

**Characterization of *Escherichia Coli* Isolates from Patients with Urinary Tract Infections from Thabet Hospital-Tulkarm, Palestine**

توصيف الإشريكية القولونية المعزولة من المرضى الذين يعانون من التهابات المسالك البولية في مستشفى ثابت- طولكرم- فلسطين

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Received: (18/6/2014), Accepted: (11/3/2015)

**Abstract**

This study aimed to analyze phylogenetic groups, the presence of class I, II, III integrons and resistance phenotype in a collection of fifty *E. coli* strains isolated from urine specimens obtained from suspected cases with urinary tract infections at Thabet Hospital, During May-December 2012. The antibiotic susceptibility testing for these isolates was done by disk diffusion method. The phylogenetic groups and class I, II and III integrons were determined by multiplex PCR. Statistical analysis was done by using Mann-Whitney U-Test (Two-tailed), Chi-square ( $\chi^2$ ) test or Fisher exact test. The results showed that 36 (72%) of the studied strains, belonged to group D, 13 (26%) strains to group A, and 1 (2%) strain belonged to group B1. Twenty one (42%) of *E. coli* strains carried class I integrons. Prevalence of class I integrons in group D and group A was 44.4% and 30.8%, respectively. There was no significant difference in the mean of antibiotic resistance score for strains belonged to group D and carried class I integrons and those belonged to group A and harbored the same integron class. Antibiotic resistance has



( $P = 6.2 \times 10^{-4}$ ). أظهرت النتائج في مخطط الشجرة (Dendrogram) تكون مجموعتين كبيرتين تعتمدان على المقاومة/الحساسية لسلاسل الإشريكية القولونية لفلوروكينولونات، وكان ارتباط مقاومة العزلات لمضاد ميثوبريم- السلفاميثوكسازول مع المجموعة (D) ذا فرق معنوي إحصائي مقارنة مع عزلات المجموعة (A) ( $P < 0.05$ ). وأظهر التحليل الجزيئي ل 50 من سلالات الإشريكية القولونية انتشاراً عالياً للمجموعة الأولى للانتجرونات، وارتفاعاً في نسبة انتشار المجموعة التطورية (D)، وارتفاعاً في متوسط سجل النقاط لمقاومة المضادات الحيوية للسلاسل التي تنتمي إلى مجموعة (D). وصفوة القول أن التهابات المسالك البولية التي تسببها مثل هذه السلالات تمثل مشكلة سريرية بسبب الخيارات العلاجية المحدودة.

**الكلمات المفتاحية:** الإشريكية القولونية الممرضة للجهاز البولي، الانتجرونات، سجل النقاط لمقاومة المضادات الحيوية، الإشريكية القولونية، المجموعات التطورية.

## Introduction

*Escherichia coli* (*E. coli*) is considered as one of the most abundant facultative anaerobic bacteria of the human and of many animals intestinal microflora. It is one of the most common clinical isolate recovered in clinical microbiology laboratories. Strains of this microorganism are classified into three major groups according to their biological significance to humans: harmless commensal strains, intestinal pathogenic strains, and extraintestinal pathogenic strains, (Russo & Johnson, 2000). *E. coli* is considered the most frequent cause of urinary tract infections (UTI). Other different infections in humans are also included. It is also responsible for an enormous burden of morbidity, mortality, and health care costs (Russo & Johnson, 2000).

Uropathogenic *E. coli* (UPEC) strains are the most commonly isolated organisms in community-acquired UTIs (70 to 90%) and among the most commonly isolated in nosocomially acquired UTIs (50%) including catheter-associated UTIs (CAUTI). *E. coli* has been identified as the causative agent in 90% of all case of UTI in ambulatory patients (Jacobsen, & et al. 2008). The clinical management of UTIs has become very complicated due to the emergence of resistance to most commonly used antimicrobial agents, particularly among UPEC strains (Karaca, & et al. 2005). A noticeable decrease in the occurrence of UPEC strains that are sensitive to cephalosporins, quinolones, fluoroquinolones, and trimethoprim, which has special clinical importance because of the



The aims of this study were to investigate the pattern of antimicrobial resistance, phylogenetic groups and integron classes in a collection of *E. coli* strains isolated from suspected cases of urinary tract infections at Thabet Hospital. In addition, to investigate associations between multi-drug resistance, existence of integrons and Phylogenetic group. Integrons detection has not been investigated previously from *E. coli* in Palestine.

