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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%), Rafampin (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 icturition 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.

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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 antihistamines 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

Table 1: Primer Sets Used For the Detection of Resistance Genes of *E.coli* Involved In UTI.

Primer	Primer sequence (5′ ⟶ 3′)
16S rRNA	F-CGAGTGGCGGACGGGTGAGT R-GTGGA TCGACATCGTTTACGGC
Int I	F-GGTCAAGGATCTGGATTTCG R-ACATGCGTGTAAATCATCGTC
IntII	F-CACGGATATGCGACAAAAAGGT R-GTAGCAAACGAGTGACGAAATG
Int II	F-AGTGGGTGGCGAATGAGTG R-TGTTCTTGTATCGGCAGGTG
ISECP1	F-ATCTAACATCAAATGCAGG R-AGACTGCTTCTCACACAT
IS26	F-TCACTCCACGATTTACCGCT R-CTTACCAGGCGCATTTCGCC
ISCR1	F-GTCGCTGCGAGGATTGTCATC R-CTCGCTTGAGGCGTTGCAT
IS903	F-CATATGAAATCATCTGCGC R-CCGTAGCGGGTTGTGTTTTC

Int-integrons; IS-insertionelement; F-forward; R-reverse

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 amined 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 Demonized 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, Rafampin, 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, Cprifloxacin, Impenien, Meropenien, Amikcin and Levofloxacin 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

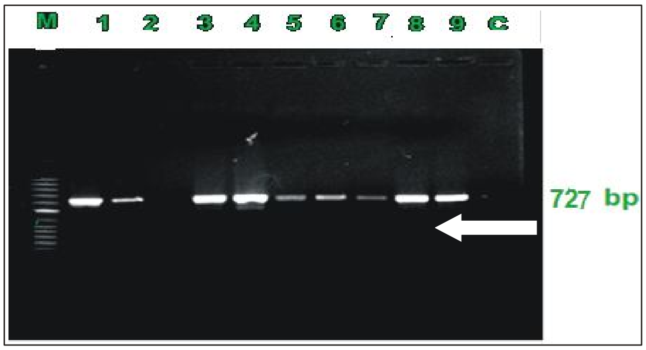

Fig. 1: Amplification of 16sRNA of *E.coli* isolates by PCR. Lanesorder: M, DNA marker (100-1500bp); Lanes, 11-9 positive 16sRNA and C, negative control.

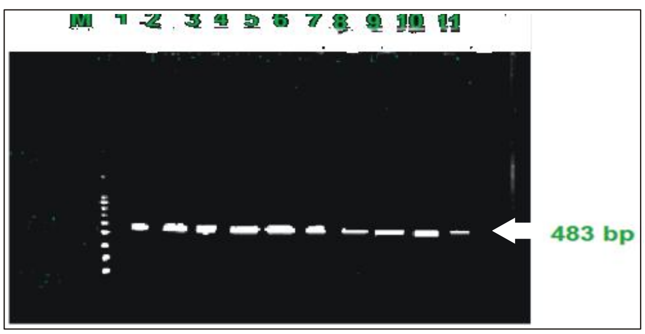

Fig. 2: Detection of class1 integrons from *E.coli* local isolates by PCR. Lanes order: M, DNA marker (100-1500bp); Lanes 1-10, positive class 1 integrons and C, a negative control.

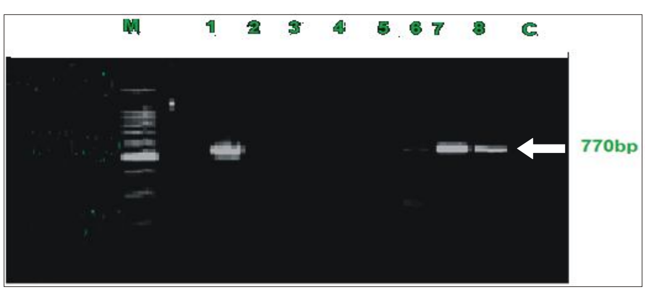

Fig. 3: Detection of class 2 integrons of *E .coli* local isolates by PCR. Lanes order: M, DNA marker (100-1500bp); Lanes 1-10 positive of class 2 integrons and C, a negative control.

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

Fourty 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

Fig. 4: Detection of ISCP1 from *E.coli* local isolates by PCR. Lanes order: M, DNA marker (100-1500bp); Lanes 1 and 3, positive of ISCP 1, while lanes 2, 4 and 8; negative results and C, a negative control.

Fig. 5: Detection of ISCR1 from *E.coli* local isolates by PCR. Lanes order: M, DNA marker (100-1500bp), Lane 1, positive of ISCR 1, lanes 2-8, negative results and C, a negative control.

Fig. 6: Detection of IS26 from *E.coli* local isolates by PCR. Lanes order: M, DNA marker (100 -1500bp); Lanes 1, 3, 6 and 7; positive results of 1500bp); Lanes1, 3, 6 and 7; positive results of IS26; lanes 2, 4, 5; negative results and C, a negative control.

Fig. 7: Detection of IS903 from *E.coli* local isolates by PCR. Lanes order: M, DNA marker (100-1500bp), Lanes 1, positive of IS903; lanes 2-8, negative results and C, a negative control.

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 Cefotxaime 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).

Amoicillin-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 high susceptibility of *E.coli* to Imipenem may be due to the limited use of this type of antibiotic and / or to administration routes by either intravenous or intramuscular (Kanj and Kanafani, 2011). The resistance to Imipenem was 12% (Fehan, 2019). No resistance among *E.coli* isolates to Imipenem was seen (Aljanaby and Aljanaby, 2018).

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 Meropenien, 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

Meropenien (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 s 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 e plain 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 intI 3 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.

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