

Original Article

Incidence of temonera, sulphuhydryl variables and cefotaximase genes associated with β -lactamase producing escherichia coli in clinical isolates

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Abstract

Background: the occurrence of the different types of Extended spectrum beta Lactamase producing *Escherichia coli* with the, Sulphurhydryl variable, Temonera and the Cefotaximase have been on the rise **Aim:** The study was to determine the prevalence of extended spectrum beta lactamase gene resistance across the clinical isolates of hospitalized patients. **Materials and Method:** Three hundred and fifty isolates of *Escherichia coli* were received from different clinical specimens. The susceptibility profile of the isolates against 10 different antibiotics was examined, the MICs (Minimum Inhibitory Concentration) for ceftazidime were also determined using micro-broth dilution assay. Isolates showing MIC \geq 6 μ g/ml for ceftazidime were screened for ESBL (PCT)phenotypic confirmatory test and subjected to PCR (polymerase chain reaction) to further. **Results:** By disk diffusion test, there was resistance to ceftazidime and cefotaxime were 180(51.4%) and 120 (34.2%) respectively. However, all strains were susceptible to imipenem. 250 isolates showed MICs \geq 6 μ g/ml for ceftazidime of which 180 (72%) were positive for extended spectrum beta lactamase. The prevalence of Sulphurhydryl variable, Temonera and the Cefotaximase among these isolates were 17.1%, 6.6% and 17%, respectively. **Conclusion:** For the identification of extended spectrum beta lactamase producing isolates it is recommended that clinical laboratories adopt simple test based on Cincinal laboratory standard institute recommendation for confirming extended spectrum beta lactamase production in enterobacteriaceae species.

Keywords: Extended spectrum beta Lactamase, bla TEM, SHV and CTX-M, Cephalosporins.

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Introduction

Antimicrobial resistance is a growing problem in many bacterial pathogens and is of particular concern for hospital-acquired nosocomial infections [1]. *Escherichia coli* is an important pathogen that causes urinary tract infections (UTIs), pneumoniae, and intra-abdominal infections in hospitalized immunocompromised patients with severe underlying diseases [2]. Resistance of *Escherichia coli* to broad spectrum antibiotics such as extended spectrum cephalosporins due to plasmid mediated enzymes (extended spectrum β -lactamases: ESBLs) results in treatment failure of infections caused by these isolates [3].

Difficult treatment of these infections may allow ESBL producing pathogens to remain within the environment and patients for the long period of time and to spread easily within and between hospitals. Within a few years of the commercial release of β -lactams, gram negative bacilli (especially *Escherichia coli*) that harbored mutated versions of the potent TEM and SHV enzymes were detected. These and other newly detected β -lactamases (for example CTX-M) hydrolyze β -lactam antibiotics containing the oxymino side-chain [4]. CTX-M preferentially hydrolyze cefotaxime and based on the changes in amino acids sequences identities is divided into five groups [5]. strong ESBL activity which can efficiently

hydrolyzes penicillins and cephalosporins. Because of inappropriate usage of antibiotic in treatment of infection caused by ESBL producing pathogens, it seems that studies about correct detection and antibiotic resistance pattern of these organisms are necessary. In recent years a few studies were done about the *E. coli* isolates producing ESBLs in our country [7, 12].

Also despite of importance of PER type β -lactamase, there is not any information concerning *E. coli* isolates harboring these *bla* gene in Yola south Nigeria. Therefore, the present study was carried out to determine the prevalence of the genes encoding SHV, TEM, and CTX-M are responsible for drug resistance.

Materials and Methods

A total of three hundred and fifty (350) patient samples were collected, all from the following biological specimens, Urine(180), Sputum(20) Blood (25), Stool (50) High Vaginal Swabs (20) Wound swabs (30), and Abscess (25). Blood samples were collected into clean blood culture bottles and incubated anaerobically, urine samples were also collected into sterile universal bottles, sputum and stool samples were also collected into clean universal sterile containers and sent to the laboratory for microbiological processing.

Standard microbiological methods according to (CLI) specimens were cultured aerobically and aerobically all at 37°C except otherwise stated.

Susceptibility Profile

The standard disk diffusion pour plate method according to Kirby buer was used, the various antibiotics used were, Ceftazidime, Cefotaxime, Ceftriaxome, Cefixime (30 μ g), Gentamycine, Ampicillin, Imipenem (10 μ g), Tetracycline (10 μ g), Aztronam (30 μ g), and Augumentin (30 μ g). zones were reported as sensitive and resistance according to the width of the zone of inhibition.