## **Materials and Methods**

### ***Sample collection***

Fifty isolates of *E. coli* were recovered from urine specimens obtained from suspected cases of urinary tract infections of inpatients and outpatients at Thabet Hospital, Tulkarm-Palestine, during May-December 2012. These isolates were identified in the laboratory of Thabet Hospital and also were confirmed in the microbiology laboratories at An-Najah National University-Nablus. The isolates were cultured on MacConkey and/or EMB agars, Gram stain, motility test and biochemical tests such as IMViC tests and H<sub>2</sub>S production were carried out on all isolates.

### ***Antibiotic resistance***

Antimicrobial susceptibility was determined according to the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method (CLSI, 2010). All *E. coli* isolates were examined for resistance to tetracycline (TE) 30µg, streptomycin (S) 10µg, gentamicin (CN) 10µg, kanamycin (K) 30µg, nalidixic acid (NA) 30µg, norfloxacin (NOR) 10µg, ciprofloxacin (CIP) 10µg, ofloxacin (OFX) 5µg, Levofloxacin (LEV) 5µg, ceftriaxone (CRO) 30µg, ceftazidime (CAZ) 30µg, cefazolin (CZ) 30 µg, trimethoprim/sulfamethoxazole (SXT) 1.25 /23.75µg and erythromycin (E) 15 µg. All antibiotics used in this study were purchased from Oxoid. Zones of inhibition were determined in accordance with procedures of the Clinical and Laboratory Standard Institute (CLSI, 2010).



### Detection class I, II and III integrons

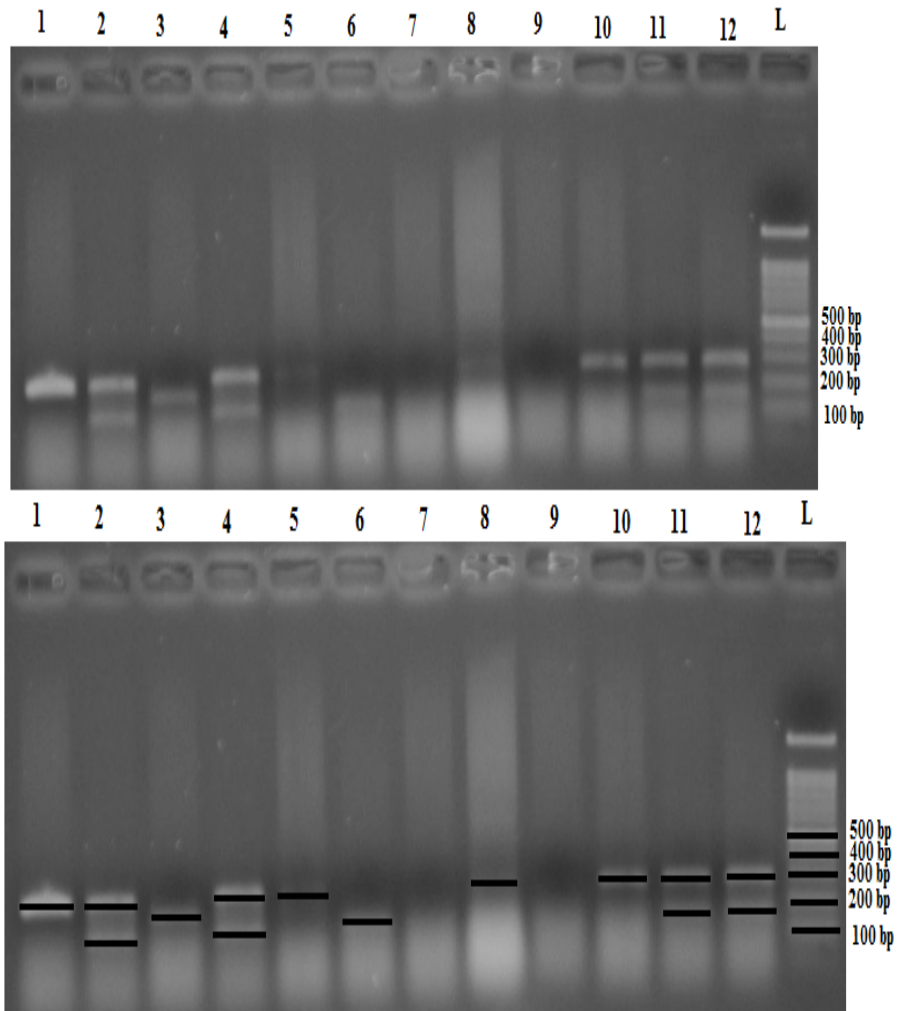
All *E. coli* isolates were screened for the presence of integrase genes *intI1* and *intI2* and *intI3* using primers described previously (Shibata, & *et al.* 2003). Primer *intI1* (forward: 5'-GCA TCC TCG GTT TTC TGG-3', reverse: 5'-GGT GTG GCG GGC TTC GTG-3' expected size amplicon is 457bp), *intI2* (forward: 5'-CAC GGA TAT GCG ACA AAA AGG T-3', reverse: 5'-GTA GCA AAC GAG TGA CGA AAT G-3' expected size amplicon is 789bp) and *intI3* (forward: 5'-ATC TGC CAA ACC TGA CTG-3', reverse: 5'-CGA ATG CCC CAA CAA CTC-3' expected size amplicon is 922bp). PCR reaction mix was performed as described in phylogenetic group determination. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 4min at 94°C followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 56°C for 40 s and extension at 72°C for 40 s, with a final extension step at 72°C for 5 min. The PCR products (10 µl) were analyzed by electrophoresis on 1.5% agarose gel.

