Prevalence of ESBL production among E.coli causing urinary tract infection

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Abstract

Urinary Tract Infections (UTIs) caused by Escherichia coli (E.coli) producing extended-spectrum β -lactamase (ESBL) are more expensive to treat than ESBL negative E.coli. Estimation the prevalence of ESBL-production among urinary E.coli isolates is essential due to its great effect on the choice of proper antimicrobials. Accordingly, the aim of this study was to detect the prevalence of ESBL-producing E. coli isolated from urine samples in Kirkuk/Iraq. 150 midstream urine samples collected from different hospitals in Kirkuk city. E.coli isolated and identified by microscopical examination, biochemical test and vitek 2 system. Phenotypic detection of ESBL was performed using double disk synergy method, also genotypic detection of ESBL genes (CTX-M) was performed using PCR technique. In this study 40 E.coli was detected, the incidence rate of ESBL-producing E.coli was 34(85%) .Remarkably ,dissemination of blaCTX-M gene was 33 (82.5%) among E.coli isolates and the gel electrophoresis showed that the molecular weight of amplified gene was 321 bp .

Keywords: Extended Spectrum Beta-lactamase, E.coli, Urinary tract infection ,CTX-M gene.

1. Introduction

UTIs are one of the most common microbial diseases and represent an important public health problem with large economic implications[1].The presence of bacteria more than 10^5 /ml in urine indicate the occurrence of UTI[2]. Among the bacterial species, *E.coli* accounts for 80% to 85% of the infections, followed by other enterobacteriaceae family members[3] *E.coli* are gram-negative bacilli that are facultatively anaerobic, non-spore-forming, and motile by peritrichous flagella in some strains[4]. *E.coli* uses several mechanisms to avoid the effects of antimicrobial agents, including alteration of the target protein in the cell wall, increasing the expression of drug efflux pumps, reducing the permeability of the outer membrane and production of Extended –Spectrum β-lactamases (ESBLs)[5]. ESBLs Producing bacteria can hydrolyze β-lactams antibiotics like penicillin and the cephalosporin group, but it is inactivated by clavulanic acid[6]. ESBL producing bacteria show coresistance to several other classes of antibiotics, resulting in the limitation of therapeutic options[7], ESBLs are divided into three categories: CTX-M, SHV, and TEM. The CTX-M genotype is the most common prevalence, have the ability to move between different bacterial population[8]. Antibiotic resistance is on the rise globally, owing to factors such as incomplete antibiotic courses ,inappropriate antibiotic use, prolonged hospital stays and self-medication. Resistance mechanisms such as ESBL production and Multi Drug Resistance (MDR) cause treatment failure[9].

2. Materials and Methods

Sample collection

A total of 40 *E.coli* isolated from urine samples. These samples were collected from different hospitals in Kirkuk city during the period from November 2021 to March 2022.

Identification of bacterial isolates

E.coli isolates were diagnosed depending on morphological features on eosin methylene blue agar, macConkey agar and blood agar, microscopic examination (Gram-stain), biochemical tests (Indole, Citrate utilizing, ,Methyl red, Voges-Proskauer, catalase, ,oxidase and urease tests) and finally identified by the Vitek 2 system[10].

Phenotypic detection of ESBLs production

Double –disk synergy test were used to detect ESBL production *E.coli* and as follow: The bacterial suspension was adjusted to 0.5 McFarland standardized suspension before being inoculated onto a Muller-Hinton agar plate by using a sterile cotton swab Then the β-lactam inhibitor cefotaxime-clavulanic acid (20/10) disk and cefotaxime disk were placed at a distance of 20 mm from each other (center to center) and incubated the plate for 24 hours at 37℃. A positive ESBL production meant that the zone produced by the cefotaxime-clavulic acid disk was ≥5 mm larger than the cefotaxime without inhibitor [11].

DNA extraction

Genomic DNA was extracted according to the DNA purification kit supplemented by the manufacturing company (Geneaid, Taiwan). Ananodrop spectrophotometer (AcTGen,Taiwan) was used to evaluation the purity and concentration of the extracted DNA samples .Then all DNA samples were stored at -20℃.

Molecular detection of β -lactamse (CTX-M) gene

β-lactmase gene(CTX-M) in UPEC isolates was

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detected using polymerase chain reaction assay(PCR) .The extracted DNA was used as a template for PCR amplification of uropathogenic *E.coli* CTX-M gene by forward primer F:5-TCAGCGAGTTGAGATCAAAAA-3/,and reverse primer R:5-AATGCTTTACCCAGCGTCAG-3/. PCR reaction were carried out in the total volume of 25µL, the reaction mixture was contained 5 µL AccuPower®PCR premix (Bioneer,korea),5 µL template DNA,1 µL F-primer,1 µL R-primer and 13 µL deionized nuclease free water. The conditions used for PCR assay are as follow :5 minutes of initial denaturation at 95℃ (1 cycle) ,followed by 35 cycles of denaturation DNA at 95℃ for 30 second ,annealing at 57℃ for 30 second and , extention at 72℃ for 40 second and final extention at 72℃ for 5 minutes (1 cycle).

Gel electrophoresis

The PCR products were separated by 1% agarose gel electrophoresis with 2 µL of eithidium bromide in 1X (TBE) buffer using DNA ladder (100-1000)bp (Bioneer ,Korea) at 90 volte for 80 minutes, the DNA was visualized under UV Transilluminater.

