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Prevalence of Class-1 Integron Genes among Multi-Drug Resistance of Uropathogenic *Escherichia coli* from Pregnant Women

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ABSTRACT

The first aim of this study was determination of the prevalence of urinary tract infections (UTI) among pregnant women in Basra- city. They were diagnosed by VITEK-2 System. *Escherichia coli* was the major pathogen (52.5%) causing UTI, followed by less percentages pathogens through*Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *proteus mirabilis* and *Enterobacter*spp. The second aim was investigation the frequency of integron genes intI-1, intI- 2 and intI-3 of *E. coli* strain and their association with antibiotic resistance used in this study. A total 42(52.5%) *E. coli* isolates from urine samples were tested againt to 10 different antibiotics and that most strains (90.4%) were as Multi- Drug Resistances at significant differences ($p< 0.05$). Among 42 isolates of MDR- *E. coli* , 31(74%) had the integron genes. The most common of strains had integron genes class-1 (intI-1) at percentage 25(60%), wherease integron genes class-2 (intI-2) and class-3 (intI-3) at percentages 6(14%) and (0%) respectively. Therefore, preventive strategies are necessary to restrict further dissemination of resistant strains.

Keywords:*E. coli*, UTI, pregnant women, multi-drug resistances, class-1 integron gene.

Introduction:

Urinary tract infection (UTI) was one of the most common infection that was diagnosed inhospitalized patients and outpatients in the world. Also, World Health Organization (WHO) were recorded about 150 millions individual infected with UTI each year[1]. Furthermore, these UTI can lead to significant mortality in the worldwide [2]. The infection cases of UTI in pregnant women were highly increased comparison with men because hormonal changes may change normal function of urinary tract [3]. Urinary tract infections are classified on their target sites by microbial species: such as bacteriuria (urine infection) or pyelonephritis (Kidney infection) and cystitis (bladder infection). Also, these infections can be asymptomatic or associated with symptoms [4]. The international studies were recorded that about 80-90% of acute UTI in the pregnant women were caused by *Escherichia coli*[5,6]. In addition, other Gram- negative rods such as *proteus mirabilis* and *Klebsiella pneumonia* were also common. Gram-positive bacteria such as *Streptococcus* group-B and *Staphylococcus saprophyticus*were less common in pregnant women in UTI [7]. Therefore, this study was aimed for isolation and detection of Multi- Drug Resistance (MDR) of *E. coli* from urinary tract infection of pregnant women in outpatients at Basra- city and determine the frequency of Integron genes (intI-1, intI-2 and intI-3) among isolates of MDR- *E. coli*.

Materials and Methods

Study design:
A total 85 women were diagnosed as urinary tract infection by obstetrician obstetrician and gynaecologist at private clinic- Basra city. These women were with an age range of 25-80 years.

Urine sample culture:
Urine samples were obtained by sterile container and cultured on Blood Agar, MacConkey Agar media, then were incubated at 37° c for 24-48 h. In addition, diagnosis of UTI was made when there were at least 10^5 organisms/ml of urine [8]. Isolates were identified by Gram staining and convention biochemical methods [9].

Bacterial diagnosis :
All bacterial isolates were diagnosed by biochemically with API-20E System and VITEK-2 Compact system (Bio Merien- France) according to the manufacture instruction. Isolates were stored in maintenance medium at 4° c until they were used.

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Urine nitrite test:
The Nitrite Test of urine was made by using Dipstick Test. A positive test indicate that cause of the UTI is Gram-negative bacteria [10].

Antibiotic sensitivity testing:
The antibiotics sensitivity test were achieved for all isolates by using Disc Diffusion Method and the results were analyzed according to the Clinical and Laboratory Standards Institute (CLSI, 2018)[11]. Table (1) was shown the antibiotics using in the present study.

Detection of MDR- *E. coli* isolates:
MDR- *E. coli* isolates were determined according to CLSI (2006). Any isolate resistance at least for three classes of different antibiotics was considered as MDR- isolate.

Identification of the integron genes:
Table (2) was shown detection of integron genes (intI-1, intI-2 and intI-3) for all isolates of MDR- *E. coli* using Polymerase Chain Reaction (PCR) through set of primers given in that table . The thermal cyclers conditions for intI-1 and intI-2 genes were as following : 96℃ for 30 second, 55℃ for 1min. and 70℃ for 3 min, followed by 25cycles of 96℃ for 15 min, 55℃ for 30 min and 70℃ for 3 min. Wherase PCR for intI -3 gene was performed at 95℃ for 5 min. Then, they were followed at 94℃-2 minutes, 57℃-1 minute and 72℃- 1.5 minute for 30 cycles [12].

Data analysis:
The program of Statistical Package for Social Sciences (SPSS)- 18 version was used for analyzing the results of antibiotics and resistance genes. The Chi-square test was used for analyzing at significant differences ($p< 0.05$).

