ISTANBUL UNIVERSITY PRESS

RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.984487
İst Tıp Fak Derg 2022 / J Ist Faculty Med 2022

HIGH PREVALENCE OF CLASS I AND CLASS II INTEGRONS IN UROPATHOGENIC *E. COLI* STRAINS (UPECs) AND THEIR RELATIONSHIP WITH ANTIBIOTIC RESISTANCE, PHYLOGENY AND VIRULENCE

ÜROPATOJEN *E. COLI* (UPEC) SUŞLARINDA SINIF I VE SINIF II İNTEGRONLARIN YÜKSEK PREVALANSI İLE ANTİBİYOTİK DİRENCİ, FİLOGRUP VE VİRULANS İLİŞKİSİ

Defne GÜMÜŞ[1], Fatma KALAYCI YÜKSEK[1], Firdevs Deniz CAMADAN[2], Merve ORAL[2], Aslı Ceren MACUNLUOĞLU[3], Mine ANĞ KÜÇÜKER[1]

[1]İstanbul Yeni Yüzyıl University, Faculty of Medicine, Department of Medical Microbiology, İstanbul, Turkey
[2]İstanbul Yeni Yüzyıl University, Faculty of Arts and Science, Department of Molecular Biology and Genetics, İstanbul, Turkey
[3]Uludağ University, Institute of Health Sciences, Department of Biostatistics, Bursa, Turkey

ORCID IDs of the authors: D.G. 0000-0003-4070-6924; F.K.Y. 0000-0002-0028-5646; F.D.C. 0000-0003-3057-6038; M.O. 0000-0002-7510-8441; A.C.M. 0000-0002-6802-5998; M.A.K. 0000-0002-4809-3985

Cite this article as: Gumus D, Kalayci Yuksek F, Camadan FD, Oral M, Macunluoglu AC, Ang Kucuker M. High prevalence of class I and class II integrons in uropathogenic E. coli strains (UPECS) and their relationship with antibiotic resistance, phylogeny and virulence. J Ist Faculty Med 2022;85(1):77-85. doi: 10.26650/IUITFD.984487

ABSTRACT

Objective: Integrons, which are highly effective in capturing, integrating and expressing gene cassettes, play an important role in the dissemination of multiple antibiotic resistances. This study investigated the correlations of integrons in uropathogenic *E. coli* (UPEC) with antibiotic resistance, virulence and phylogeny and also the relationships of phylogroups with virulence and antibiotic resistance.

Materials and Methods: Fifty UPECs isolated from uncomplicated cystitis and uncomplicated pyelonephritis were investigated to detect the presence of class I, II and III integrons and phylogenetic grouping by the PCR method. Their statistical relationship with antibiotic resistance and virulence genes were investigated using our previous findings.

Results: Among 50 UPEC strains, 37 (74%), 22 (44%) and only one (2%) strain was shown to harbor class I, class II and class III integrons, respectively. Twenty one (42%) strains were found to carry both class I and class II integrons. The majority of the strains were grouped as phylogroup B2 (38%) and phylogroup E (38%). The presence of integrons was in association with only ampicillin resistance (p=0.014). Integrons was found to be related neither to virulence genes nor phylogroups; however, the presence of *PAI* (p<0.001), *ompT* (p=0.035), and *usp* (p<0.001) genes was found

ÖZET

Amaç: Gen kasetlerinin yakalanması, integrasyonu ve ekspresyonunda oldukça etkin olan integronlar, çoğul antibiyotik direncinin yayılımında önemli bir rol oynarlar. Bu çalışmada, üropatojen *E. coli* (UPEC) suşlarında integron varlığının antibiyotik direnci, virülans ve filogenetik gruplar ile ve filogrupların, virulans ve antibiyotik direnci ile ilişkisi araştırılmıştır.

Gereç ve Yöntemler: Komplike olmayan sistit ve komplike olmayan piyelonefrit etkeni olarak izole edilen 50 UPEC suşu sınıf I, II ve III integronların varlığı ve filogruplarının belirlenmesi amacıyla PCR yöntemi ile incelenmiştir. Elde edilen sonuçlar istatistiki ilişki açısından daha önceki araştırmamızdan elde ettiğimiz bulgular ile incelenmiştir.

