DETERMINATION OF THE MARKERS OF MULTI DRUGS RESISTANT GENES IN ESCHERICHIA COLI ISOLATED FROM URINARY TRACT INFECTION

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Abstract

The urinary tract is the most common site of E. coli infection and more than 90% of all uncomplicated urinary tract infections (UTIs) are caused by E. coli infection. The recurrence rate after a first E. coli infection is 44% over 12 months. E. coli UTIs are caused by uropathogenic strains of E. coli. This study is designed to investigate the prevalence of E. coli in local patients suffering from UTIs and to determine the genes associated with multi-durgs antibiotic resistance of E. coli and designing an alternative therapy by using species specific bacteriophages derived from stools and urine of patients. This study was carried out using 820 samples comprised of 100 stool and 620 urine from patients with UTIs. Out of the total number, there were four hundred twenty samples had E.coli (51%) as determined by microbiological, biochemical and vitek 2 system and confirmed by 16sRNA. Infection was found to be. More frequent in females (73.17%) than in males (28.82%). The antibiotic sensitivity tests were carried out on E.coli isolates against 16 different antibiotics. Results revealed resistance at high ratios for nine antibiotics: Cefotaxime (96.56%), Azithromycin (90%), Augmentin (86%), Etrapanem (85%), Cefipime (82.81%), Ceftriaxone (84.37%), Raffampin (83%) Ceftazidime (81.25%) and Norflo acin (61%). On the other hand, a lower percentage of antibiotic resistance was seen with Nitrification (59.37%), Doxycycline (45.31%), Ciprofloxacin (40%), Impenien (18.75%), Meropenien (12.50%), Amikcin (4.68%) and Levoflaxacin (2.50%). Presence of integrons and the insertion (IS) elements in resistant E. coli were examined by PCR and sequencing. Class 1 integrons were detected in all antibiotics resistant E. coli isolates, while only 40% E. coli isolates had class 2 integrons and none had class 3 integrons gene. Additional, ISECP1, ISCR1, IS26 and IS903 appeared in 10%, 5%, 20% and 5%, respectively , of tested resistant isolates.

Key words: Urinary tract infection, E. coli, antibiotic resistance, integrons, insertion elements, 16srRNA.

Introduction

Urinary tract infection (UTI) is defined as the presence of microbial pathogens in the urinary tract (Ahmed, 2014). The infection of the bladder and urethra are referred to as the infection of the lower urinary tract whereas the kidney and ureter infection is an indication of upper urinary tract infection. UTIs can be classified as uncomplicated or complicated based on the factor that triggers the infection primary or recurrent depending on the nature of occurrence. UTI can be a symptomatic or symptomatic, characterized by a wide spectrum of symptoms ranging from mild burning icteritition to bacteremia, sepsis, or even death (Gawad et al., 2018). Microorganisms routinely isolated from urine during UTIs include Staphylococcus spp., Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp., Proteus spp, Enterobacter spp., Citrobacter spp., Enterococci spp. and Candida spp. (Nguyen, 2008). Escherichia coli strains belonging to enterobacteriaceae family are normal habitant of gastrointestinal tract in the wide range of warm blooded hosts. Many E. coli strains are harmless while some are pathogenic, meaning that they can cause illness, either diarrhea or infections in the intestinal sites such as urinary tract infections (UTI) in human.
Despite *E. coli* is a chief composing of the normal intestinal flora, but it identified as very active opportunistic pathogen associated with UTIs. It is causing more than 90% of all cases of UTI (Forsyth et al., 2018).

*Escherichia coli* is greater global common health concern, because it is becoming resistant to currently available antibiotics (Subash et al., 2014). Across the United States and Canada, urinary tract isolates of *E. coli* from out-patient clinics showed increased resistance to antibiotics (Zhanel et al., 2000). *E. coli* is characterized by its characteristic of multidrug resistance (MDR) (Nepal et al., 2017). It is characterized by high resistance to antibiotics as a result of possessing resistance enzymes such as β- lactamases that give resistance to beta- lactams, aminoglycosides, antimicrobials, and quinolones.

**Materials and Methods**

**Collection of Urine and Stool Samples**

The study carried out on 820 midstream urine and stool samples were collected in sterile cups from patients attending Central Teaching hospital of pediatrics, Yarmouk, Ibn-balady, Fatima Al-Zhraa hospitals, private urology clinic and central public health laboratory during the period from December 2018 to August 2019. Samples were collected and transported to the laboratory during one hour by using a cold box.

**DNA Extraction and Detection of Integrons**

DNA extraction was performed with DNA and plasmid extraction kits (Promega, USA) using a pre-treatment protocol for Gram-negative bacteria and following the manufacturer’s instruction.

The quantity and quality of the DNA was analyzed using a Nano drop 1000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA).

