8% of `12,059 *K. pneumonia* isolates were positive for ESBL and 0.4% of 71,488 *E.coli* isolates were positive. The serious problems of antibiotic abuse and poor drug policy in our study area probably explains the high percentage of ESBL producers obtained in our study isolates.

The use of CIVA medium for quick identification of ESBL producers earlier reported by Carlos and Sliva (2000) was also confirmed in this study. The use of this medium in our study yielded results in less than 24hours as opposed to other methods of 48 hours. This is of great importance as it leads to a reduction in the time the patient will have to wait before obtaining proper diagnosis. This reduction in time will also encourage routine ESBL screening and detection.

The ESBL producers were obtained from different states. In some states, few sample types were obtained. Rivers state had blood, urine and urinogenital swab while Cross River State had blood, high vaginal swab, urine and wound swab. From all the state based on simple percentage, the highest yield came from Delta State (30.5%) the lowest was from Rivers State (9.4%). This is likely due to the small variety of samples obtained from the state in this study. Among Edo, Delta and Akwa Ibom, the state with the lowest percentage occurrence of ESBL was Akwa Ibom with 12.5%. This line of investigation will be followed in subsequent studies to clearly elucidate the reason for the wide margins in percentage prevalence despite the closeness in number of samples obtained in each of the study sites. Using univariate analysis, results of fitting logistic regression on each variable indicate significant association of ESBL production with isolate type ($p<0.01$) and states ($P<0.001$) based on likelihood ratio test. There was no evidence of such association ($p=0.60$) for source and patient location ($p=0.50$). Using multivariable logistic regression model, association was observed in isolate type and state of origin. As in univeriate analysis, there was no significant association between source and patient location. Furthermore, isolates from *E.coli* samples were 1.83 (95% CI:1.20-2.79) times as likely to be ESBL positive as those of *K. pneumoniae*. Compared to Akwa Ibom, other states such as Edo, Delta and Cross River were 2.02(95%

CI:1.50-3.89), 3.2(9.5% CI:169-6.06) and 3.14(95% CI:1.61-6.14) times respectively as likely to have ESBL positive result. Pairwise interactions between in/out-patient, sources isolates and state were not found to be significant (p>0.05). Since blood samples did not give any isolate they were excluded from the analysis.

The work of Muzaheed *et al.,* (2008) confirmed that the difference between isolates from out-patient and in-patient was not very wide. It was observed that in-patient gave resistance of 75%, while out-patient gave 97%. Some studies have earlier revealed that the ESBL problem mainly resides in intensive care units and they serve as a reservoir of infections. The prolonged hospital stay invariably encourages antibiotics resistance due to usage of the cephalosporin. This may due to the fact that the cephalosporin are among the most frequently prescribed antibiotics. Our poor antibiotic policy has also promoted the development of resistance. The high rate of resistance in out-patient cases as observed in this study, further affirms this fact.

5. CONCLUSION

The study has revealed the prevalence of ESBL-producing organisms in the study area. This is believed to be the rationale among other factors, the problem of antibiotic resistance experienced in this part of the world. The impact of this serious public health threat cannot be glossed over. Concerted efforts must be made by researchers to carry out further research with view to completely understand the mode of action of these enzymes and how they can be effectively controlled. However, Government on its part must implement the findings of the various research efforts. The importance of this cannot be overemphasized because tomorrow's problem is actually a product of today's inactions.

REFERENCES

[1] Chidima, C., Peter, M., Ujam, T. & Ejikeugwu, C. (2018). Incidence of community acquired ESBL- producing bacteria among asymptomatic University students in Amambra State, Nigeria. *European Journal of Biological Research, 8*(3), 138-147.

[2] Raji, A., Jamal, W., Ojemhen, O. & Rotimi, O. (2013). Point survillienne of antibiotic resistance in *Enterobacteriaceae* isolates from patients in a Lagos Teaching Hospital, Nigeria. *Journal of Infection and Public Health, 6*, 431-437.

