Cross Class Resistance to Non Beta Lactam Antimicrobial in Extended Spectrum Beta Lactamases Producing *Escherihia coli*-A Concern to Health Care Practioners.

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ABSTRACT: Extended Spectrum Beta lactamases are group of enzymes capable of Hydrolyzing the third generation cephalosporines thus rendering them ineffective for treatment. This study was carried out to determine the susceptibility profile of extended Spectrum Beta lactamases (ESBL) producing and non ESBL producing strains of *E. coli.* A total of 180 raw sewage samples were collected between September and December 2011 in Sagamu, Ogun State, Nigeria and were examined for the presence of *Escherichia coli* using standard Microbiological technique. The isolated *Escherichia coli* were later screened for production of extended Spectrum Beta lactamase enzyme using double disk synergy test method. Antimicrobial susceptibility patterns of both ESBL producing and non ESBL producing strains were evaluated using disk diffusion method. A total of 61(39.20%) strains of *Escherichia coli* were isolated from the samples. 20(32.8%) of the isolated *Escherichia coli* produced ESBL enzyme. Antimicrobial susceptibility studies performed on the twenty ESBL producers and twenty non ESBLs producers showed that the ESBL producers had significantly reduced susceptibility compared with the non ESBL producers with an alarming trend of associated resistance to gentamicin (75%), Nitrofurantion (70%), Erythromycin (70%), Ciprofloxacin (55%), Sulphamethoxazole (45%), Clindamycin (50%) and Amikacin (35%). Our findings confirm the cross class resistance to non Beta lactam antimicrobials in Extended spectrum Beta lactamases producing *Escherichia coli*. It is therefore very imperative for the clinicians to always request for the susceptibility test result of this isolate before the commencement of treatment.
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1.Introduction

Extended Spectrum beta lactamase enzymes were first recognized in a single strain of *Klebsiella pneumoniae* isolated in Germany (Knothe *et al.,*1983). Since then, several types of ESBLs have been described (Jacoby and Medeiros, 1991). Resistance to extended spectrum cephalosporins can occur in *Escherichia coli* and *Klebsiella pneumoniae* via the production of extended spectrum Beta lactamases that are capable of hydrolyzing the oxyiminocephalo-sporins and monobactams (Bradford, 2004) thus making therapy very difficult for clinicians by rendering the antibiotic ineffective (Kim *et al.,* 2002, Lin *et al.,* 2004).These enzymes are encoded by plasmid genes and are often located within transponson and integrons thereby facilitating their association with other transferable genetic determinants of resistance to cotrimoxazole, aminoglycosides or tetracyclines among others (Lautenbach *et al,* 2001). These types of organism are important sources of nosocomial and community acquired infections (Borer *et al*., 2002.,Canton and Coque, 2006).Clinicians, microbiologists, infection control practitioners and hospital epidemiologists are concerned about ESBL producing bacteria because of the increasing incidence of such infections, the limitations of effective antimicrobial drug therapy and adverse patient outcomes (Rossi *et al.,* 2006).There has also been reports of the growing concern of the organisms producing extended spectrum beta lactamases showing cross class resistance to non Beta lactams Antimicrobials (Paterson, 2000., Procop *et al*., 2003).This confers additional resistance to other antimicrobial classes including fluoroquinolones and aminoglycosides, which may result in therapeutic failures and possibly life threatening bacterial infections (Hunter *et al.,* 2010) thus reinforcing the reason why it is important to detect ESBL producing isolates for proper interpretation of susceptibility tests and subsequent antimicrobial therapy (Paterson *et al.,*2000., NCCLS 2001., Hadziyannis *et al.,* 2000). This study was therefore designed to compare the frequency of resistance to non Beta lactam antibiotics in ESBL producing and non ESBL producing strains of *Escherichia coli.*

2.Materials and Methods

2.1 Collection of Raw Sewage

A total of 180 raw sewage were collected from different sources of the samples in and around Sagamu, Ogun State, Nigeria. 18 samples each of this raw sewage were collected from 10 different collection sites at different time for a period of 4months.

2.2 Bacteriological Analysis

An MPN presumptive test in Mac Conkey broth, MPN confirmatory test in brilliant green bile lactose broth and complete test using EMB agar followed by biochemical tests were performed for isolation and confirmation of *Esherichia coli* (Cheesbrough, 2006).

