

Phenotypic Characterization and Comparative Study on ESBL-producing *E. coli* of Clinical Origin

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Abstract: Extended-spectrum β -lactamases (ESBLs) continue to be a major challenge in clinical setups worldwide, conferring resistance to the expanded-spectrum cephalosporins. The present study focused on the prevalence of ESBL-producing *E. coli* clinical isolates among patients diagnosed of Wound and Urinary Tract Infections attending Federal Teaching Hospital Abakaliki. A total of one hundred and ninety two clinical isolates of *E. coli* was studied for their susceptibility patterns to cephalosporin antibiotics and detection of ESBL producers was carried out by double disc synergy test (DDST) and Brilliance ESBL Agar. Of the 192 isolates tested for their antibiogram, 19(9.9%), 41(21.4%), 132(68.7%); 48(25.0%) and 144(75.0%) isolates were from wound, high vaginal swab, urine, male and female respectively. The isolates showed higher susceptibility to cefepime (a 4th-generation cephalosporin) with percentage susceptibility of 78.9, 85.4 and 73.5 to the isolates from wound, HVS and urine respectively. Higher resistance was recorded among the 3rd-generation cephalosporins which include Cefotaxime (63.4%), Ceftriaxone (57.9%), and Cefpodoxime (73.7%). Among the resistant isolates of *E. coli*, 20 isolates were phenotypically confirmed ESBL producers by the DDST and Brilliance ESBL Agar methods. Ten (10) ESBL producing *E. coli* was confirmed using DDST method whereas 15 ESBL producing *E. coli* was confirmed using the Brilliance ESBL Agar. Brilliance ESBL Agar was found to be better than DDST in the detection of ESBLs. Continuous monitoring of drug resistance and regulating the use of cephalosporin drugs in our hospitals is vital for proper infectious disease management and treatment.

Keywords: ESBLs, *E. coli*, Patients, DDST and Brilliance ESBL Agar.

Introduction

Extended-spectrum β -lactamase (ESBL)-producing Gram-negative bacteria are emerging pathogens. 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 (Lautenbach *et al.*, 2001; Paterson, 2006). The rapidity of the development and spread of resistance is a complex process that is influenced by selective pressure, pre-

existence of resistance genes and poor use of infection control measures in our hospitals. Extended spectrum cephalosporins (including the third generation cephalosporins) gained widespread clinical use in the early 1980s and were developed because of the increasing prevalence of ampicillin-hydrolyzing β -lactamases such as TEM-1, TEM-2 and SHV-1 enzymes in *Enterobacteriaceae*, notably in *Escherichia coli* (Mark and Paul, 2003). The production of beta-lactamase enzymes by Gram-negative bacteria is one of the most important mechanisms of resistance to beta-lactam antibiotics.

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ESBLs therefore, are acquired plasmid-mediated beta-lactamases that hydrolysis broader spectrum beta-lactam antibiotics including the oxyimino-3rd-generation cephalosporins and the penicillins (Paterson and Bonomo, 2005). Some data supported that foreign travel as well as household and community exposure may be important risk factors leading to colonization of the human intestinal tract by ESBL-producing *E. coli* (Laupland *et al.*, 2008; Valverde *et al.*, 2008). Person-to-person transmission and acquisition from a common source, probably related to food and poor hygiene clearly contribute to ESBL dissemination within families or community (Rodriguez-Bano *et al.*, 2008). Similarly, other studies have reported a high prevalence of ESBL-producing bacteria in certain communities. The high prevalence of these bacteria in healthy carriers may be an important source of community-acquired infections, including urinary tract infections (UTIs). However, whether these ESBL-producing bacteria can be causative infectious agents in the carrier has not yet been confirmed (Niki *et al.*, 2011). Clinical strains of *Escherichia coli* producing ESBLs have high prevalence of ESBL-producing *Enterobacteriaceae* in hospitals in African countries including Morocco, South Africa and Nigeria (Ibadene *et al.*, 2008; Sekhsokh *et al.*, 2008; Pitout *et al.*, 1998; Aibinu *et al.*, 2003). Arpin *et al.*, (2009) detected a variety of β -lactamases among the isolates of *E. coli* and *K. pneumoniae*, namely SHV-, CTX-M-, OXA- and TEM-type enzymes. The CTX-M type was the most common ESBL in our setting. In Nigeria, these enzymes have been reported in *Enterobacter* species from human patients in Lagos (Aibinu *et al.*, 2003). Chau and Obegbunam (2007) reported the presence of high resistant extended spectrum beta-lactamase producing *Escherichia coli* in Enugu State. ESBLs have been implicated in a variety of stubborn bacterial-related infections such as bacteraemia, pneumonia, UTIs and wound infections (Iroha *et al.*, 2010; Ejikeugwu *et al.*, 2013). Hence, the present study is designed to evaluate the prevalence of ESBL-producing strains of *Escherichia coli* in the hospital and compare the efficiency of Brilliance ESBL Agar and Double Disc Synergy Test in the detection of ESBLs.