Table 1. Antimicrobial categoriesproposed against *E. coli* isolates

Antimicrobial categories	**Drug disc/ Potency (µg)**
Aminoglycosides	Streptomycin (S)(10 µg), Amikacin (AK)(30 µg)
Tetracyclin	Tetracyclin(TE) (30 µg)
Quinolones	Nalidixic acid (NA)(30 µg) Ciprofloxacin (CIP)(5 µg)
Phenicol derivatives	Chloramphenicol (C)(30 µg)
Cephalosporins	Cefotaxime (CTX)(30 µg) Ceftriaxone (CRO)(30 µg)
Penicillins/β-lactamase inhibitors	Ampicillin (AMP)(10 µg)
Monobactam	Aztreonam (AZT)(30 µg)

Table 2. Primer pairs sequences and product size

Primers	**Sequence (5 to 3)**	**Product size (bp)**
intI-1	F: GGTCAAGGATCTGGATTTCG R: ACATGCGTGTAAATCATCGTC	436

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intI-2	F: CACGGATATGCGACAAAAAGG R: TGTAGCAAACGAGTGACGAAATG	788
intI-3	F: AGTGGGTGGCGAATGAGTG R: TGTTCTTGTATCGGCAGGTG	600

Results

Prevalence of *Escherichia coli* in UTI

Eighty five of women patients with clinical symptoms of UTI in this study were diagnosed by obstetrician and gynaercologist at private clinic- Basra city and 85 urine samples were collected from them. 80 cases had positive urine culture and 5 cases didn't have any bacteria. In the figure (1), the frequencies of all isolates were diagnosed from positive urine cultures . The most common pathogens isolated were*E. coli* 42(52%) followed by *Klebsiella pneumonia* 11(13.7%), *Staphylococcus aureus* 8(10%), *Pseudomonas aeruginosa* 7(8.7%), *Staphylococcus epidermidis* 5(6.2%), *Proteus mirabilis* 4(5%) and *Enterobacter* spp. 3(3.7%).

Patterns of antibiotics resistance for *E. coli* :

Table (3) shows patterns of antibiotics resistance against *E. coli* isolates. Most isolates of *E. coli* were high resistance 42(52.5%) at ($p < 0.05$) toward most antibiotics used as treatment in the hospitals. addition, this table (3) explained most *E. coli* isolates 38(90.4%) were determined as MDR- isolates (isolates resistance for three classes of different antibiotics).

Distribution of integron genes among MDR- *E. coli* isolates

Among 42 isolates of MDR- *E. coli* , 31(74%) had the integron genes. The intI-1 and intI-2 genes were identified with percentage 25(60%) and 6(14%) respectively, whereas the intI-3 gene was not identified in any isolates. Thes results were shown in Figure (2,3).

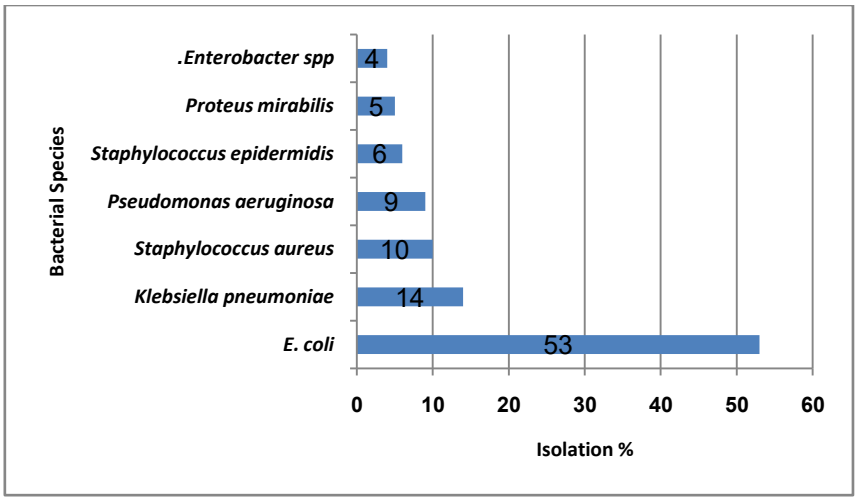

Figure 1. Frequency of the bacterial isolates from urine samples

Table 3. Patterns of antibiotics resistance for *E. coli*

Antibiotic	Diffusion zone(mm)	Total number of isolate		
		Sensitive S(%)	Intermediate I(%)	Resistant R(%)

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Streptomycin	≤ 11	0	22(52.3%)	20(47.6%)
Amikacin	≤ 11	4(9.5%)	4(9.5%)	34(80.9%)
Tetracycline	≤ 11	0	0	42(100%)
Nalidixic acid	≤ 11	6(14.2%)	0	36(85.7%)
Ciprofloxacin	≤ 15	4(9.5%)	15(35.7%)	23(54.7%)
Chloramphenicol	≤ 12	7(16.6%)	0	35(83.3%)
Cefotaxime	≤ 14	2(4.7%)	3(7.1%)	37(88%)
Ceftriaxone	≤ 13	9(21.4%)	0	33(78.5%)
Ampicillin	≤ 13	0	0	100(100%)
Aztreonam	≤ 12	7(16.6%)	5(11.9%)	30(71.4%)