### Statistical analysis

Comparisons of proportions were tested using the Chi<sup>2</sup> ( $\chi^2$ ) test or Fisher exact test. Comparisons of aggregate antibiotic resistance scores were done by using the Mann-Whitney U-test.  $P < 0.05$  values were considered statistically significant.

### Results

Analysis of data showed that the majority of the studied isolates, namely 36 (72%) strains belonged to group D. Thirteen strains (26%) were assigned to group A, while 1 strain (2%) was belonged to group B1 (Figure 1). There was a significant difference between the prevalence of these phylogenetic groups at  $P < 0.001$ .



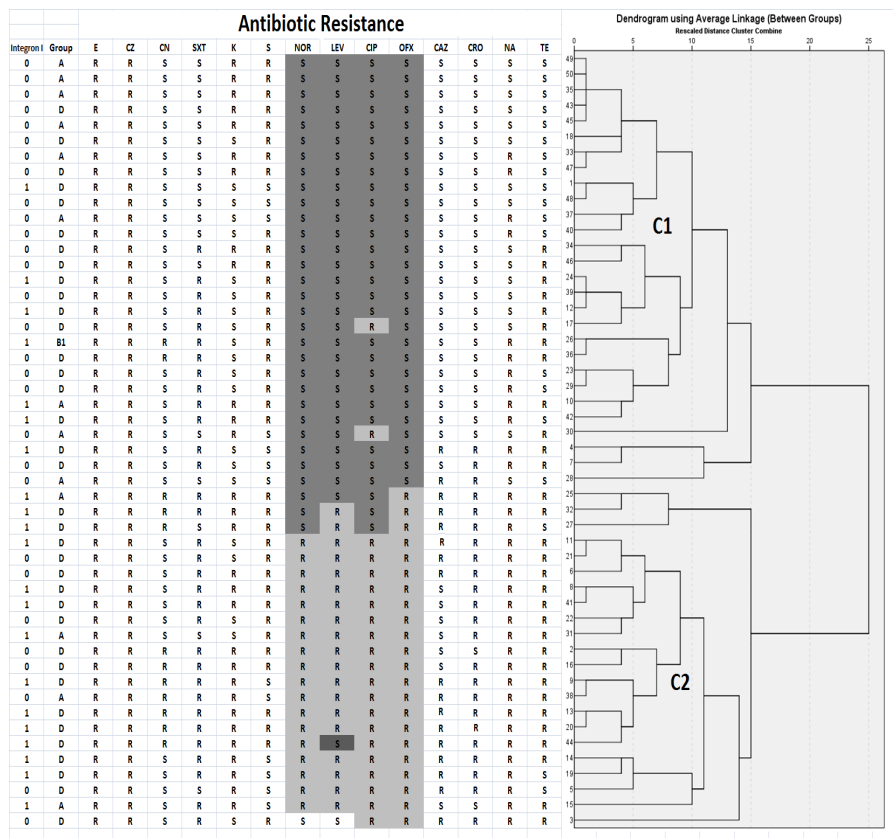
**Figure (1):** Triplex PCR profiles specific for *E. coli* phylogenetic groups. Lanes 1, 2, 4, 5, 8, 10, 11 and 12 belonged to phylogenetic group D; lanes 3, 7 and 9 belonged to phylogenetic group A; lane 6 belonged to phylogenetic group B1, and lane L: Ladder.



