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Occurrence of Extended Spectrum Beta Lactamase Producings Resistant *Escherichia coli* and *Klebsiella pneumoniae* in Clinical Isolates and Associated Risk Factors

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Abstract: A total of 420 clinical isolates of *Escherichia coli* (235) and *Klebsiella pneumoniae* (185) were collected from 2 tertiary hospitals in Enugu to determine the presence of extended spectrum β-lactamase enzymes and its associated risk factors. These isolates were obtained from different sources namely, urinary tract catheters (106), intensive care unit (bacteremia) (52), umbilical catheter (41), abdominal surgery site (91), antibiotic exposure (wound) (57) and arterial catheter (73). They were characterized using standard microbiological techniques. Production of ESBL was determined using the Double Disc Synergy Test (DDST) method and susceptibility of ESBL positive isolates to Gram negative antibiotic discs was carried out using disc diffusion technique. The result of this study showed that out of 420 clinical isolates of bacteria tested for ESBL enzyme production, 105 (44.6%) isolates of *Escherichia coli* comprising of 62 (41.3%) from University of Nigeria Teaching Hospital Enugu (UNTH) and 43 (57.8%) from Enugu State University teaching hospital Enugu (ESUTH) expresses ESBL enzymes. Also, 62 (33.5%) clinical isolates of *K. pneumoniae* comprising of 24 (20%) from UNTH and 38 (61.2%) from ESUTH yielded ESBL enzymes. The highest occurrence of ESBL enzyme were from umbilical catheter 39 (36.7%) where, 28 (26.4%) were from *E. coli* and 11 (10.3%) from *K. pneumoniae*, while the least were from the antibiotic exposure patients (bacteremia) 20 (35.0%), where 15 (26.3%) were from *E. coli* and 5 (8.7%) from *K. pneumoniae*. The susceptibility pattern of ESBL positive strains showed that they were multi-drug resistant except in ESUTH were these organisms were sensitive to ceftriaxone and ofloxacin (57.1%). The study suggests, the routine check for clinical isolates of ESBL production with particular emphasis on patients attending the ICU and those in invasive treatment is recommended.

Key words: Enzymes, clinical isolates, risk factors, occurrence, agar, antibiotic disc

INTRODUCTION

Emerging resistance to antimicrobial drugs increases morbidity and mortality by hampering the provision of effective chemotherapy and makes treatment more costly (Cohen, 1992; Tenover and Hugles, 1996; Acar, 1997). During the past 2 decades, antibiotic resistant mutant strains that produce Extended Spectrum β-lactamase enzymes (ESβLs) have emerged among the Enterobactericeae, predominantly *Escherichia coli* and *Klebsiella pneumoniae*. The emergence of such strains has important clinical and therapeutic implications and they are often derived from TEM and SHV enzymes by mutations (Bush *et al*., 1995). The 1st plasmid-transferable β-lactamase was discovered and named TEM-1 after temoniera, the Greek patient that harboured the *E. coli* isolates from which the enzymes was obtained (Knothe *et al*., 1983). ESBLs have spread widely and constitute a major cause of nosocomial infections associated with high mortality rates, particularly in serious infections such as septicemia (Kim *et al*., 2002). In Africa, ESBLs have been reported in Egypt (Shannon *et al*., 1990), Tunisia, Senegal (Sow *et al*., 1997), Nigeria (Olusegun *et al*., 2005), South Africa (Essack *et al*., 2001), Kenya (Kariuki *et al*., 2001) etc. from different clinical sources. However, information concerning ESBL production and colonization associated with risk factors are lacking in Nigeria. In the present study, we investigated the occurrence of ESBL production in *E. coli* and *K. pneumonia* and risk factors associated with ESBL production and colonization in 2 tertiary hospitals in Enugu, Nigeria.

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MATERIALS AND METHODS

Collection of clinical isolates: A total of 420 clinical bacteria isolate comprising of *Escherichia coli* (235) and *klebsiella pneumoniae* (185) were collected from 2 tertiary hospitals namely University of Nigeria Teaching Hospitals (UNTH) Ituku Ozalla, Enugu and Enugu State University Teaching Hospital Enugu (ESUTH). One hundred and six clinical isolates were collected from urinary tract catheter, 52 from ICU (Intensive care unit (bacteremia), 41 from umbilical catheter, 57 from antibiotic exposure (wound), 91 from abdominal surgery site and 73 from arterial catheters. All the clinical isolates were identified and characterized using standard microbiological identification techniques (Chessbrough, 2000).