Materials and Methods

Study area: This prospective study was conducted in the Department of Applied Microbiology at Ebonyi State University, Abakaliki, Nigeria. A total of 192 isolates were obtained from clinical samples from October 2014 to October 2015. Among 192 isolates, 48 were from male patients and 144 isolates were from female patients diagnosed of wound and UTIs; and who attended the Federal Teaching Hospital Abakaliki, (FETHA) Ebonyi State for medical attention.

Bacteriology: All the clinical samples of wound swabs and urine samples were inoculated on MacConkey agar (MAC), Chocolate agar (CA) and Cystein Lactose Electrolyte Deficient (CLED) medium (Oxoid, UK), and incubated at 37°C for 18-24 hrs. Suspected bacterial colonies were sub-cultured onto freshly prepared MAC, CLED and CA for the isolation of pure cultures of the bacteria. *E. coli* isolates were identified based on colony morphology and biochemical reactions using standard microbiological conventional identification techniques (Cheesbrough, 2010; Stephan and William, 2006).

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility testing was performed by the modified Kirby-Bauer disc diffusion method (Bauer *et al.*, 1990). Various antimicrobial discs were used which include antimicrobials for screening of ESBL-producing bacteria. Single antibiotic disks of Ceftazidime (30 μ g), Cefotaxime (30 μ g), Ceftriaxone (30 μ g), Cefpodoxime (10 μ g), Aztreonam (30 μ g), Cefepime (30 μ g) and Amoxicillin plus Clavulanic acid 30) (Oxoid, UK) were used for determining the susceptibility profile of the test bacterium. The results were interpreted as per the Clinical Laboratory Standard Institute (CLSI) recommendations (CLSI, 2006).

Screening for ESBL production: Bacterial isolates that showed decreased susceptibility (intermediate by CLSI criteria) to any of the third generation cephalosporins including ceftazidime and cefotaxime were suspected to produce ESBL and further tested for ESBL production phenotypically. According to CLSI guidelines, strains showing zone of inhibition of ≤ 22 mm for ceftazidime, ≤ 27 mm for cefotaxime, and ≤ 25 mm for ceftriaxone were selected for ESBL confirmatory test.

Phenotypic determination of ESBL using Double Disc Synergy Test (DDST):

The *E. coli* isolated colonies were inoculated in peptone water at 37°C for 2–6 h. The turbidity was adjusted to 0.5 McFarland standards and lawn culture was made on Mueller-Hinton agar using sterile swab stick. Amoxicillin plus Clavulanic acid disk (20 μ g and 10 μ g, respectively) was placed in the centre of plate. A disc of cefotaxime (30 μ g) and ceftazidime (30 μ g), were placed at a distance of 15 mm away from the centrally placed disc (Amoxicillin plus Clavulanic acid disk). The plate was incubated at 37°C overnight. An increase of ≥ 5 mm in the inhibition zone diameter for either of the cephalosporins (ceftazidime and cefotaxime) tested in combination with amoxicillin-clavulanic acid versus its zone when tested alone confirms ESBL production phenotypically (Pituot *et al.*, 2004).