Screening for ESBL

ESBL production ability of isolates showing MICs \geq 6 μ g/ml for ceftazidime was examined by using phenotypic confirmatory test (PCT). In brief, pairs of discs containing cefotaxime ESBLs in *Klebsiella pneumoniae* from Iran Iran J Basic Med Sci, Vol. 13, No. 3, Summer 2010 113 (30 μ g) and ceftazidime (30 μ g) with and without clavulanic acid (10 μ g) were placed on opposite sides (at a distance of 20-30 mm) of the same inoculated plate containing Muller Hinton agar (BBL-Becton Dickinson). A positive test result was defined as \geq 5 mm increase in zone diameter compared to a disk without clavulanic acid [16].

DNA Extraction And PCR

ESBL positive isolates were cultured in LB (Luria-Berneti) broth at 37 °C overnight and plasmid DNA was extracted according to the published method of Johnson and Woodford [17].

Specific primers and annealing temperature for amplifying the *bla*SHV, *bla*TEM, and *bla*CTX genes by PCR were shown in Table 1. PCR was carried out in solution containing 200 μ M concentration of dNTPs, 10 Pmol of each primer, 0.8 mM/ μ l MgCl₂, 0.5 U *Taq* polymerase and 50 ng DNA template in a final volume of 25 μ l *Escherichia coli* 6681 containing *bla*SHV, *bla*CTX-M and *bla*TEM gene (Kindly provided by Patrice Nordmann) were used as controls.

PCR-RFLP

The CTX, PER and TEM ESBL amplified genes were characterized by PCR-Restriction Fragment Length Polymorphism (RFLP). This analysis was performed using *Pst*I for TEM and PER and *Pvu*II for CTX-M ESBL amplified genes. Restriction fragments were analyzed using gel electrophoresis in a 1% (W/V) agarose gel.

Statistical Analysis

Parametric methods (t-test) were used for statistical analysis of the data obtained from drug susceptibility testing and also using standard percentage occurrence for the calculations of data received

Results

The susceptibility profile showed a remarkable 100% susceptibility of the isolate to Imipenem and there was also the resistance to Ceftazidime and Cefotaxime with (51.4 % and 34.2 %) 180 and 120 respectively, the resistance pattern across board showed a (83%) resistance pattern of the isolates to tetracycline and Ampicillin. The resistance pattern for Ceftriaxome (23%), Cefixime (16%), Gentamycine (32%) Aztronam (18%), and Augumentin (29%). A total of 181 isolates (52%) were seen to be resistant one or more of the third generation Cephalosporin. 250 isolates showed MICs \geq 6 μ g/ml for ceftazidime of which 180 (72%) The MICs of ceftazidime in ESBLs producing isolates ranged from 6 to > 512 μ g/ml, 63% of which showed MICs \geq 128 μ g/ml. The ESBL-producing isolates were recovered mostly from urine (n = 180), stool (n = 50) and wound (n = 30) specimens. Table 1 showed the results of percentage resistance to antibiotics in ESBL positive isolates.

Discussion

Escherichia coli is a known pathogenic organism that has caused sever nosocomial, urinary tract infection, blood borne disease and gastro enteritis which has led to sever morbidity and mortality. Following the extensive use and constant abuse of the extended spectrum beta lactam agent, the outbreaks of infection caused by extended spectrum beta Lactamase producing *Escherichia coli* have been widely reported throughout the world [18]. The production of ESBLs is a major threat to the use of new generation of cephalosporins [19, 20]. Long hospitalization, diabetes, age over 60 and previous antibiotic treatment have been reported as the risk factors to acquire infections with ESBL strains [21].

Table 1 Showing the Percentage resistance pattern of the isolates across the various antibiotic

Isolate	CAZ(%)	CTX(%)	AMP(%)	AZT(%)	CN(%)	AUG(%)	IMP(%)	TET(%)	CRO(%)	ZOZ (%)
Escherichia coli	51.4	34.2	84	18	32	29	0	84	23	16

CAZ: Ceftazidime, CTX: Cefotaxime, AMP: Ampicillin, CN: Gentamycin, AUG: Augmentin, IMP: Imipenem, TET: Tetracycline, CRO: Ceftriaxone, ZOZ: Cefoxurime.