Preliminary studies

Determination of susceptibility patterns of the bacterial isolates: Thirty eight gram of molten Mueller Hinton agar was prepared by dissolving in 1000 mL of distilled water and heated over a bunsen burner flame for 10 min. A 20 mL volume each of the agar was dispensed into Bijou bottles and autoclaved at 121°C for 15 min. They were poured into a Petri dish and incubated for 18-24 h at 37°C to ascertain sterility. A 0.5 MacFarland equivalent standard of each of the test organism were inoculated in Mueller Hinton agar plate using sterile inoculation loop. These were allowed for 30 min to pre diffuse into the agar. The antibiotic discs (ceftazidime -30 μg, cefepime -30 μg and cefotaxime -30 μg, ceftriaxone 30 μg, Oxoid, UK) were then placed on the surface of the agar plates. The plates were incubated for 18-24 h at 37°C and the radial zones of inhibition were taken according to CLSI criteria. All the isolates that showed resistance to any of the test antibiotics above were subjected to double disc synergy test for phenotypic characterization and identification of ESBL.

Double Disc Synergy Test (DDST): Ninteen gram of molten Mueller Hinton agar was prepared by dissolving in 500 mL of distilled water and heated over a bunsen burner flame for 10 min. A 20 mL volume each of the agar was dispensed into Bijou bottles and autoclaved at 121°C for 15 min. They were poured into Petri dishes after autoclaving and incubated for 18-24 h at 37°C to ascertain sterility. A 20 mL volume each of molten Mueller Hinton agar was prepared and dispensed aseptically into Petri dishes. A suspension of each of the test organism equivalent to 0.5 MacFarland equivalents standards was inoculated on the surface of each of the molten Mueller Hinton agar plates using sterile inoculation loop. These were allowed for 30 min to pre diffuse into the agar. A combination disc (Amoxicillin -20 μg and clavulanic acid -10 μg, Oxoid, UK) was placed at the centre of the inoculated plate and a single β-lactam antibiotic disc (ceftazidime -30 μg and cefotaxime -30 μg, Oxoid, UK) were each placed 15 mm apart center to center on both sides of the plates. The plates were then incubated for 18-24 h at 37°C. The radial zones of inhibition were taken according to CLSI criteria. All isolates whose radial zone of inhibition increased from 5 mm and above in the presence of the combination disc (Amoxicillin -20 μg and clavulanic acid -10 μg,) as against its zone of inhibition when tested alone, was suspected to express ESBLs enzymes phenotypically.

Determination of the resistance patterns of esbl positive isolates to antibiotics discs: Ninteen gram of molten Mueller Hinton agar was prepared by dissolving in 500 mL of distilled water and heated over a Bunsen burner flame. A 20 mL volume each of the agar was dispensed into Bijou bottles and autoclaved at 121°C for 15 min. They were poured into Petri discs and incubated for 18-24 h at 37°C to ascertain sterility. A 0.5 MacFarland equivalent standard of each of the test organism were inoculated on the surface of molten Mueller Hinton agar plate using sterile inoculation loop. These were allowed for 30 min to pre diffuse into the agar. The following antibiotic discs (Amikacin 30 μg Ampicillin 10 μg, sulphamethoxazole/ trimethoprim 25 μg, ciprofloxacin 5 μg, ofloxacin 5 μg and Gentamicin 10 μg, Oxoid, UK) were then placed on the surface of the agar plates. The plates were incubated for 18-24 h at 37°C and the radial zones of inhibition was taken according to CLSI criteria.

RESULTS

The results of this study are presented in Table 1-4. Table 1 shows the percentage of ESBL occurrence from different isolates of *E. coli* and *K. pneumoniae* from the 2 hospitals. The result revealed that ESBL occurrence from *E. coli* and *K. pneumoniae* was higher in ESUTH than UNTH, while *E. coli* has the greatest frequency of occurrence from the 2 hospitals. Generally, the percentage of occurrence was 105 (44. 6%) in *E. coli* and 62 (33.5%) in *K. pneumoniae* (Table 1). Table 2 shows the percentage of ESBL occurrence from different risk factors. The highest occurrence of ESBL from *E. coli* and *K. pneumoniae* were from urinary tract catheter followed by abdominal surgery site, arterial catheter, antibiotic exposure (wound), ICU (bactaeremia) and umbilical catheter. The susceptibility patterns of ESBL positive strains shows that these organisms were multi-drug resistant (Table 3 and 4).