ESBL Testing using Brilliance ESBL Agar. Code: PO5302: Brilliance ESBL Agar (pale, off white, semi-opaque gel medium) is a chromogenic screening agar plate for detection of ESBL-producing organisms. The medium provides presumptive identification of ESBL-Producing *E. coli* direct from clinical samples or isolates in 24 hours. Brilliance ESBL Agar was inoculated directly with isolated colony of *E. coli* prepared as a liquid suspension approximately equivalent to 0.5 McFarland turbidity standards. The medium was allowed to warm to room temperature before inoculation. The inoculated plates were incubated for 18-24 hours at 37°C. Negative plates were re-incubated for additional 24 hours. Blue or Pink colonies were recorded as presumptive positive ESBL-producing *E. coli* (CLSI, 2006; Thermo Scientific Oxoid Microbiology, 2013).

Results

The distribution of *E. coli* isolated from patient attending FETHA are shown in the Table 1. Out of 192 clinical isolates of *E. coli* used for this study, 48 (25.0 %) were isolated from male, while 144 (75.0

%) were from female. Moreover, the site of isolation of the isolates are wound 19 (9.9 %), high virginal swab 41 (21.4 %) and urine 132 (68.7 %). The results of antimicrobial susceptibility test of the *E. coli* isolates to some commonly used cephalosporin antibiotics are shown in Table 2. The results showed that cefepime are most effective drugs against the *E. coli* isolates. Higher levels of resistance by the *E. coli* isolates were recorded for ceftazidime, cefotaxime, ceftriaxone, cefpodoxime and aztreonam. Phenotypic characterization of ESBLs-producing *E. coli* isolated from patient attending FETHA was shown in Table 3. Among the resistant isolates of *E. coli*, 15 were ESBL producers detected by DDST and Brilliance ESBL Agar. Ten ESBL producing *E. coli* was confirmed using DDST whereas 15 ESBL producing *E. coli* was confirmed using Brilliance ESBL Agar. Figure 1 shows a keyhole effect produced by *E. coli* isolate expressing ESBL using DDST method. This appearance is the characteristic for ESBL-producing bacteria due to the synergistic effect produced between amoxicillin-clavulanic acid (a beta-lactamase inhibitor) and the third generation cephalosporins such as ceftazidime and cefotaxime.

Table 1: Distribution of *E. coli* isolated from patient attending FETHA

Sample site	No of Isolates (%)	No +ve for Male (%)	No +ve for Female (%)
Wound Swab	19 (9.9)	7 (36.8)	12(63.2)
High Virginal Swab	41(21.4)	-(0.0)	41(100)
Urine	132(68.7)	41(31.1)	91(68.9)
Total	192 (100)	48(25.0)	144(75.0)

Table 2: Antibiotic profile of *E. coli* isolated from patient attending FETHA

Antibiotics	Wound Swab		High Virginal Swab		Urine	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Cefepime	15(78.9)	4 (21.1)	35(85.4)	6 (14.6)	97(73.5)	35(26.5)
Ceftazidime	12(63.2)	7 (36.8)	25(61.0)	16(39.0)	70(53.0)	62(47.0)
Ceftriaxone	8 (42.1)	11(57.9)	19(46.3)	22(53.7)	92(69.7)	40(30.3)
Cefpodoxime	5 (26.3)	14(73.7)	22(53.7)	19(46.3)	69(52.3)	63(47.7)
Aztronam	9 (47.4)	10(52.6)	24(58.5)	17(41.5)	80(60.6)	52(39.4)
Cefotaxime	7 (36.8)	12(63.2)	15(36.6)	26(63.4)	50(37.9)	82(62.1)

Table 3: Phenotypic characterization of ESBLs- producing *E. coli* isolated from patient attending FETHA

Sample site	Brilliance ESBL Agar		Double Disk Synergy Test	
	No +ve Male;	No +ve Female	No +ve Male;	No +ve Female
Wound Swab	-	-	-	-
High Virginal Swab	-	4	-	3
Urine	4	7	2	5
Total		15		10