**Detection of class 1, class 2 and class 3 integrons and 16sRNA**

*E. coli* DNA amplification was performed in a DNA thermal cycler Gene Amp® PCR system 2700 (Applied Biosystems Division, Foster City, CA, USA) in a final volume of 20 containing 6 µl of DNA extract mixed with 6 µl of Deionized sterile water, 10 µl of Green Master Mix (1), 1 µl of each primer and 2 µl of DNA polymerase (Promega, USA). The conditions of the amplification were as follows: initial denaturation at 94°C for 10 min, followed by 30 cycles of DNA denaturation at 94°C for 45 s, primer annealing at 62°C (intI 1 and intI 2) or 60°C (intI 3 and 16sRNA) for 35 s, primer extension at 72°C for 2 min and a final elongation at 72°C for 7 min. Positive and negative control (Saenz et al., 2004) were included in all PCR assays and 1 kb ladder (Invitrogen) was used as a molecular size standard. After amplification, PCR products were separated by electrophoresis on 1% agarose gel in 1 TBE buffer, stained with ethidium bromide and visualized by UV transillumination.

**Detection of Insertion Sequences**

DNA extracts were aminated for the detection of different insertion sequences associated with ESBL genes, performing PCRs assays using the specific primers and showed in table 1 (Eckert et al., 2006). The PCRs were performed in a final volume of volume of 20 containing 6 µl of DNA extract mixed with 6 µl of Demozone sterile water, 10 µl of Green Master Mix (1), 1 µl of each primer and 2 µl of DNA polymerase (Promega, USA), in a DNA thermal cycler GeneAmp® PCR system 2700 (Applied Biosystems Division, Foster City, CA, USA). Amplification conditions were modified in order to improve the specificity using an initial denaturation at 94°C for 12 min, followed by 35 cycles of DNA denaturation at 94°C for 1 min and primer annealing temperature depending on the IS primer extension at 72°C for 2 min and a final elongation at 72°C for 10 min. PCR products were separated by electrophoresis on 1% agarose gels and were visualized under UV light after staining with ethidium bromide.

**Results**

**Antibiotic Resistance of Ecoli Isolates**

Out of the total 820 samples collected from UTI patients, there were 420 *E. coli* isolates (51%) resisted a wide range of nine antibiotics cefotaxime, Aztromycin,
Augmentin, Etrapanem, Cefipime, Ceftriaxone, Rifampin, ceftazidime and Norfloxacin at higher ratios of 96.56%, 90%, 86%, 85%, 82.81%, 84.37%, 83%, 81.25% and 61%, respectively. Lower levels of antibiotic resistance were observed for Nitrofucation, Doxycycline, Ciprofloxacin, Imipenien, Meropenien, Amikcin and Levofoxacin at 59.37%, 45.31%, 40%, 18.75%, 12.50%, 68% and 2.50%. Accordingly, antibiotic resistance of *E.coli* isolates was divided into two groups based on the number of resistant antibiotics. The first group included isolates resisted to 6, 7, 8 or 9 antibiotics with ratios of 29.76%, 23.80%, 19.04% and 13.57%, respectively. The second group of *E.coli* resisted 10 or 16 antibiotics at ratios of 7.85% and 5.95%, respectively. After isolation and quantitation of genomic DNA from *E.coli* isolates, it was run on 0.8% agarose gel to assess its quality.

Presence of 16SrRNA by PCR amplification and specific primers. All isolates (100%) were positive for 16sRNA with amplified DNA bands of 723bp as shown in 1.

**Detection of Class 1, 2, 3 Integrons Resistant Genes**

Forty out of 200 antibiotics resistant *E.coli* isolates had class 1 Integrons (100%) after amplification of 483bp of specific gene Sequences as shown in fig. 2. While only 16 *E.coli* isolates had class 2 integrons with specific amplicon size of 483 after PCR amplification and gel electrophoresis fig. 3. On other hand, The class 3 integrons gene was absent in all tested local *E.coli* isolates. When there the exacerbated antibiotic resistant was associated with the differential detection/presence of positive integrons resistant genes is not yet clear.

**Detection of insertion elements (IS) in local *E.coli* isolates**

The IS elements tested were ISECP1, ISCR1, IS26 and IS903 by PCR amplification using specific primers. Only two isolates (13.32%) had ISECP1 fig. 4 and one...
isolate (6.66%) appeared to have ISCR1 fig. 5. Four
isolates (26.66%) had IS26 fig. 6, while IS903 appeared
in one isolate only (6.66%) fig. 7.

Discussion

Results of this study of multiple antibiotic resistance
to the third generation of cephalosporin represented by
Cefotaxime and Ceftriaxone was at higher ratios of
96.56% and 84.37%, respectively. These results were in
consistent with those reported by Mussa and Al-Mathkury,
(2018). They attributed the resistance for these two
antibiotics due to the fact that the bacteria used natural
efflux systems. Doxycycline bacteriostatic antibiotic
belongs to the tetracycline group that acts by inhibiting
protein synthesis. Tested UPEC Ecoli isolates showed
there a 45% resistance against Doxycycline. Resistance
development of E.coli isolates to Doxycycline could be
explained by active efflux bacteria due to decrease in
antibiotic concentration inside bacterial cell the second
less common change of target location (Kapoor, 2017).

