Al-Kufa University Journal for Biology / VOL.11 / NO.1 / Year: 2019
Print ISSN: 2073-8854 Online ISSN: 2311-6544

Extended Spectrum β-lactamases (ESBL) producing *Escherichia coli* (*E.coli*)

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Abstract:

A total of 40 *Escherichia coli* (*E.coli*) isolates were recovered from women urinary tract infections (UTIs) in Al-Hilla teaching hospital, and private clinics. Isolates were identified to species level with a VITEK-2 system. Among the 40 women of UTI, 27(67.5%) had been preceded by a prior UTI and 13(32.5%) had not. The susceptibility to 4 antibiotics were tested using disc diffusion test, resulting in 9(33.3%), 7(26%), 3(11.1%), 1(4%) were resistant to ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, and nitrofurantion, respectively with prior UTI. For detection extended spectrum β-lactamase (ESBL) producing *E.coli*, all 40 isolates were tested by using double-disk test method, and then confirmed by using combination disk test. The results showed that 10 (25%) were β-lactamase producing isolates.

Key word : *E.coli*, VITEK-2 system , ESBL .

Introduction:

The pathogens causing urinary tract infections (UTIs) are almost always predictable, with *E.coli* the primary etiologic agent among both outpatients and inpatients [1]. The vast majority of UTIs arise in female outpatients, many of whom are treated empirically by physicians if their symptoms suggest acute uncomplicated bacterial cystitis [2]. Uropathogenic *E.coli* (UPEC) strain of *E.coli* is the most common pathogen isolated from the patients with UTI, often from the patient's own intestinal flora. Strain-specific virulence factors and host susceptibility give them the ability to colonize the urinary tract epithelia selectively, ascending through the urethra, bladder, possibly kidney tissue and cavities, triggering inflammatory reactions [3]. Guidelines recently published by the Infectious Diseases Society of America (IDSA) recommend trimethoprimsulfamethoxazole (SXT) as initial therapy for women with acute uncomplicated bacterial cystitis, but only in communities where the prevalence of SXT resistance is less than 10 to 20% [4]. The emergence of extended-spectrum β-lactamase (ESBL) producing bacteria, particularly *E.coli* and *Klebsiella pneumoniae* (*K. pneumoniae*), is now a critical concern for the development of therapies against bacterial infection. Since the early 1980s, the number of nosocomial infections by ESBL-producing, gram-negative bacteria has been increasing worldwide, and β-lactamase production has become a major causative agent for increasing resistance to antibiotics [5]. The ESBL genes are mostly plasmid encoded, and most ESBLs can be divided into 3 genotypes: TEM, SHV, and CTX-M [6]. The major ESBL producer was *K. pneumoniae* before 2000, and the predominant ESBL genotypes were TEM and SHV [7]. *E. coli* has now become an important ESBL carrier. In addition, a genotype CTX-M has become more prevalent worldwide compared to the TEM and SHV genotypes [8]. During the 1990s, ESBL-producing organisms were described mainly as members of the TEM- and SHV-β-lactamase families in *E. coli* and *K. pneumoniae* causing nosocomial outbreaks [9]. The emergence of *E. coli* isolates with multiple-antibiotic-resistant phenotypes, involving

co-resistance to four or more unrelated families of antibiotics, has been previously reported and is considered a serious health concern [10]. Transference of resistance determinants by mobile genetic elements including plasmids, transposons, and gene cassettes in integrons [11] and the alteration in *mar* locus regulation are important factors that can contribute to the increase in multiresistant bacteria.

The aim of study:

Extended spectrum β-lactamases (ESBLs) have emerged as a major threat worldwide with limited treatment options; hence the present study was aimed to determine the occurrence of ESBLs in *E.coli* isolates from women patients with UTI.

Materials and methods:

Bacterial isolates:

Fourty *E.coli* isolates were obtained from urine samples in Al-Hilla/Iraq during the period from May to July 2014. Urine samples were collected from women patients Al-Hilla Marjan teaching hospital, and private clinics in Al-Hilla city. Clinical isolates were identified as *E.coli* based on their morphology, Gram-staining. Vitek 2 system was performed to identify species level of *E.coli* isolates.