3. Result and discussion

Atotal of 40 *E.coli* isolates from 150 urine samples were collected from patients admitted to different Hospitalis in Kirkuk city.The results of our study are in agreenment with previous study ,which revealed that *Ecoli* formed 35% of the organisms, which were isolated in the urine culture[4], while disagreement with the result of other study, that showed *E.coli* formed 67.6% of the isolates[12]. *E.coli* isolates were identified according to their morphological features ,biochemical test and lastly by the vitek -2 system .*E.coli* isolates were produce metallic green sheen on eosin EMB,pink color on macConkey agar and beige color on blood agar. On microscopic examination , all *E.coli* isolates were gram negative rod and non spore-forming ,while biochemical test showed that all *E.coli* isolates were positive to indol,methyle-red and catalase while negative to Voges- proskauer ,citrate and urease test ,on kliglller iron agar , *E.coli* produce acid/acid no H2S with gas formation[13].

Phenotypic detection of β-lactamase

Double disk synergy test (DDST) were used for detection the capability of *E.coli* isolates for ESBL production .The incidence rate of ESBL-producing E.coli was 34(85%) as in Fig.1. Many mechanisms of resistance to β-lactam antibiotics exist among Enterobacteriaceae members; nevertheless, β-lactamases are the most prevalent and clinically relevant mechanism of resistance to β-lactam antibiotics within this bacterial group[14] .This result was approximate with the results of prevous studies about isolation *E.coli* producing ESBL, which showed that 85% and 85.24% of *E.coli* isolates were ESBL positive respectively[15,16] .While our result disagreement with the prevous study,which showed that 38.9 % of *E.coli* isolates were ESBL positive[17] .

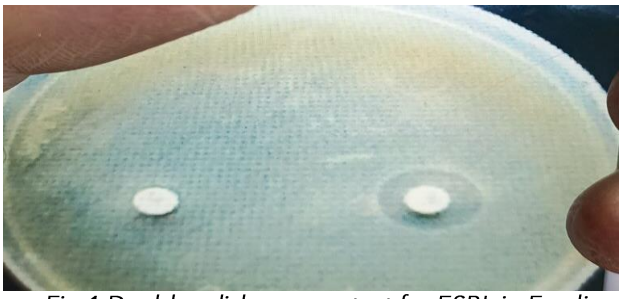

Fig.1:Double –disk synergy test for ESBL in E.coli

Genotypic detection of ESBL (CTX-M)

CTX-M gene detection was performed under optimal condition by using PCR technique. From a total 40 extracted DNA samples were used for screening the CTX-M gene .The Result showed that 82.5% of the isolates had this gene as in Fig.2 these result agreed with the prevous study carried by Jena *et al* (2017)[18],which showed that 82.6% of the isolates carried CTX-M gene, while our study disagreement with the study Ojer-Usoz *et al*(2017)[19], which showed 21.4% of *E.coli* isolates carried this gene.

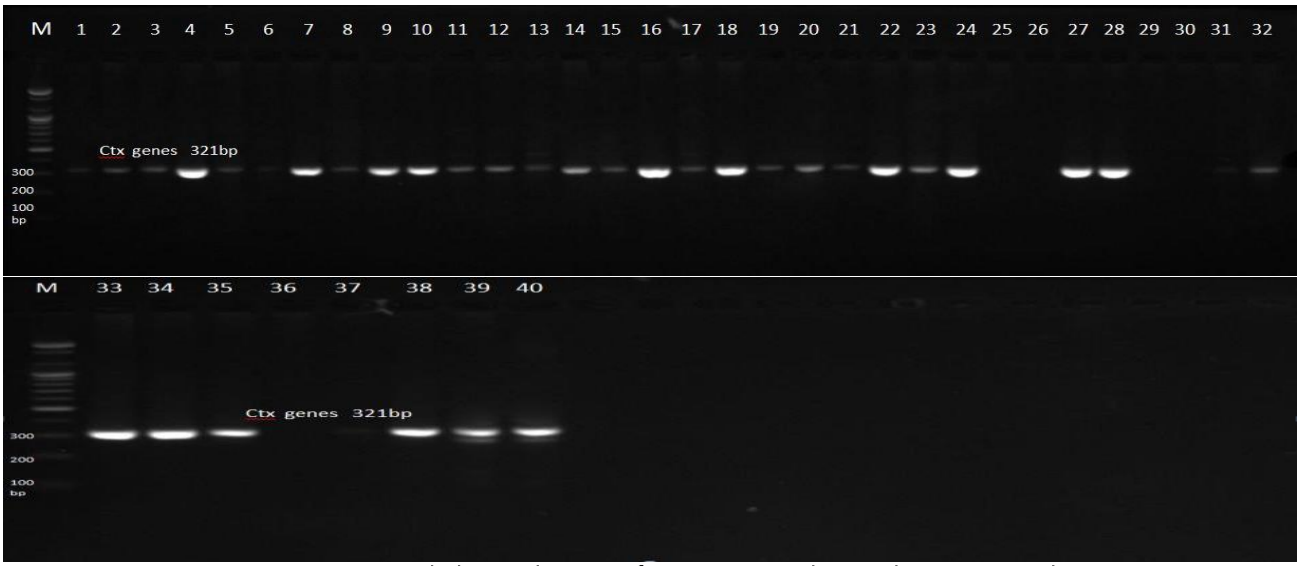

Fig.2: Agarose gel electrophoresis of CTX-M gene.The product size is 321bp

4. Conclusion

Our study showed the prevalence of the ESBL production *E.coli* isolated from UTI was (85%) and CTX-M gene was 82.5% .Rapid diagnosis of resistant strains is essential in order to choose effective treatment options and to prevent the increase of resistance .It is also advised that the treatment of UTIs should be done according to the resistance and susceptibility pattern in order to avoid drug resistance and treatment failure ,which can lead to infection complications.

5. References

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