Bulgular: Elli UPEC suşunun 37'sinin (%74) sınıf I integron, 22'sinin (%44) sınıf II integron ve bir suşun (%2) ise sınıf III integron taşıdığı belirlenmiştir. Yirmi bir (%42) suşun ise sınıf I ve sınıf II integronları birlikte taşıdığı gösterilmiştir. Suşların çoğu B2 (%38) ve E (%38) filogruplarında sınıflandırılmıştır. İntegronların varlığı ile sadece ampisilin direnci ilişkili bulunmuştur (p=0,014). İntegronların varlığı ile virulans genleri veya filogruplar arasında bir ilişki bulunmamıştır; ancak *PAI* (p<0,001), *ompT* (p=0,035) ve *usp* (p<0,001) genlerinin varlığı ile B2 filogrubu arasında anlamlı ilişkili bulunmuştur. Filogrup E ile ko-trimoksazol direnci arasında

Corresponding author/İletişim kurulacak yazar: defne.gumus@yeniyuzyil.edu.tr

Submitted/Başvuru: 14.04.2021 • **Revision Requested/Revizyon Talebi:** 01.06.2021 •
Last Revision Received/Son Revizyon: 02.07.2021 • **Accepted/Kabul:** 10.08.2021 • **Published Online/Online Yayın:** 12.01.2021

to be significantly related to phylogroup B2. Phylogroup E was found to be statistically significantly correlated with co-trimoxazole resistance (p=0.043).

Conclusion: Consistent with previous studies, our results have proven that there is a strong association between antibiotic resistance and the presence of integrons (especially class I) in UPEC strains, and it has been shown that integrons became very prevalent globally.

Keywords: Uropathogenic *E. coli*, phylogroups, integrons, virulence factors, antibiotic resistance

anlamlı ilişkili bulunmuştur (p=0,043).

Sonuç: Bulgularımız literatürle de uyumlu olarak UPEC suşlarında antibiyotik direnci ile integronların (özellikle sınıf I) varlığı arasında güçlü bir ilişki olduğunu kanıtlamış ve tüm dünya genelinde integronların yaygın görüldüğü anlaşılmıştır.

Anahtar Kelimeler: Üropatojen *E. coli*, filogruplar, integronlar, virulans faktörleri, antibiyotik direnci

INTRODUCTION

Over the last decades, investigations have shown that integrons are highly effective in capturing, integrating and expressing gene cassettes, and play an important role in the dissemination of multiple antibiotic resistances within microbial populations. Integrons lack the specific mobilization machinery, but they are associated with insertion sequences, transposons and/or plasmids (1-4).

Integrons have an integrase gene (intI), an attachment site (attI), and a promoter region (Pc) which induces the expression of integrated gene cassettes (1, 5-7). Among the five different classes of integrons, class I and class II integrons are the most common and clinically important. Integrons are very common (22-95%) in enteric bacteria isolated from various infections, although they are also found to be present in commensal bacteria. Studies have shown that integrons horizontally transfer more than 130 different gene cassettes, which encode antibiotic resistance (8, 9).

Escherichia coli are classified into different pathogenicity groups according to specific virulence factors. One of these pathogroups is known as uropathogenic *E. coli* (UPEC). As one of the primary etiological agents of urinary tract infections (UTIs), UPECs account for 80-90% cases of community-acquired UTIs and 40-50 % of hospital-acquired UTIs. It is well known that UTIs are one of the most common infectious diseases, with nearly 150 million new cases diagnosed annually all around the world (7, 10, 11). It has been shown that UPEC is a heterogeneous group of strains composed of several virulence assortments and phylogroups. The majority of various virulence traits of UPECs are fimbrial and/or afimbrial adhesins, iron uptake systems such as siderophores, exotoxins and bacteriocins (12-16).

According to the presence/absence of *chuA* and *yjaA* and DNA fragment TspE4.C2, *E. coli* strains are classified into four main phylogroups (A, B1, B2 or D) (17); however in the last decade four new phylogroups were identified which have caused *E. coli* strains to be classified into eight groups (A, B1, B2, C, D, E, F and *Escherichia* clade I) (18). Previous studies have shown that UPECs are mostly grouped as phylogenetic groups B2 and D, and virulence factors described for UPECs are shown to be related to phylogenetic group B2 (6, 7, 19).

This study investigated the correlations of integrons in UPECs with antibiotic resistance, virulence and phylogeny and also the relationship between phylogroups and virulence.

MATERIALS AND METHODS

Strains

Fifty UPEC strains isolated from patients with different uncomplicated cystitis and uncomplicated pyelonephritis were included. These strains were isolated within the context of our previous study in which their virulence genes (*afa*, *aer*, *cnf1*, *sfa/foc*, *pap3/4*, *PAI*, *iroN*, *ompT*, *hly*, *usp*, *pap1/2*) and antibiotic susceptibilities [ampicillin (AMP), amoxicillin/clavulanate (AMC), cefuroxime (CXM), ceftriaxone (CRO), cefixime (CFX), gentamicin (GN), ciprofloxacin (CIP), ofloxacin (OFX), co-trimoxazole (SXT), nitrofurantoin (NIT) and aztreonam (ATM)] were investigated back then (15). We kept the bacteria at -80^{0}C for further examinations.

