Original Article

EXTENDED SPECTRUM BETA - LACTAMASE (EBSL) IN *E.COLI* ISOLATED FROM A TERTIARY HOSPITAL IN ENUGU STATE, NIGERIA

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ABSTRACT

Objective: The frequency of extended spectrum ß - lactamase (ESBL) producing *E. coli* in a tertiary hospital in Enugu, Enugu State, Nigeria was studied.

Methodology: Clinical specimens were collected from patients attending the Medical Microbiology laboratory unit of the University of Nigeria Teaching Hospital (UNTH), Enugu. Isolates of *E. coli* were obtained from various specimen types namely, urine (19), blood (25), wound (52), and sputum (32). Susceptibility studies were carried out using agar diffusion method by Kirby and Bauer, while ESBL detection on these isolates was carried out on Mueller Hinton agar using the double disc diffusion method.

Results: The frequency of ESBL producing *E. coli* among the clinical isolates was 11.4% (15) and ESBL producing organisms were isolated more frequently from blood (6) followed by wound (5), urine (3) and sputum (1).

Conclusions: Urgent measures to avert the further spread of ESBL producing organisms in Enugu, Enugu State, Nigeria is a compelling necessity.

KEYWORDS: ESBL, Isolate, Resistance, Sensitivity, Antibiotic discs, Frequency, Plasmid.

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INTRODUCTION

Escherichia coli is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infections (UTI).[1] These infections can sometimes progress to cause serious infections such as blood poisoning disease which can be life threatening. ESBL producing organisms are very difficult to eliminate because of their multi–drug resistance to various classes of antibiotics such as cephalosporins, monobactam, carbapenems, ciprofloxacin, erythromycin and ß-lactam ß-lactamase inhibitor combination.[2] The most prevalent mechanisms of bacteria resistance among gram negatives are the production of ß-lactamase enzymes, alteration in the penicillin binding proteins, outer membrane

permeability and combination of multiple mechanism of resistance.[2] Extended spectrum â – lactamase (ESBL) enzyme produced mostly by the Enterobacteriaceae have emerged as a serious nosocomial pathogen globally.[3] They are chromosomally or plasmid mediated and occur as a result of a spontaneous mutation that takes place in the serine active site of the old beta lactamase enzyme adding 4-6 new amino acids that have extended their hydrolytic substrate.[4] These enzymes comprise diversity and are included in the 2nd group of Bush classification.[5]

ESBL was first isolated from a gram – negative organism (*Klebsiella pneumoniae*) in 1983[6]. Resistance to expanded – spectrum cephalosporin's and other antimicrobial agents among clinical isolates of gram – negative bacteria is on the rise world wide and this can be attributed to the indiscriminate use of these drugs.[7,8] Recent studies in Taiwan Europe and in the USA have demonstrated a high frequency of antimicrobial resistant bacteria and a trend of increasing resistance under continued antibiotic selective pressure.[9] Our study was designed to investigate the frequency of ESBL in the University of Nigeria Teaching Hospital (UNTH) Enugu, bearing in mind that no such studies have been carried out in this part of our country.

METHODOLOGY

Test Isolates: Clinical specimen namely urine (19), blood (25), wound (52) and sputum (32) were collected from patients attending the Medical Microbiology laboratory unit of the University of Nigeria Teaching Hospital (UNTH) Enugu, between February and June 2007. These isolates were characterized and identified based on colony appearance on MacConkey agar, staining reaction and standard biochemical tests.[10]

Antibiotic Susceptibility Studies: The antibiotic sensitivity test was performed by disc diffusion technique using commercially available discs on Mueller Hinton agar plates. The discs includes perfloxacin (5µg), augumentin (25µg), ampicillin (30µg), streptomycin (10µg), gentamicin (30µg), streptomycin (10µg) Nalidixic and (30µg), septrin (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefotaxime (30µg) amikacin (30µg) ceporex (15µg), ofloxacin (10µg), and ciprofloxacin (10µg). Molten Mueller Hinton agar media were prepared and dispensed aseptically into 150 Petri–dishes. A 0.1ml suspension of each *E. coli* isolate, equivalent to 0.5 McFarland standards was aseptically seeded into Mueller Hinton agar plates respectively. This was allowed to stand for one hour to solidify. The antibiotic paper discs were aseptically placed on the surface of the molten Mueller Hinton agar and allowed for 30 minutes to pre-diffuse. The set up was done in triplicate for each isolate, with a control plate containing no antibiotic disc. These were incubated for 18 – 24 hours at 37^0C, after which the radial zones of inhibitions were taken.

Double Disc Synergy test: Mueller Hinton agar plates were prepared and inoculated with standard inoculum of 0.5 McFarland equivalents standard of *E. coli.* 30µg disc of ceftriaxone, cefotaxime and ceftazidime antibiotics were placed on the agar at a distance of 15mm center to center from a combination disc (amoxicillin 20µg and clavulanic acid 10µg). *E. coli* ATCC 25922 was used as a negative control while *E. coli* ATCC 35218 was used as a positive control. ESBL production was interpreted if the zone around the test antibiotic disc increased towards the center disc (Augmentin).