[3] Lee, H., Bae, K. & Lee, S. (2011). New definitions of extended spectrum β-lactamase conferring worldwide emerging antibiotic resistance. *Medical Resource and Review, 32*,216-232.

[4] Agrawal, P., Ghosh, A., Kumar, S. & Kapil, K. (2008). Prevalence of extended spectrum β-Lactamases among *E.coli* and *K.pneumoniae* isolates in tertiary care hospital. *Indian Journal of Pathology and Microbiology, 51*, 139-142.

[5] Frieamann, R., Raveh, D., Zartzer, E. & Yinnon, M. (2009). Prospective evaluation of colonization with extended spectrum β-Lactamase (ESBL) producing *Enterobacteriaceae* among patients at hospital admission and of subsequent colonization with ESBL producing *Enterobacteriaceae* among patients during hospitalization. *Infection Control Hospital Epidemiology, 30*,534-542.

[6] Iroha, R., Okoye, E., Osigwe, A. & Nwakaeze, E. (2017). Isolation, phenoptypics characterization and prevalence of ESBL producing *Echerichia coli* and *Kebsiella* species from orthopedic Hospital Enugu (NOHE), South-East Nigeria. *Journal of Pharma Care Health Systems, 4*(4), 72-84.

[7] Ejikeugwu, C., Ikegbunam, M., Ugwu, C. & Iroha, I. (2013). Phenoptypic detection of *Klebsiella pneumoniae* strains producing extended spectrum β-Lactamase (ESBL) enzymes. *Scholars Academic Journal of Bioscience, 1*(1), 20-23.

[8] Paterson, L. & Bonomo, A. (2005). Extended spectrum beta lactamase: A clinical update. *Clinical Microbiology Reviews, 18*(4), 657-686.

[9] Jemima, A. & Verghese, S. (2008). Multiplex PCR for bla (CTX-M) and bla (SHV) in the extended spectrum beta lactamase (ESBL) producing gram negative isolates. *Indian Journal of Medical Research, 128*(3), 313-317.

[10] Marty, L. & Jarlier, V. (1998). Surveillance of multi- resistant bacteria; justification of the role of the laboratory, indicators and recent French data. *Pathological Biology, 46*, 18-27.

[11] Albertini, T., Benoit, C. & Jarlier, V. (2002). Surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) and Enterobacteriaceae producing extended spectrum beta lactamase (ESBLs) in Northern France: A give year multicentre study. *Journal of Hospital Infections*, *52*, 107-113.

[12] Health Protection Agency (HPA) (2006). Laboratory detection and reporting of bacteria with extended spectrum beta Lactamases: National, standard method QSOP 51, Issue 2. Retrieved May 1, 2011 from: http//www.hpa-standardmethods.org.uk/pdfsops.asp.

[13] Fang, H., Lundberg, C. & Struwe, J. (2004). Molecular epidemiological analysis of *E.coli* isolates producing extended spectrum beta lactamases for identification of nosocomial outbreaks in Stockholm, *Sweeden. Journal of Clinical Microbiology, 42*(12), 5917-5920.

[14] Carlos, P. & Silva, M. (2000). Elaboration and evaluation of a new screening medium for detection and presumptive identification of extended spectrum beta lactamases producing organisms. *Brazillian Journal of Microbiology*, *31*, 271-274.

[15] Muzaheed, Y., Dul, M. & Paterson, l. (2008). High prevalence of CTX-M-15 producing *Klebsiella pneumoniae* among in-patients with urinary tract infection in Southern India. *Journal of Antimicrobial Chemotherapy*, *61*(6), 1393-1394.

[16] Kiratisin, P., Apisarhthanardk, A. & Mundy, L. (2007). The emergency of novel ceftazidime CTX-M extended spectrum beta lactamase, CTX-M-55 in both community onset and hospital acquired infections in Thailand. *Diagnostic Microbiology and Infectious Diseases*, *58*, 349-355.