Table 1: Percentage of ESBL occurrence from different clinical isolates of *E. coli* and *K. pneumoniae* from ESUTH and UNTH Enugu

No. *E. coli* isolates from each hospital	No. *K. pneumoniae* isolates from each hospital	Percentage of ESBL occurrence from				Total percentage of ESBL occurrence	
		E. coli in UNTH	*K. pneumoniae* from UNTH	*E. coli* in ESUTH	*K. pneumoniae* in ESUTH	*E. coli* from both hospitals	*K. pneumoniae* from both hospitals
ESUTH = 83	ESUTH = 62	62 (41.3%)	24 (20%)	43 (51.8%)	38 (61.2%)	105 (44.6%)	62 (33. 5%)
UNTH = 150	UNTH = 125	-	-	-	-	-	-
Total = 233	Total = 187	-	-	-	-	-	-

Table 2: Percentage of ESBL occurrence from different risk factors

No. isolates from each specimen	Percentage of ESBL		
	Occurrence from both isolates	Positive *E.coli* from each specimen	Positive *K. pneumoniae* from each specimen
Urinary tract catheter-106	39 (36.7)	28 (26.4)	11 (10.3)
Abdominal surgery site-91	34 (37.3)	21 (23.0)	13 (14.2)
Arterial catheter-73	30 (41.0)	18 (24.6)	12 (16.4)
Antibiotic exposure (wound)-57	20 (35.0)	15 (26.3)	05 (08.7)
ICU (bacteremia)-52	20 (38.4)	12 (23.0)	08 (15.3)
Umbilical catheter-41	20 (48.7)	10 (24.3)	10 (24.3)

Table 3: Percentage resistance patterns of gram-negative antibiotic discs against clinical isolates of EBSL positive *E. coli* and *K. pneumoniae* from UNTH Enugu

No. isolates	Susceptibility patterns	Percentage zone of inhibition IZD (MM)					
		CN	CRO	SXT	AMP	CIP	OFX
Escherichia coli	Susceptibility	10.2	15.3	13.6	8.5	12.7	12.3
	Intermediate	0.8	1.2	0.0	0.0	0.4	0.0
	Resistant	80.0	74.5	76.4	93.5	77.0	87.7
K. pneumoniae (24)	Susceptibility	19.5	16.1	4.2	22.1	18.2	15.7
	Intermediate	6.8	13.1	5.1	6.8	8.5	13.1
	Resistant	74.7	83.8	90.7	61.1	74.3	72.2

CN: Gentamicin; CRO: Ceftriaxone; SXT: Sulphomethoxazole/Trimethoprim; AMP: Ampicillin; CIP: Ciprofloxacin; OFX: Ofloxacin; UNTH: University of Nigeria, Teaching Hospital, Enugu

Table 4: Percentage resistance pattern of gram negative antibiotic disc against EBSL positive clinical isolates of *E. coli* and *K. pneumoniae* from ESUTH, Enugu

No of isolates	Susceptibility patterns	Percentage zone of inhibition IZD (MM)					
		CN	CRO	SXT	AMP	CIP	OFX
E.coli (43)	Susceptibility	3.17	57.10	4.7	30.1	39.7	57.1
	Intermediate	9.50	6.00	6.3	14.2	11.1	3.1
	Resistant	87.40	36.70	91.0	65.7	50.2	39.8
Klebsiella pneumoniae (38)	Susceptibility	30.40	6.52	36.9	36.9	30.4	47.8
	Intermediate	4.30	19.60	23.9	23.9	4.3	6.5
	Resistant	65.20	73.90	39.1	56.5	65.2	45.6

CN: Gentamicin; CRO: Ceftriaxone; SXT: Sulphomethoxazole/Trimethoprim; AMP: Ampicillin; CIP: Ciprofloxacin; OFX: Ofloxacin; ESUTH: Enugu State University of Science and Technology, Teaching Hospital

Isolates from UNTH were all resistant to the antibiotics while those from ESUTH were relatively sensitive to ceftriaxone (57.1%) and ofloxacin (57.1%).