Antimicrobial Susceptibility Test:

1- Disc diffusion test (DD test):

The antimicrobial susceptibility patterns of isolates to different antimicrobial agents was determined and interpreted according to [11]. Disk diffusion test was used against 4 antibiotics, the following antimicrobial agents were obtained (from Oxoid, U.K) as standard reference disks as known potency for laboratory use: Ampicillin (l0mg), trimethoprim-sulfamethoxazol (1.25/ 23.75mg), ciprofloxacin (5mg), and nitrofurantion (300mg).

2- Detection of ESBL:

2-1- with disk diffusion method:

The methods proposed by [12] were followed, using 30mg disks of cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO) or aztreonam (AZT) (BBL, Becton Dickinson, USA) at a distance of 15mm from a disk of amoxicillin/clavulanic acid (AMC) (20/10mg). Positive result is indicated when the inhibition zones around any of the cephalosporin disks are augmented in the direction of the disk containing clavulanic acid.

2-2 Combination disk test (CDT):

The test was performed with Mueller-Hinton agar plates, and disks containing 30 µg of cefotaxime or ceftazidime with and without 10 µg of clavulanic acid. The test is positive if zone diameters increased by ≥5 mm for either cefotaxime, ceftazidime, when tested in combination with clavulanic acid versus its zone when tested alone, as indicated by the manufacturer or CLSI [11].

Results and Discussion:

A total of 40 *Escherichia coli* (*E.coli*) isolates were recovered from women urinary tract infections (UTIs) in Al-Hilla teaching hospital, and private clinics. Among the 40 woman of UTI, 27(67.5%) had been preceded by a prior UTI. The bacterial adhesion to urinary tract mucosa is a critical virulence factor. Type P

fimbriae mediate adhesion through recognition of digalactoside type residues (Galα1→Gal1β) of P-type antigens of blood groups. These receptors are expressed in epithelial cells of the urinary tract [13].

In the present study, resistance to ampicillin occurred in 9 (33.3%) patients with a prior history of UTI, and 6 (46.1%) isolates among patients without a prior history. The mechanism of resistance to β-lactam antibiotic is mostly due to either production of β-lactamases that hydrolyze β-lactam ring, or lack of penicillin receptors on cell wall and / or alteration in their permeability to β-lactam antibiotics preventing the uptaking of them [14]. Hassan *et al*, 2010 [15] found that all *E.coli* isolates were resistant to ampicillin, carbenicillin and ceftazidime, most isolates of *E.coli* were resistant to cefotaxime (78%), ceftriaxone (93%), ciprofloxacin (82%), levofloxacin (67%) and amoxicillin-clavulanic acid (67%).

Results showed that resistance to trimethoprim-sulfamethoxazol (TMP-SMZ) took place in 7 (26%) isolates with a prior history of UTI, and 5 (38.4%) isolates among patients without a prior history. Trimethoprim is a tetrahydrofolate reductase inhibitor that, when added to sulfamethoxazole, provides a second step block in the folate biosynthetic pathway. TMP-SMZ proved to be bactericidal [16]. Blocking folate metabolism at 2 sites decreased the emergence of resistance, nevertheless, resistance to TMP-SMZ has occurred because of amino acid substitutions in both enzymes, Plasmids (e.g., pSK41) carry the altered genes, which facilitate the spread of TMP-SMZ resistance. Exogenous thymidine will render TMP-SMZ inactive, because it bypasses the double biosynthetic blockade. Wright *et al*, 1999 [17] showed that resistance to TMP-SMX was present in 67 (15%) of 448 urinary coliform isolates.

In this study, resistance to ciprofloxacin found in 3 (11.1%) isolates with a prior history of UTI, and 1 (8%) isolates among those without a prior history. Ciprofloxacin, is bacteriocidal drug, affecting Gram positive and Gram negative bacteria. Generally anti Gram negative activity is more closely associated with DNA gyrase inhibition, whereas anti Gram positive activity is more closely associated with bacterial topoisomerase IV inhibition. DNAgyrase, and bacterial topoisomerase IV, are vital for dicating the proper topology of DNA which is important for protein biosynthesis, DNA replication and DNA repair, this interference with DNA transcription, replication, and repair will promote its cleavage, leading to rapid bacterial cell death. Also resistance to ciprofloxacin is most commonly associated with genetic-based alterations in the topoisomerases, resulting in decreased drug binding. Moreover, decreased porin presence and also decreases their uptake. Active uptake seems not to be a significant factor in their absorption although it is believed to contribute to distribution and excretion, and particularly with efflux of these agents from bacterial cells before they reach their intercellular targets [18]. Arslan *et al*, 2005 [19] showed that resistance to ciprofloxacin was present in 32 (42%) of 77 isolates from community-acquired urinary tract infections in Turkey.