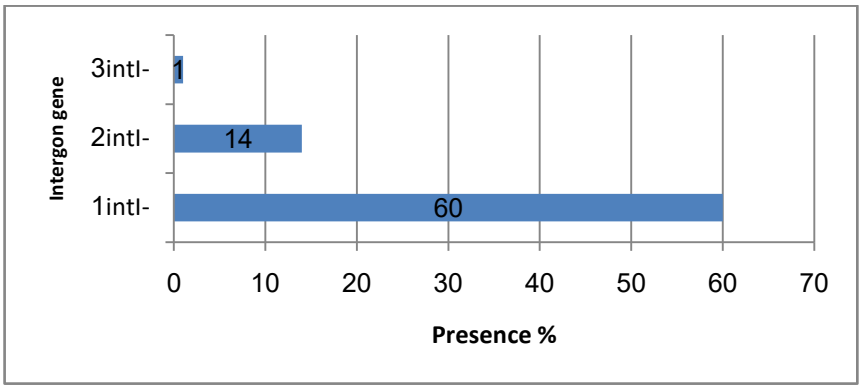

Figure 2. Percentages of integron genes for MDR- *E. coli*

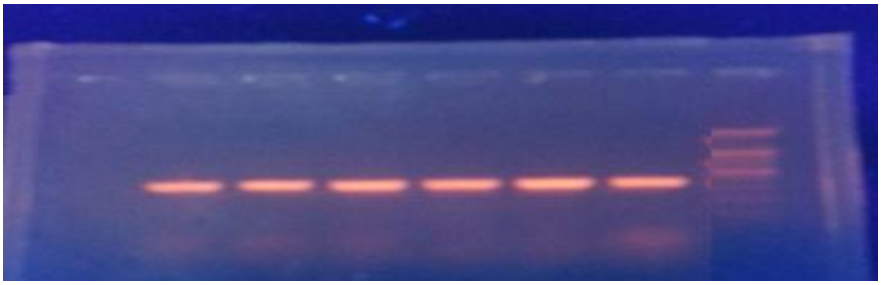

Figure 3.PCR amplification of intI-1 genesfor MDR- *E. coli* Lane L: DNA ladder, lan1-10: amplified genes of intI-1 (fragments of 483bp)

Discussion

This study was the first for investigation the relationship between uropathogenic of MDR-*E. coli* isolates from pregnant women with integron genes (intI-1,2,3). The urinary tract infection was represented as most common infections that affected for all age groups and they are the most frequent cause of morbidity in pregnant women [13]. In this study descries high percentage of pregnant women at (30-45) ages infected with UTI because these ages of women are more susceptible to change in anatomical and physiological features [14]. 80 urine samples which were processed for screening of

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UTI, the most predominant isolate was 52.5% isolates of *E. coli* at significant differences ($p < o.o1$) from another bacterial types. Identification *E. coli* isolates were done by biochemical tests, VITEK-2 System and urine nitrite test because VITEK-2 System is very accurance for identification of the pathogenic bacteria [15]. Also, urine nitrite test was considered as a rapid screening test for bacteriuria[14]. The present study was explained most *E. coli* isolates as causative agents. In study in Iran, it was founded most common of urinary tract infections by *E. coli* isolates especially in women [16]. In addition, these results were similar with another studies in Iraq [17,18]. These uropathogens have the ability to bind to the bladder epithelium and form biofilm- like intracellular bacterial that are responsible for colonization and protect their organisms from antibiotics [19]. In this study, it shower a high risk of antibiotic resistance of *E. coli* isolates in pregnant women and about 38(90.4%) *E. coli* isolates were determined as MDR . These results were agreement with the findings of previous studies that carried out at different countries in the world; for example in Iraq [20,21]. Several studies showed the antibiotic resistance among Gram- negative bacteria, including *E. coli*, has become a serious problem in worldwide. Also, the integrons are considered as the source of transferring drug resistance between pathogens [22]. Previous studies are confirmed that theseintegrons especially class-1 integron can be considered as transferring integrons for antibiotics resistance through MDR- *E. coli* isolates [23,24]. present study was explained that the most common of MDR- *E. coli* isolates had the integron genes, especially intI-1 in 25(60%) comparing with integron intI-2 and intI-3(14%) and (0%) respectively. MDR- *E. coli* isolates were encoded by resistance genes exist on the integrons and they were considered as active genetic elements to transfer resistance between the bacterial isolates.In our study, similar to another studies [25,26]. For example, another studywas achieved by Karimi *et al. al*.,(2012)[27], he studied antibiotics resistance for 12 antibiotics against 175 *E. coli* isolates and he was founded most resistance isolates carrying class-1 integron genes.

Conclusion:

The present study showed that most local isolates of *E. coli* from pregnant women in Basra city had ability as MDR and almost these isolates were carried class-1 integron genes. Also, these genes can be transferred to other clinical strains and they increase urinary tract infections.

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