DISCUSSION

Extended Spectrum β-lactamases (EsβLs) refers to β-lactamase enzymes produced by Gram negative organisms that confer resistance against broad spectrum β-lactam antibiotics such as cefotaxime, ceftriaxone, ceftaxidime and aztreonam, which normally have activity against Gram-negative bacilli (Moosden, 1997). Reports of ESBL-producing strains have been appearing for about 2 decades now. However, laboratories in our environment have been slow to embrace ESBL detection methods, in part because the clinical importance of identifying such strains remains elusive and perhaps because of dearth of information on this new β-lactamase enzyme group, now evolving worldwide. However, there are now increasing clinical evidence that shows the importance of detecting these strains in our environment.

The result of this study demonstrated that ESBL production was detected in 39.76% (167 of 420) of the isolates comprising of 105 (44.6%) *E. coli* and 62 (33.5%) *K. pneumoniae*. In Europe, the prevalence of ESBL production among isolates of Enterobacteriaceae varied from country to country, <1% of *E. coli* in the Netherlands, while in France as many as 40% of *K. pneumoniae* were found to be positive for ESBL enzyme (Bradford, 2001). The fact that the present

research recorded prevalence rate of 44.6% (*E. coli*) and 33.5% (*K. pneumonia*) clearly indicates high prevalence of ESBL producing organisms in eastern Nigeria. This underlines, the urgent need for immediate intervention especially in the area of enlightening the clinicians in eastern Nigeria on the necessity for routine ESBL screening to avert future escalation.

The highest occurrence of ESBL producing *Klebsiella pneumoniae* and *E.coli* in the present study were from catheter 39 (36.7%) and the least was from antibiotic exposure (wound) 20 (35.0%) (Table 2). ESBL enzymes are known to be isolated most frequently from intensive care unit especially from patients that are receiving invasive treatments. Multi resistance infections in ICU patients are a major cause of morbidity, increased length of ICU admission and mortality. Several studies and surveillance programs indicated the highest prevalence of nosocomial infections including methicillin resistant *Staphylococcus aureus* multi-resistant *Pseudomonas aeruginosa Acinotobacter* sp. and ESBL producing Enterbactericeae (*Klebsiella pneumoniae* and *Escherichia coli*) among ICU patients. Rates of ESBL infection by *K. pneumoniae* in the ICU is high in Nigeria (Olusegun *et al*., 2005). A multi-centre, prospective, observational study (Paterson *et al*., 2000) reported that 15% of 216 consecutive cases of *K. pneumoniae* bacteraemia were due to ESBL producing organisms. Eightt four percent of ESBL *K. pneumoniae* was hospital acquired in the ICU. Sources of ESBL *K. pneumoniae* included intravascular catheter (34%), pneumonia (28%) and intra abdominal infection (19%) (Paterson *et al*., 2000). ESBL producing bacteria cause's infections of the urinary tract, bactaeremia, respiratory tract infections, catheter or device related and studies have shown that constant use of invasive treatment measures and high volume of antibiotics are potential risk factor for ESBL colonization (Shiappa *et al*., 1996). The high occurrence of ESBL observed in our study are thus, not out of place since, the sites of infections and their treatment applications such as device related, surgical related, antibiotic exposure and prolong stay in hospital ICU, are potential risk factor for ESBL infection and colonization. Consequently, patients placed on these devices as a treatment measure are at a very high risk of acquiring ESBL enzyme infections and colonization.

Of the 2 hospitals, ESUTH had the greater number of ESBL occurrence from *E. coli* and *K. pneumoniae* isolates (55.9%) than UNTH (31.0%). In Nigeria, β-lactam antibiotics are the most frequently prescribed antibiotics against aerobic Gram negative bacilli infections and selective pressure exerted by the extensive use of these β-lactam drugs especially, in treating some life threatening infections most likely result in strains developing ESBL enzymes.

CONCLUSION

Our present study demonstrated a high occurrence of ESBL enzymes in clinical isolates of *E. coli* and *K. pneumoniae* and multi drug resistance against various kinds of antibiotics. We therefore suggest, that laboratory based surveillance should be conducted on a continuous basis to detect ESBL producing Gram negative bacteria among patients, especially those at the ICU and oncology wards and those undergoing device related treatments such as arterial catheters, central venous catheters, urinary tract cathethers, gastrostomy or jejunostomy tube and umbilical catheter. Patients infected with resistant Gram negative bacteria should be separated from other patients so as to reduce the risk of transmission of the resistant organisms and the indiscriminate use of antibiotics should be discontinued especially with the cephalosporins.

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