Amoxicillin-clavulanic acid resulted in antibiotic
resistance up to 86%. In contrast, only 34% of E.coli
resisted the same drug (Khadum et al., 2018). The causes
of Resistance were due to a change in the permeability
of the outer membrane of the bacterial cell and its
possession of efflux and ToIC-AcrAB systems.

The resistance to Nitro furan was found to be 59.37%.
It induces its action by rendering bacterial flavoproteins
to in activate or altering bacterial ribosomal proteins and
other macromolecules. It was reported that the resistance
to Nitrofuran was 45.5% (Hooton et al., 2004; Reis et
al., 2016 and Abdu et al., 2018). In contrast, the
resistance rate was as low as 4% and 37.5% (Khadum
et al., 2018 and Ateba et al., 2020), respectively.

The high susceptibility to Quinolones might be due to
its binding ability to gyrase and topoisomerase IV to disrupt
enzymes function leading to DNA damage (Aldred et
al., 2014). A resistance rate of 50-70% against Levoflo
acin was reported in Baghdad city (Naji et al., 2017). In
Pakistan, the resistance to Levofloxacin was 80.8% (Altaf
et al., 2019). The excessive of treatment and inappropriate
use of these antibiotics may led to developing resistance
(Gupta et al., 2011).

Imipenem is antibiotic that act by the inhibition of
peptidoglycan synthesis in bacterial cell walls through the
inhibition of essential enzymes (transpeptidases) involved
in the terminal stages of peptidoglycan biosynthesis and
highly resistant to β-lactamase (Papp-Wallace et al.,
2011).

The second most active antibiotic against tested
UPEC was Amikacin with a resistance rate of 4%. These
results were consistent with the previously reported
resistance of 5.8% (Al-Samarai et al., 2016). Amikacin
is aminoglycoside antibiotics that acts by inhibiting protein
synthesis through a modification at the ribosomes (Gad
et al., 2011).

In this study the resistance to Meropenen,
Aztromycin, Etrapenem and Rafampin were at 12.5%,
90%, 85% and 80, respectively. Another study on the
antimicrobial resistance by Binod et al., (2018) in Nepal
recorded a low percentage of resistance against
Meropenem (12.5%). No resistance among *E. coli* isolates to Meropenem, Etrapenem were seen (Paula, 2017).

The multiple antibiotic resistance among *E. coli* isolates is relatively of high incidence which may be due to the high genome diversity with the ability to gain or lose genes through a horizontal gene transfer, therefore, the antibiotic resistance genes can transfer from one organism to another which contributes to increase the rate of emerging of resistance (Rasko et al., 2008). It was shown that 76.51% of *E. coli* isolates resisted a multiple antimicrobials of different structural classes and considered multidrug resistant (Karlowsky et al., 2003).

**Detection of *E. coli* Multi antibiotic Resistant Genes**

In this study class-II introngs appeared in 10% of isolates and no class-III integrons was detected. In Iran, integrons 1, 2 and 3 appeared in 22.05%, 33.34% and 6.25% of *E. coli* isolated from children with UTI, respectively (Rezaee et al., 2011). Integrons were detected in fluoroquinolones, β-lactams, aminoglycosides, trimethoprim and chloramphenicol (Maguire, 2001). Since IS elements were shown to be responsible for the rapid transmission of bacterial multidrug resistance in the environment (Zinser, 2002). IS26 was detected in 20% of local *E. coli* isolates involved in UTI (this study).

Thus, the coexistence of diverse types of integrons and IS sequence suggest possible risk for the dissemination of resistant genes among different microbial agents and environment (Zhang, 2009). Integrons may confer resistance for antibacterial isolates due to the presence of *sul1* (Zhao et al., 2001). This mechanism may explain how a multiple resistance for various antibiotics was acquired simultaneously such as aminoglycosides, cephalosporin, the penicillin and trimethoprim (White et al., 2002).

**Conclusion**

In this study, it is clear that the genes responsible for conferring multiantibiotic resistance were located on integrons and IS elements. Integrons class1 was present in all tested *E. coli*, while the prevalence of integrons class2 appeared at a much lower ratio but int3 was absent. Detection of IS elements showed that IS26 was distributed in 20% of isolates. The combination ISCP1, IS903, ISCR1 distributed in 5% of isolates. Whether multi antibiotic resistance is conferred by a certain single gene rather another or even the combination of multiple resistant genes synergistic effects are still uncertain.

Different environments and, therefore, additional investigations regarding the genetic composition of these integrons and insertion sequences are encouraged, to understand the role of these mobile elements in the spread of multidrug-resistant bacteria.

**References**


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