Twenty one (42%) of *E. coli* strains contained class I integrons. Class II and III were not detected in these strains. Strains carried class I integrons showed a higher mean antibiotic resistance score (9.8) than those without class I integrons (6.6). The comparison of intra-group percentages revealed that class I integrons were most prevalent (44.4%) in group D isolates compared to 30.8% in group A. These results showed no significant difference in the mean antibiotic resistance score (10) for strains belonged to group D and carried class I integrons and those belonged to group A (9.5).

Seven different classes of antibiotics were used including: Quinolones (nalidixic acid), Cephalosporines (ceftriaxone, ceftazidime, cefazolin), Tetracyclines (tetracycline), Fluoroquinolones (ofloxacin, ciprofloxacin, levofloxacin, norfloxacin), Aminoglycosides (streptomycin, kanamycin, gentamicin), Sulfonamides (trimethoprim/sulphamethoxazole) and Macrolides (erythromycin). The rates of resistance of *E. coli* isolates to different antibiotics tested are presented in Table 1. Antibiotic resistance has ranged from 24% for gentamicin to 100% for cefazolin and erythromycin. Forty resistance patterns were observed in the UPEC isolates with profile streptomycin- kanamycin- cefazolin- erythromycin, being the most predominant (n=5) indicating a striking diversity of resistance patterns among uropathogenic strains in this hospital. Also results showed that 98% of strains were multidrug resistant (MDR). Gentamicin, norfloxacin and levofloxacin were the most effective drugs in general. Group D isolates showed high resistant rate compared with isolates belonged to group A. The correlation between the median antibiotic resistance scores and the phylogenetic group was examined. The median antibiotic resistance scores were 8.4 and 6.5 for strains belonged to group D and group A, respectively. Differences were statistically significant ( $P = 6.2 \times 10^{-4}$ ). The prevalence of antibiotic resistance tested in each strain ranged from 14.3% to 100%. It was found that 61.5% (8/13) of strains belonged to group A were resistant to 5 or less antibiotics, while 66.7% (24/36) of strains belonged to phylogenetic group D were resistant to 6 or more antibiotics. Based on resistant patterns to fluoroquinolones isolates were grouped into 2 large clusters. The clustering pattern was

independent of phylogenetic groups (Figure 2). The results also showed strong association of trimethoprim/sulphamethoxazole resistance with the D group  $P < 0.05$  (Table 1). Among fluoroquinolones and/or quinolones-resistant strains, the frequencies of groups D and A, were 75.0% (27/36) and 61.5% (8/13), respectively.



**Figure (2):** Dendrogram of 50 uropathogenic *E. coli* strains isolated from urine samples based on the UPGMA method derived from analysis of the antibiotic resistance profile, phylogenetic groups and class I integrons.

C: Cluster; R: resistant; S: sensitive.

**Table (1):** Antibiotic resistance of 50 *E. coli* urinary isolates recovered from Thabet Hospital, Tulkarm, Palestine.