Table 2 showing the primer names across the tables and the annealing temperature

Primer name	Sequence (5' to 3')	Size (bp)	Annealing temperature	Gene	References
CTX-MA	CGCTTTGCGATGTGCAG	550	63°C	^{bla} CTX-M	35
CTX-MB	ACCGCGATATCGTTGGT				
TEM-A	GAGTATTCAACATTTCCGTGTC	848	43°C	^{bla} TEM	36
TEM-B	TAATCAGTGAGGCACCTATCTC				
SWSHV-A	AAGATCCACTATCGCCAGCAG	231	60°C	^{bla} SHV	36
SWSHV-B	ATTCAGTTCCGTTTCCAGCGG				

Table 3 Showing the percentage occurrence of the genes across the biological specimens

Samples	Total	ESBL PCT (+)%	^{Bla} TEM	^{Bla} CTX-M	^{Bla} SHV
URINE	180	78(43.3)	20	12	18
SPUTUM	20	18 (90)	8	3	6
BLOOD	25	14(56)	6	1	4
STOOL	50	30(60)	10	2	16
SWAB(HVS)	20	15(75)	5	-	10
WOUND SWAB	30	15(50)	10	1	6
ABSCESS	25	10(40)	-	4	-
TOTAL	350	180	59	23	60

PCT: phenotypic test, ESBL: extended spectrum beta lactamase

In our study antimicrobial susceptibility testing showed that 250 isolates showed MICs $\geq 6 \mu\text{g/ml}$ for ceftazidime of which 180 (72%) were positive for ESBL in PCT. ESBLs in other countries in our region such as India (97.1%), Turkey (57%) and South Korea (30%) (22-24). However, previous studies from Iran about ESBL positive strains of *Escherichia coli* is rare; Feizabadi *et al* in 2006 detected 44.5% ESBL positive rate among clinical *Escherichia coli* isolated from clinical specimens in Tehran [8]. The rates of ESBL producing *Escherichia coli* isolated from Tehran reported by Aminzadeh *et al* was 52.5% in 2008 [9]. Ramazanzadeh *et al* reported 34.8% of ESBL producing strains of Gram-negative bacteria isolated from Kurdistan [10]. In 2009 Bazzaz *et al* also indicated that the prevalence of ESBL producing strains of *E. coli* and *K. pneumonia* was 59.2% [11]. As 180 of 250 ceftazidime resistant isolates were ESBL positive in this study, it appears that ESBL production has a significant role in resistance to cephalosporines rather than other mechanisms of resistance such as loss of porins and efflux pumps in our research [25-26]. The MICs of ceftazidime for the majority of ESBL positive isolates which 180 (72%) was $>32 \mu\text{g/ml}$. However, these isolates were susceptible to imipenem. So, the best coverage against ESBL-producing isolates was obtained with imipenem (0% resistance). Since ESBL genes are generally plasmids mediated, many of the organisms that harbor ESBLs also are resistant to other classes of antibiotics such as aminoglycosides, fluroquinolons, tetracyclines, chloramphenicol and sulfonamides [27]. These results obtained from past issues on this confirms the prevalence of occurrence in our results and our study: the rate of resistance to

cephalosporines, aminoglycosides and fluroquinolons in ESBL positive strains is higher compared to all of studied isolates. In accordance with our results, 41% of ESBL positive strains were resistant to ciprofloxacin which is lower than Lautenbach's report (60%) [28].

The prevalence of *bla*SHV and *bla*TEM genes in this study was 17.1% and 17% respectively which are different from the results of the multi-national study group (67% and 16%, respectively) [30]. In Iran, Feizabadi *et al* in 2009 showed that 69.7% of *Escherichia coli* isolated from Tehran were ESBL positive and the prevalence of *bla*TEM, *bla*SHV, *bla*CTX-M-I and *bla*CTX-M-III among these isolates was 54%, 67.4%, 46.51% and 29%, respectively [12].

Conclusion

The emergence and increased spread of ESBL-producing *Escherichia coli* strains is worrisome and usage of Cephalosporins against these isolates is ineffective. As imipenem is the drug of choice for serious infection disease these days, prolong and extensive use of this drug in treatment of infection caused by resistant isolates will enhance. Because of this problem prudent use of β -lactam antibiotics containing an oxyimino group and consistent application of basic infection control procedures in treatment centers is necessary. Due to importance of ESBL producing organisms and difficult treatment of infections caused by these bacteria, for rapid identification of ESBL producing isolates clinical laboratories should adopt simple test based on CLSI recommendation for confirming ESBL production in enterobacteracea species. Laboratory

services should be available to support every infection control program. Unfortunately there is rare co-operation between clinical settings and laboratories in Nigeria. We should always remember that effective treatment of serious infections will only be achieved by close cooperation between clinical and laboratory staff.

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