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# Resistant Pattern and Molecular Characterization of Extended-Spectrum β -Lactamse-Producing *Escherichia Coli in Skaka/ Saudi Arabia*

KEYWORDS: E.coli, ESBL, Antimicrobial, Vitek2,Skaka, Saudi Arabia.


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**ABSTRACT** *In this study 82 isolates of E.coli were identified and subjected to antibacterial activity against 18 different antibiotics.PCR analysis was used to isolate five ESBL genesandto confirm the identification of β -lactamase's producers observed during Kirby–Bauer's disc diffusion method using indicator antibiotics.PCR identified the presence of CTX-M, TEM, SHV, CTX-M +TEM, CTX-M+SHV at the following sequences 39.0%, 18.3%, 12.2%, 6.2% and 1.2% respectively. 23.2% of the ESBL isolates did not express any of the above resistance genes. All the ESBL producing* **Escherichia** *colistrains were resistant to ampicillin, cefazolin, ceftazidime, ceftriaxone and cefepime while imipenem,meropenem and amikacin were highly active in all the isolates, the other antibiotics expressed different degree of activity as shown in table (3) .This result confirmed the existence of high level of multi drug resistance( MDR) withinthe extended-spectrum β -lactamase* **Escherichia** *coli in Skaka and the predominance of CTX-M gene.*

**Introduction:**
The introduction of antimicrobial agents into clinical practice was accompanied by the problem of antibiotic resistance. Currently, resistance to antibiotics poses a major problem in both hospital and community settings throughout the world(1). Many infectious diseases causing death in humans were brought under control by the expanded use of these antimicrobial agents. The resistance to antibiotics representsa major clinical problem all over the world, potentially leading to treatment failure or even patient death where resistant bacteria are etiological agents of severe infections (2).Extended-spectrum β -lactamase (ESBL)-producing Escherichiacoli and Klebsiellapneumoniae have rapidlyspread worldwide and pose a serious threat for health care-associated (HA) infection (3). The fundamental characteristic of ESBLs is their ability to hydrolyze oxyiminocephalosporins and aztreonam while being inhibitedby β -lactamase inhibitors (4,5).It wasshown that exposure to oxyiminocephalosporins and transferfrom another hospital were among identified risk factors associated with infection due to ESBL producers(6). In recent years non- TEMAND non-SHV plasmid mediated ESBLs have been reported mainly the CTX-Menzyme which is recognized as a rapidly growing family of ESBLs (7).CTX-M extended-spectrum beta-lactamases have emerged as the most common type of ESBL globally, their incidence easily surpassing those of SHV and TEM ESBLs in most locales. (8) Many of the CTX-M enzymes hydrolyze cefotaxime efficiently than ceftazidime, the opposite to the case for many of the more familiar TEM and SHV enzymes (9, 10).

**Methodology:**
Eighty two extended spectrum β -lactamase producingE.coli strains were recovered from different specimens collected from Patients attending princeAbd/Al RhmanSidery Hospital during the period October to March 2013. Antimicrobial susceptibility testing using Kirby–Bauer's disc diffusion method according to CLSI guidelines was adopted. The following antibiotics were used as indicators for the detection of ESBL enzyme, using double disc diffusion test, ceftriaxone (30µg),cefotaxime (30 µg), ceftazidime (30 µg). Zone diameter for possible ESBL-producing E.coli strains was calculated. Antibacterial activity of all the isolates against 18 different antibiotics using Vitek2 Compact analyzer was done.

**DNA extraction**
All the E.coli isolates (82) which were classified as ESBL producers were selected for molecular characterization. Genomic DNA was extracted from overnight cultures using PrepMan Ultra Sample Preparation Reagent kit supplied by Bio System Company according to the manufacturer instructions. The cell was suspended into 100µL of the reagent in a microcentrifuge screw-cap tube. The tube was vortexed for 30 seconds, heated for 10 minutes at 100 C° then 50 µL of the supernatant was transferred into a second tube. The DNA was frozen at -20 to be used when needed.