2.3 Phenotypic Determination of ESBL Enzymes

This was carried out as described by Afiukwa *et al.* (2010) using double disc synergy test. Briefly, a sterile Mueller Hinton agar was prepared and a 0.5 MacFarland equivalent standard of the test organisms was streaked on the surface of the agar with a sterile loop and allowed for 15-20 minutes to prediffuse. An Augmentin which is a combination of Clavulanic acid (20µg) and amoxicillin (10µg) was placed at the centre of a petridish and cefotaxime (30µg), ceftaxidime (30µg), aztreonam (30µg), ciprofloxacin (30µg) were placed 15mm apart centre to centre on the plates with a sterile forceps. These were incubated at 37°C for 18 – 24h.An enhanced zone of inhibition from 5mm above in the presence of Augmentin is regarded as positive for Phenotypic production of ESBL enzyme.

2.4 Antibiotic Susceptibility Testing

The medium used was Mueller Hinton Agar(Oxoid,U.K).The bacterial inoculum were adjusted to 0.5McFarland turbidimetric standard and inoculated onto the medium using flooding method. For each antibiotics, three replicate plates were prepared against the test organisms (ESBL producing *E. coli* and non ESBL producing *E. coli*). Antibiotic susceptibility of each of the isolates were evaluated by the agar disc diffusion method using the following antibiotic disc viz; gentamicin (10µg), Erythromycin (5µg), Ciprofloxacin (5µg), Imipenem (10µg), Amikacin (10µg), Nitrofurantion (50µg), Sulphamethoxazole (25µg), Clindamycin (20µg). These were then incubated at 37°C for 24hour after which the interpretation of the zones of inhibition were done using NCCLS(2001) interpretative charts as resistant, sensitive and intermediate.

3. Results

Out of 180 Sewage samples examined for the presence of *Escherichia coli*, 61(39.2%) showed the presence of the organisms. 20(32.8%) of the isolated *Escherichia coli* produced ESBL enzyme while the remaining 41(67.2%) were non ESBL producers.

The susceptibility profiles of the ESBL producing and non ESBL producing strains of *Escherichia coli* determined shows that the ESBLs producers were significantly more resistant to the non Beta lactams antibiotics tested ($P<0.05$) (table 2). The association between Extended spectrum Beta lactamases enzyme and non Beta lactam antibiotics were statistically evaluated and this was found to be significantly associated ($X^2c = 58.8$, $X^2t = 3.84$, $P<0.05$) (table 4) and this lead to the rejection of the null hypothesis which states that no association exist between beta lactamases enzyme and non Beta lactam antibiotic resistance. X^2c is the chi square calculated and X^2t is the chi square tabulated.

Table 1. Comparison of the susceptibility profiles of ESBL producing and non ESBL producing *Escherichia coli*

Antibiotics	ESBL Producers		Non ESBL Producers	
	Susceptible N (%)	Resistant N(%)	Susceptible N (%)	Resistant N(%)
Gentamicin	5(25%)	15(75%)	19(95%)	1(5%)
Nitorfurantoin	6(30%)	14(70%)	19(95%)	1(5%)
Erythromycin	6(30%)	14(70%)	17(85%)	3(15%)
Ciprofloxacin	9(45%)	11(55%)	14(70%)	6(30%)
Imipenem	20(100%)	--------	20(100%)	-------
Amikacin	13(65%)	7(35%)	19(95%)	1(5%)
Sulphamethoxazole	11(55%)	9(45%)	17(85%)	3(15%)
Clindamycin	10(50%)	10(50%)	18(90%)	2(10%)

N = Number of isolates, % = Percentage of isolates

Table 2. Relative Evaluation of the significancy of the resistance pattern of ESBL and Non ESBL Producers to Non Betalactam Antibiotics.

Antibiotics	ESBL Producers Mean of ± resistance isolates	Non ESBL Producers SEM of the resistance isolates	t – value	P value
Gentamicin	15.00 ± 0.00	1.00 ± 0.00	93.0	<0.05
Nitorfurantoin	14.00 ± 0.00	1.00 ± 0.00	86.3	<0.05
Erythromycin	14.00 ± 0.00	3.00 ± 1.41	11.0	<0.05
Ciprofloxacin	11.00 ± 0.00	6.00 ± 1.41	5.0	<0.05
Imipenem	--------	--------	------	-------
Amikacin	7.00 ± 1.00	1.00 ± 0.00	6.0	<0.05
Sulphamethoxazole	9.00 ± 1.41	3.00 ± 0.00	6.0	<0.05
Clindamycin	10.00 ± 0.00	2.00 ± 0.71	16.0	<0.05

Table 3. Sensitivity Pattern of ESBL and Non ESBL Producers to Non Beta lactam Antibiotics