Detection of integrons and phylogroups

The DNA template from UPECs was prepared from overnight cultures in Tryptic soy broth (TSB) at 37°C. An extraction kit (GeneDireX, Taiwan) was used according to the manufacturer's instructions.

We investigated the presence of class I, class II and class III integrons on both genomic and plasmid DNAs as suggested in Ren et al. (20). For this purpose, all extracted DNAs were examined by multiplex polymerase chain reaction (PCR) for the presence of *intI*, *intII* and *intIII* genes (20).

Primers used in this research are shown in Table 1 (20, 21). A master mix kit (Genemark, Taiwan) was used in PCR assays. Mixtures (25 µL last volume) were prepared according to the manufacturer's suggestions (Genemark, Taiwan): 5 µL master mix, 2 µL DNA, 2 µL each primer (1 µL for each primer from 10 pmol concentration) and nuclease-free water.

Table 1: Primers used in integron PCR analysis

Genes	Primer sequence	Amplicon size	Reference
Class I int F	5′-CCT CCC GCA CGA TGA TC-3′	280 bp	(20, 21)
Class I int R	5′-TCC ACG CAT CGT CAG GC-3′		
Class II int F	5′- GTA GCA AAC GAG TGA CGA AAT G-3′	780 bp	(20)
Class II int R	5′- CAC GGA TAT GCG ACA AAA AGG T-3′		
Class III int F	5′ -GCC TCC GGC AGC GAC TTT CAG-3′	976 bp	(20)
Class III int R	5′-ACG GAT CTG CCA AAC CTG ACT-3′		

The reaction conditions for PCR amplification were as follows: initial denaturation for 4 min at 94°C; degradation for 45 sec at 94°C; annealing for 45 sec at 57°C; elongation for 55 sec at 72°C; final elongation for 4 min at 72°C. These reactions were carried out for 30 cycles (Prima Trio high media thermal cycler, Mumbai, India) (20). The amplicons were stored at -20°C.

We could not find any strain harboring *intIII* gene for use as a positive control in PCR assays. Therefore, DNA sequence analysis was performed for confirmation of class III integron positive DNA samples by using the ABI3730 XL Genetic Analyzer device (GATC Biotech AG, Germany).

According to the study of Clermont et al., (18), phylogroups of UPEC strains were determined via two sequential PCR assays. In the first stage, phylogroups were determined according to the presence/absence of TspE4.C2, *chuA*, *yjaA* and *arpA* genes. Second multiplex PCR assays using *trpAgpC*, *ArpAgpE,* and *trpBA* primers were performed to distinguish the phylogroups D from E, E from *Escherichia* clade I, and A from C (18). All primers are shown in Table 2.

A Master mix kit (Genemark, Taiwan) was used in PCR. Mixtures (25 µL last volume) were prepared according to the manufacturer's suggestions (Genemark, Taiwan): 5 µL master mix, 2 µL DNA, 2 µL each primer (1 µL for each primer from 10 pmol concentration), and nuclease-free water.

The reaction conditions for PCR amplification were as follows: initial denaturation for 4 min at 94°C; degradation for 30 sec at 94°C; annealing for 30 sec at 56°C; elongation for 45 sec at 72°C; final elongation for 5 min at 72°C. These reactions were carried out for 30 cycles (Prima Trio high media thermal cycler, Mumbai, India) (18). The amplicons were stored at -20°C.

All amplified products were separated by agarose gel electrophoresis in a 1.5% agarose gel stained with ethidium bromide (0.5 µg/mL), visualized under UV light. After that products were electrophoresed for 40 min under 80