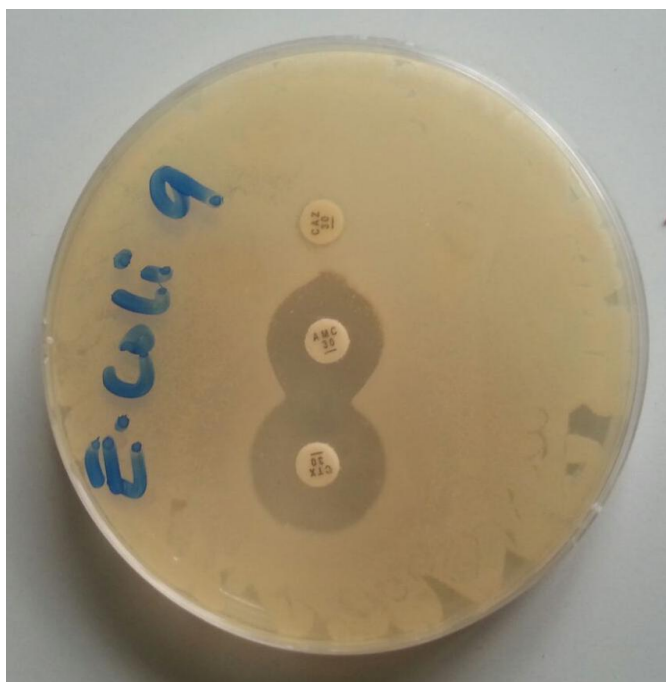


Figure 1. Illustration of ESBL positive plate.

Discussions

Extended-spectrum β -lactamases (ESBLs) are a rapidly evolving group of β -lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam yet are inhibited by clavulanic acid. Typically, they derive from genes for TEM and SHV-type beta-lactamases by mutations that alter the amino acid configuration around the active site of these earlier β -lactamases. This extends the spectrum of β -lactam antibiotics susceptible to hydrolysis by these enzymes. ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species. However, it can still be chromosomal mediated. There is no doubt that ESBL-producing organisms are of enormous clinical and microbiological significance. Such bacteria are associated with severe infections such as bacteraemia, intra-abdominal infection, wound infection, urinary tract infections (particularly in the community setting), and respiratory tract infections (Pituot *et al.*, 2004). A total of one hundred and ninety two (192) isolates of *E. coli* was studied for their susceptibility patterns to cephalosporin antibiotics and detection of ESBL producers by double disc synergy test (DDST) and Brilliance ESBL Agar. Of the 192 isolates tested for their antibiogram, 19 (9.9%), 41 (21.4%), and 132 (68.7%) isolates were from wound, high vaginal swab, and urine samples respectively while 48 (25.0%) and 144 (75.0%) isolates were from male and female patients respectively. The *E. coli* isolates showed higher susceptibility to 4th-generation cephalosporins (cefepime). This is akin with previous works in which cefepime was reported as the drug of choice for the treatment of bacterial infections caused by

ESBL-producing organisms (Lye *et al.*, 2008; Walsh *et al.*, 2005 and Paterson, *et al.*, 2005). Higher resistance of the *E. coli* isolates were also recorded among the 3rd-generation cephalosporins which includes Cefotaxime (63.4%), Ceftriaxone (57.9%), and Cefpodoxime (73.7%). Higher levels of resistance of ESBL-producing *E. coli* isolates as obtainable in this study have also been previously reported (Bradford, 2001). Among the resistant isolates of *E. coli*, 15 were ESBL producers detected by DDST and Brilliance ESBL Agar. Our findings agreed with the research work done by Aibinu *et al.*, 2003, Chau and Obegbunam, 2007 and Iroha *et al.*, 2010, where they found ESBL-producing *E. coli* among patients diagnosed of wound infections, high vaginal infections and UTIs in Nigeria. Overall, 10 ESBL producing *E. coli* was confirmed using the DDST method whereas 15 ESBL producing *E. coli* was confirmed using the Brilliance ESBL Agar. The false negative results in ESBL detection phenotypically observed using DDST is as a result of co-production of other related enzymes such as metallo-beta-lactamases (Walsh *et al.*, 2005), plasmid-mediated and chromosomal inducible AmpC beta-lactamase in the test bacteria other than ESBLs (Jacoby *et al.*, 2005). However, the brilliant result observed using Brilliance ESBL Agar is as a result of formulation with antibiotic and chromogenic mixture which inhibits every other enzymes present and allows the production of ESBL enzyme only to grow and produce colour pigments. In this present study, Brilliance ESBL Agar was found to be better than DDST in the phenotypic detection of ESBLs. Continuous monitoring of drug resistance, proper susceptibility studies and regulating the use of

cephalosporin drugs in our hospitals is important in the containment of the emergence and spread of ESBL-producing bacteria in the hospital environment.

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