Antibiotics	ESBL Producers Mean of ± resistance isolates	Non ESBL Producers SEM of the resistance isolates	t – value	P value
Gentamicin	5.00 ± 0.00	19.00 ± 1.00	-14	<0.05
Nitorfurantoin	5.00 ± 1.00	19.00 ± 1.00	-9.9	<0.05
Erythromycin	5.00 ± 0.00	17.00 ± 1.00	-8.5	<0.05
Ciprofloxacin	9.00 ± 0.00	14.00 ± 1.00	-5.0	<0.05
Imipenem	20.00 ± 0.00	20.00 ± 1.00	0.0	>0.05
Amikacin	13.00 ± 0.00	19.00 ± 1.00	-6.0	<0.05
Sulphamethoxazole	11.00 ± 1.00	17.00 ± 0.00	-6.0	<0.05
Clindamycin	10.00 ± 0.00	18.00 ± 1.00	-8.0	<0.05

Table 4. Association between Extended spectrum Beta lactamase and Antibiotic resistance

Beta lactamase reaction	Antibiotic Sensitivity test result		Total
	Sensitive	Resistant	
Positive	80	80	160
Negative	143	17	160
Total	223	97	320

$X^2c = 58.8$, $X^2t = 3.84$, $X^2c > X^2t$ hence null hypothesis was rejected.

4. Discussion

Over the past decade, ESBL producing organisms have emerged as serious nosocomial pathogens throughout the world (Livermore and Yuan, 1996). Outbreaks due to this type of pathogen among the most critically ill patients in intensive care units has been reported (Jacoby,1997).Continued mismanaged selective pressure however contributed towards the emergence of multiple drug resistance bacteria and that has been regarded as an inevitable genetic response to misappropriated exposures of microbial populations to antimicrobial therapy (Sheikh *et al.,* 2003). The high detection rate of *Escherichia coli* in sewage samples was expected because these samples provide an excellent growth condition for these bacterial (Oshoma *et al.,* 2009). This study disclosed an elevated decreased susceptibility of ESBL producing *Escherichia coli* to all the tested antibiotics (Procop *et al.,*2003; Paterson *et al.,* 2000a, Paterson *et al.,* 2000b). The decreased susceptibility of ESBL producing *Escherichia coli* to the tested antibiotics may be due to the multidrug resistance gene in plasmids that they are harbouring (Rooney *et al.,* 2009). However,the non ESBL producing *Escherichia coli* were significantly susceptible to the various antibiotics tested. This may be due to the lack of mutation that has occurred in the active serine site of ESBL producing organisms (Afiukwa *et al.,* 2010). Results showed that all the twenty extended spectrum Beta lactamase producing *Escherichia coli* isolates were found to be multidrug resistant. Antibiotic resistance among isolates of *Escherichia coli* in the present study was comparable to reports from other parts of the world, which also revealed multiple drug resistance among gram negative rods (Oplustil *et al.,* 2001., Winokur *et al.,*2001., Afiukwa *et al.,* 2010). Antibiotic susceptibility test

results of the above isolates also revealed an alarming trend of associated resistance to gentamicin (75%), Nitrofurantoin (70%), Erythromycin (70%), Ciprofloxacin (55%), Sulphamethoxazole (45%), Clondamycin (50%), Amikacin (35%).Such resistance has been reported in recent surveys from Canada, Italy, Spain, Greece and UK and are due to the bla_{CTX-M} genes that are found in association with genetic structures such as sul 1 type integron (Padmini *et al.,* 2008). This structures is genetically linked to class 1 integrons known to integrate antibiotic-resistant gene cassette responsible for resistance to B-lactams, aminoglycosides, chloramphenicol, sulphonamids and to a lesser exlentvifampicin (Pitout *et al.,* 2005). In the present study, we demonstrated significant differences in the susceptibility profiles between ESBL producing and non ESBL producing *E.coli* for gentamicin, Nitrofurantion, Erythromycin, Ciprofloxacin, Amikacin, Sulphamethoxazole and clindamycin except for Imipenem where no significant difference was observed in the susceptibility patterns of both organisms. These finding support the hypothesis that ESBL producing strains of *Escherichia coli* are more likely to have *diminished* susceptibility to non B – lactam antibiotics compared with non ESBL producing isolates of *Escherichia coli*. Our finding is also similar to that of Procop *et al* (2003).Therefore, it is imperative to use the antimicrobial susceptibility profile of the individual isolates to guide treatment.

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