During this study, resistance to nitrofurantion was 1 (4%) among patients with a prior history of UTI, and there was no resistance to nitrofurantoin among patients without a prior history. Nitrofurans are a group of compounds characterized by the presence of one or more nitro-groups on a nitroaromatic or nitroheterocyclic backbone. Examples of compounds belonging to this group include furazolidone, nitrofurazone and nitrofurantoin: drugs that all display antimicrobial activity and are used clinically to treat different types of infections. Nitrofurantoin is taken orally, rapidly absorbed and excreted in the urine to generate high therapeutic concentrations

[20]. Nitrofurantoin is prescribed for use against uncomplicated lower urinary tract infections (UTIs). Studies of *E.coli* extracts have shown that strains resistant and susceptible to nitrofurans differ in their ability to reduce the compounds, [21] suggesting that nitrofurans need to be activated by this reducing activity to show their antibiotic effect. Furthermore, it has been shown that there are two types of nitroreductase activities in *E.coli*: one type insensitive to oxygen (type I) and the other inhibited by oxygen (type II). Chemical analyses indicate that in type I nitroreduction, the nitro-moiety of the parent compound is reduced via a sequence of intermediates, including a nitroso and hydroxylamine state, to a biologically inactive end product where one of the intermediates is thought to be responsible for toxicity. The specific identity of the bio-reactive intermediate is not known, but it binds and disrupts bacterial DNA and protein and is believed to be hydroxylamine [22].

Karlowsky *et al*, 2003 [23] Showed that TMP-SMX, ampicillin, nitrofurantoin, and ciprofloxacin, susceptibilities for urinary *E.coli* resistant isolates from female outpatients in 1998–2001 were 17.5%, 38%, 0.8%, and 2.3%, respectively. Because of the low levels of resistance for nitrofurantoin and ciprofloxacin *in vitro*, they suggested that nitrofurantoin and ciprofloxacin may be used as adequate alternative therapy for female outpatients with uncomplicated urinary tract infections when TMP-SMX cannot be used. The activity of nitrofurantoin and ciprofloxacin against TMP-SMX resistant *E.coli* may be an important consideration for patient treatment.

All 40 isolates were tested by using double-disk test method for detection extended spectrum β-lactamase (ESBL) producing *E.coli*. The results showed that 10 (25%) were β-lactamase producing isolates which showed expansion of indicator cephalosporin inhibition zone towards amoxicillin-clavulanic acid disk. And then confirmed by using combination disk test that showed the same isolates (10) ≥ 5 mm increase in cephalosporin inhibition zone with clavulanic acid. Extended spectrum β-lactamases (ESBLs) are the enzymes produced by Gram-negative bacilli that have the ability to hydrolyze β-lactam antibiotics containing an oxyimino group (third generation cephalosporins and aztreonam) and are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam [24]. ESBLs are usually plasmid-mediated β-lactamases, most commonly found in *K.pneumoniae*, *E.coli* and other Gram-negative bacilli [25]. The number of (ESBL) producing *Enterobacteriaceae* isolated from clinical material derived from either hospitalized patients or those living in the community has increased [26]. Studies indicated that the drug resistance rate of ESBLs producing *E.coli* was higher than that of non ESBLs producing *E.coli*. It was also reported that the rate of multiple drug-resistant strain was higher in ESBLs producing *E.coli* than in non ESBLs producing *E.coli*, and that multiple drugresistant strains occurred frequently in drug-resistant strains [27]. Hence more attention should be paid clinically to ESBLs producing *E.coli*, and antibiotics should be used rationally according to the results of antimicrobial susceptibility tests. Multiple drug-resistance may be related to the extensive use of spectrum β-lactamases. However, its mechanism is still uncertain [28]. Lautenbach *et al*, 2001 [29] demonstrated that Of the 33 patients with ESBL-producing *E.coli*, and *K.pneumoniae* urinary infection, 25 (75.8%) of them had infections due to *K.pneumoniae* and 8 (24.2%) had infections due to *E.coli.* Villar *et al*, 2013 [30] found that 14 of 37 *E.coli* were ESBL producers from the pregnant women.

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