RESULTS

Fifteen clinical isolates of *E. coli* were ESBL producers, making a frequency of 11.4% (15) (Table-II). ESBL producers were isolated more from blood (6), followed by wound (5), urine (3) and sputum (1). Resistance and sensitivity patterns of ESBL producing *E. coli* result shows that the organisms resistant percentage were higher to the cephalosporins followed by gentamicin, nalidixic acid and ampicillin while the sensitivity percentage was highest to streptomycin, followed by septrin, amikacin, perfloxacin, ofloxacin & ceporex . The percentage of intermediate result was low except with the cephalosporins and ciprofloxacin (Table-I).

Table-I: Percentage resistance and sensitivity patterns of ESBL producing *E. coli* isolated from UNTH Enugu.

Org. Nos.	*% Resistance*	*% Intermediate*	*% Susceptibility*	*Antibiotics*
01	63.1	4.6	32.6	Gentamicin
02	57.3	22.5	20.2	Nalidixic acid
03	32.5	62.3	65. 2	Augmentin
04	32. 5	11.6	55.9	Ceporex
05	34.8	8.7	56.5	Ofloxacin
06	32.5	18.6	48.9	Ciprofloxacin
07	37.2	6.9	60.6	Perfloxacin
08	60.5	2.3	37.2	Ampicillin
09	23.2	2.3	74.5	Septrin
10	52.8	24.7	22.5	cefotaxime
11	-	-	100	Streptomycin
12	23.9	6.5	69.6	Amikacin
13	49.4	15.7	34. 9	Ceftazidime
14	41.6	30.3	28.1	Ceftriaxone

DISCUSSION

The frequency of ESBL producing bacteria in most hospitals is probably very high especially in the developing world and may be causing serious therapeutic problems globally. This is probably because, constant failure in therapy are attributable to the presence of plasmids that harbors ESBL enzymes which easily inactivate antibiotics used in therapy.[11] Over the past decade, different types of ESBL producing Enterobacteriaceae have emerged as serious nosocomial pathogens throughout Europe.[5] The percentage of isolates expressing ESBL production is variable although a recent study from the United States reported a high percentage of ESBL (83%) where *Klebsiella pneumoniae* and *E. coli* were most frequently associated with ESBL production.[11] The presence of ESBL enzymes in these organisms is because expanded spectrum â lactamase are commonly included in the empirical antibiotic regimens for treatment of gram negative sepsis.

E. coli is known to be a common organism that is involved in serious nosocomial and community acquired infections.[12] It is responsible for the outbreak of different kinds of diarrhea, especially travelers diarrhoea in developing countries and also it is implicated in urinary tract infection.[12] Therefore, the increased use of broad spectrum cephalosporins has become one of the major factors responsible for the high rate of selection of extended spectrum beta lactamase producing microorganisms.[13] Our study shows that ESBL producing organisms are present at the UNTH, Enugu. This certainly posses a serious public health threat, because UNTH is a major tertiary hospital in Enugu metropolis serving over 85% of the populace. The rapid dissemination of this resistance enzyme to other organisms *via* plasmids, may occur , if it is not urgently controlled.

Unfortunately, information on infections caused by ESBL producing organism (*E. coli*) are limited particularly in our environment and many clinicians are yet to fully appreciate the immense significance of detecting ESBLs. These can lead to the outbreak of multi – drug resistant gram negative pathogens that could bring about expensive control efforts and therapeu-

Table II: Prevalence of ESBL producers in UNTH

Hospital	*Total no of Isolates*	*Source of collection*	*Nos of orgs. Collected from each source*	*No of ESBL from each source*	*Total not of EBSL producers*
UNTH	152	Wound	52	05	15 (11.4%)
		Urine	32	03	
		Sputum	19	01	
		Blood	25	06	

tic failures in patients who receives inappropriate antibiotics.

Recently there are reports of ESBL producing isolates of *E. coli* and *K.pneumoniae* in Western part of Nigeria.[14] In Northern France, the overall prevalence of ESBL producers was low (11.4%) from *K. pneumoniae* and was high (47.7%) from *Enterobacter aerogens* after a five year surveillance study.[15] Although, the overall frequency of ESBL – producing isolates in UNTH, as reported in present study was relatively low (11.4%), a significant increase may occur in few years time, if adequate preventive measures are not taken to suppress the spread of ESBL producing organisms in this hospital community.

The sensitivity and resistant percentage results in Table-I shows that some ESBL producing organisms (org nos. 3, 4, 5, 7, 9, 10 and 11) are sensitive to the quinolones, septrin, streptomycin and augmentin. Studies have shown that it is not advisable to administer an ESBL infected patient with any of the cephalosporin antibiotics. It is however, advised that such patients could be treated with any of the quinolones, macrolides or erythromycin which are shown to be sensitive *in vitro*. It is important to note however that some drugs have been shown to be sensitive *in vitro* are not *in vivo*[16]. This study suggests the controlled use of antibiotics in tertiary hospitals to avert the outbreak of ESBL organisms.

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