**PCR detection of ESBL GENE**
All selected multidrug resistant E.coli isolates were screened for ESBL enzymes using the primers listed in table (1). PCR reaction was adjusted to 50µl containing 3µl of total DNA (50-100 ng) as a template, 3mM MgCl 250 pmol of each primer & 250 µM each of deoxynucleotidetriphosphate (dGTP,dCTP, dATP and dTTP), and 1.5U Taq polymerase. The PCR assays for the target genes were performed by using the thermocycler as follows: Initial denaturation for 5 min at 94 C° followed by 35 cycles of denaturation at 94C° for 30-seconds, primer annealing at 62 C° and extension at 72C° for 45seconds. Tubes were held at 4C°. The PCR products were analyzed by 1.5% agarose gel electrophoresis run for 45 minutes at 80 V. The gel was visualized with ultraviolet light using a Gel documentation system.

**Table (1):ThePrimer sequences used for the amplification of ESBLs genes**

| | Gene | Primer sequences ( 5 to 3 ) | size (bp) |
|---|---|---|---|
| 1 | CTX-M- F | CVA TGT GCA GYA CCA GTA A | 585 |
| 2 | CTX-M--R | ARG TSA CCA GAA YMA GCG G | |
| 3 | TEM-F | TCCGCTCATGAGACAATAACC | 931 |
| 4 | TEM-R | TTGGTCTGACAGTTACCAATGC | |
| 5 | SHV-F | TGGTTATGCGTTATATTCGCC | 868 |
| 6 | SHV-R | GGTTAGCGTTGCCAGTGCT | |

**Result:**

**Fig (1):- Frequency of ESBLs genes among E.coli clinical isolates**

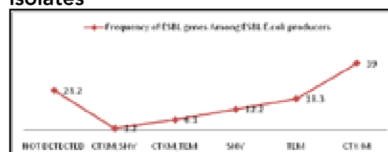

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**Fig (2):-Antibacterial activity of ESBL producers among E. coliisolates**

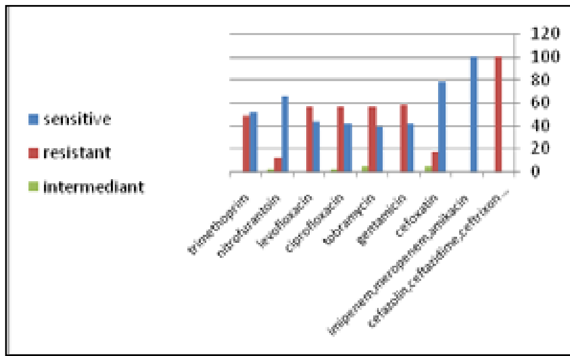


**Table (3) Frequency of ESBL genes among E.coli ESBL producers**

| Percent | Frequency | |
|---|---|---|
| 39.0 | 32 | CTX-M Valid |
| 18.3 | 15 | TEM |
| 12.2 | 10 | SHV |
| 23.2 | 19 | not detected |
| 6.1 | 5 | CTX-M , TEM |
| 1.2 | 1 | CTX-M , SHV |
| 100.0 | 82 | Total |