Group	Antibiotic	Resistant strains		Prevalence of antibiotic resistance between groups <sup>a</sup>		Aggregate antibiotic resistance scores (median) <sup>a</sup>	
		No. of samples	%	D (n=36)	A (n=13)	D 8.4 (304/36)	A 6.5 (84/13) <sup>****b</sup>
Tetracycline	Tetracycline	31	62	66.7% (24/36)	46.2% (6/13)	6.6 (237/36)	4.3 (56/13)
Quinolones	Nalidixic Acid	34	68	72.2% (26/36)	53.8% (7/13)	7.3 (261/36)	4.5 (59/13) <sup>****</sup>
Cephalosporines	Ceftriaxone	23	64	52.8% (19/36)	30.8% (4/13)	7.1 (257/36)	2.9 (38/13) <sup>****</sup>
	Ceftazidime	17	34	53.8% (14/36)	23.1% (3/13)	4.5 (163/36)	2.2 (28/13) <sup>****</sup>
	Cefazolin	50	100	100% (36/36)	100% (13/13)	8.8.4 (304/36)	6.5 (84/13)



... Continue table (1)

Group	Antibiotic	Resistant strains		Prevalence of antibiotic resistance between groups <sup>a</sup>		Aggregate antibiotic resistance scores (median) <sup>a</sup>	
		No. of samples	%	D (n=36)	A (n=13)	D 8.4 (304/36)	A 6.5 (84/13) <sup>****b</sup>
	Gentamicin	12	24	25% (9/36)	15.4% (2/13)	3.0 (108/36)	1.8 (24/13)
	Streptomycin	37	74	77.8% (28/36)	61.5% (8/13)	6.7 (241/36)	4.5 (59/13)
Macrolides	Erythromycin	50	100	100% (36/36)	100% (13/13)	8.4 (304/36)	6.5 (84/13)
Sulfonamides	Trimethoprim /Sulphamethoxazole	32	64	75% (27/36)	30.8% (4/13) <sup>**</sup>	7.2 (259/36)	3.2 (41/13) <sup>***</sup>

<sup>\*\*</sup> significant difference at  $P < 0.05$ , <sup>\*\*\*</sup> significant difference at  $P < 0.01$ ,

<sup>\*\*\*\*</sup> significant difference at value  $P < 0.001$  ( $\chi^2$  test and Fisher exact test).

<sup>a</sup>One isolate of group B1 is not included; <sup>b</sup>Mann-Whitney U-test.



(Rezaee, & *et al.* 2011), were only 27.1% of MDR isolates carried class I and class II integrons.

The antimicrobial resistance among UPEC has increased dramatically worldwide (Johnson, & *et al.* 2003). In our study, the most prevalent resistances were to nalidixic acid, ceftriaxone, cefazolin, tetracycline, ofloxacin, ciprofloxacin, streptomycin, kanamycin, trimethoprim/sulphamethoxazole and erythromycin. These antibiotics were and still the best therapeutic choices and therefore higher resistance prevalences are not surprising (Petkovsek, & *et al.* 2009). The study also showed that 98% strains of *E. coli* were multidrug resistant and isolates from UTIs showed higher resistance rates to all antibiotics. These were consistent with new report published from Palestine (Adwan, & *et al.* 2014), which showed that *E. coli* strains isolated from patients with UTIs had high resistance to different antibiotics. This high rate of resistance is most likely due to high antibiotic selective pressure resulting from inappropriate use of these antimicrobial agents in various clinical setting. The finding of high prevalence of integrons regardless of the phylogenetic group was reported by (Saeed, & *et al.* 2009). While those strains of *E. coli* under very low selective pressure, resistance emerges without integrons (Skurnik, & *et al.* 2009). Thus, the emergence of resistant strains in our area might be promoted by the lack of proper use of an antibiotics and/or the availability of antibiotics sold over the counter. These results were in contrast report published recently (Ejrnæs, & *et al.* 2011), which showed that *E. coli* strains collected from recurrent UTIs characterized by a low level of resistance. Also these finding results were consistent with previous results (Molina-López, & *et al.* 2011; Muhammad, & *et al.* 2011; Ferjani, & *et al.* 2012), which showed that UPEC strains expressed highest resistance rates to different antimicrobial agents. In addition, these results were consistent with a previous reports (Bashir, & *et al.* 2011), which showed that all the isolates were multiple drug resistant (MDR). Data of this research were consistent with previous reports (Bashir, & *et al.* 2011), which showed that phylogenetic group D isolates were more drug resistant as compared with phylogenetic group A. The emergence of 40 drug resistance patterns showed high variability among local UPEC isolates.





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