**Table (2):Resistance genes of ESBL E.coli and antibiotics cross tabulation**

| | gene | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | CTX-M | TEM | SHV | CTX-M, TEM | CTX-M,SHV | Not detected | |
| Piperacillin sensitive | 18 | 9 | 3 | 3 | 0 | 9 | 42 |
| resistant | 11 | 6 | 6 | 2 | 1 | 8 | 34 |
| intermediant | 3 | 0 | 1 | 0 | 0 | 2 | 6 |
| Cefoxitin sensitive | 23 | 13 | 10 | 5 | 1 | 12 | 64 |
| Resistant | 6 | 2 | 0 | 0 | 0 | 6 | 14 |
| intermidient | 3 | 0 | 0 | 0 | 0 | 1 | 4 |
| tobramicin sensitive | 16 | 3 | 2 | 2 | 0 | 9 | 32 |
| Resistant | 14 | 10 | 8 | 3 | 1 | 10 | 46 |
| intermediant | 2 | 2 | 0 | 0 | 0 | 0 | 4 |
| Ceftazidim resistant | 32 | 15 | 10 | 5 | 1 | 19 | 82 |
| Gentamicin sensitive | 19 | 3 | 3 | 1 | 0 | 8 | 34 |
| resistant | 3 | 12 | 7 | 4 | 1 | 11 | 48 |
| Nitrofurantoin sensitive | 21 | 8 | 6 | 4 | 0 | 15 | 54 |
| Resistant | 3 | 3 | 2 | 0 | 1 | 1 | 10 |
| intermedia | 8 | 4 | 2 | 1 | 0 | 3 | 18 |
| Trimethoprim sensitive | 15 | 7 | 5 | 3 | 0 | 12 | 42 |
| resistant | 7 | 8 | 5 | 2 | 1 | 7 | 40 |

**Discussion:**

The spread of ESBL-producing bacteria was dramatically increased worldwide, indicating that continuous monitoringsystems and effective infection-control measures areabsolutely required. Therapeutic options for infections due to ESBL producers have also become increasingly limited. Healthcare interactions, including the use of antibiotics,particularly oxyiminocephalosporins and hospital transfer is among well-defined risk factors for acquisition of ESBLproducingbacteria (11, 12, 13).CliniciansCliniciansCliniciansCliniciansdepend heavily on information from the clinical microbiology laboratory for treatment of their seriously ill patients taking into account that clinical importance of antimicrobial susceptibility tests requires that these tests be performed under optimal conditions and that laboratories have the capability to provide results for the newest antimicrobial agents. The present study has found that resistant rates ofE.coliisolate were relatively high and majorities of isolates were inactive to non β -lactam agents, especially gentamicin, ciprofloxacin, levofloxacin and tobramycin (Fig 2) resulting in a markedpercentage of MDR isolates. Between 82 ESBL-producing isolates; 76.8% carried β -lactamgenes, which can probably account for a high-level β -lactam resistantphenotype. On the other hand, AllESBL-producing E. coli isolates demonstrated high MICs of oxyiminocephalosporin while they remained in the susceptible range for carbapenems. The rates of resistance to non- β -lactam agents, including tetracycline, amikacin, gentamicin, cotrimoxazole, and ciprofloxacin, for ESBLproducing were 87.5%, 12.5%, 66.3%, 72.7%, and78.1%, respectively. Up to 72.3% of ESBL-producing E. coli expressed the MDR phenotype.All ESBL-producingisolates were subjected to PCR experiments to detect ESBL genes, including, CTX-M, TEM, SHV CTX-M +TEM, CTX-M+SHV groupswere detected in 39.0%, 18.3%, 12.2%, 6.1%, and 1.2% of theESBL-producing E. coli strains respectively. None of the five groups of ESBL genes under test were demonstrated in 23.2% of the isolates. (Table 3).Among ESBL-producingE.coli isolates; CTX-M gene was found to be the most predominant gene among the isolates this result was in accordance with a study carried out by James H., who stated that CTX-M extended-spectrum beta-lactamases (ESBLs) had emerged as the most common type of ESBLGlobally (14).TheTheTheTheCTX-M family, first described in 1992 (15), is known to be themost dominant non-TEM, non-SHV ESBL among Enterobacteriaceaeand is recognized as a rapidly growing family ofESBLs that selectively prefer to hydrolyze cefotaxime ratherthan ceftazidime (16). On this study, the prevalence of blaSHV was 12.2% this result was slightly high compared tothe study carriedby PattarachaiKiratisin, who indicate that only 3.8%of ESBL-producing E. coli isolates carried blaSHV, while 87.4%of ESBL-producing K. pneumoniaeisolates carried blaSHV.The variation may be due to their isolates, which was collected from a health care associated infection in which CTX-M family is endemic(3).

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