Table 2: Primers used in phylogroup PCR analysis

Genes	Primer sequence	Amplicon size	Reference
chuA.1bF	5′-ATGGTACCGGACGAACCACC-3′	288 bp	(18)
chuA.2bR	5′-TGCCGCCAGTACCAAAGACA-3′		
yjaA.1bF	5′-CAAACGTGAAGTGTCAGGAG-3′	211 bp	
yjaA.2bR	5′-AATGCGTTCCTCAACCTGTG-3′		
TspE4C2.1bF	5′-CACTATTCGTAAGGTCATCC-3′	152 bp	
TspE4C2.2bR	5′-AGTTTATCGCTGCGGGTCGC-3′		
AceK.fF	5′-AACGCTATTCGCCAGCTTGC-3′	400 bp	
ArpA1.rR	5′-TCTCCCCATACCGTACGCTA-3′		
trpAgpC.1	5′-AGTTTTATGCCCAGTGCGAG-3′	219 bp	
trpAgpC.2	5′-TCTGCGCCGGTCACGCCC-3′		
ArpAgpE.f	5′-GATTCCATCTTGTCAAAATATGCC-3′	301 bp	
ArpAgpE.r	5′-GAAAAGAAAAAGAATTCCCAAGAG-3′		
trpBA.f	5′-CGGCGATAAAGACATCTTCAC-3′	489 bp	
trpAgpC.1	5′-AGTTTTATGCCCAGTGCGAG-3′		

volts with 1XTBE electrophoretic liquid. The results were visualized and recorded using Hi-UV MAX transilluminator (HiMedia, India). DNA ladder (Genemark, Taiwan) labeled between 100-1000 bp was used.

Statistical analysis

The correlations of integrons with antibiotic resistance, virulence genes, and phylogroups were statistically analyzed. The categorical variables were reported as n (%) by using the Pearson Chi-Square test, and Fisher's exact test. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.) was used for statistical analysis, and a p-value <0.05 was considered as statistically significant.

This study was approved by the Istanbul Yeni Yuzyıl University Medical Faculty Research Ethics Committee (Date: 01.10.2018, No: 8).

RESULTS

Detection of integrons

Among 50 UPEC strains, 37 (74%) and 22 (44%) were shown to carry class I and class II integrons, respectively. Twenty-one (42%) of UPECs were found to possess both classes I and II integrons (Figure 1-2).

It was also shown that both *intI* and *intII* genes were encoded more frequently in plasmids rather than genomic DNA. The *intI* gene was shown to be harbored in 22 (44%) strains on genomic DNA and in 34 (68%) strains on plasmid DNA. Similarly, 14 (28%) and 19 (38%) of UPEC strains were found to possess *intII genes* in genomic and plasmid DNA, respectively. We found that only one strain was shown to carry the *intIII* gene which was encoded only on plasmid DNA. The result was confirmed with a sequence analyzing method.

Detection of phylogroups

The majority of the strains were grouped as phylogroup B2 (38%) and phylogroup E (38%), and the rest of the strains were defined as D (8%), A (2%), C (2%) and F (2%). Five (10%) strains could not be classified (Figure 3 and 4).

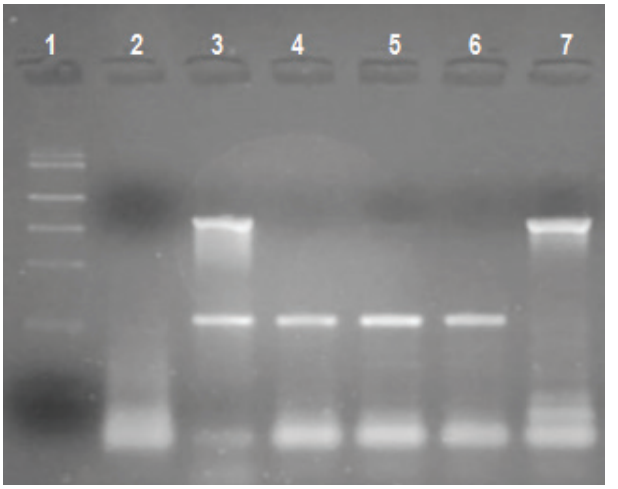

Figure 1: Agarose gel image of the *intI* and *intII* genes amplified by multiplex PCR

Well 1: DNA ladder, well 2: negative control, well 3: positive control *(intI*-280 bp *and intII*-780 bp), well 4-6: *intI* positive strains, well 7: *intII* positive strains

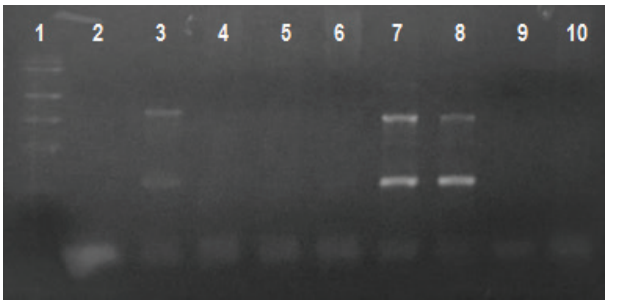

Figure 2: Agarose gel image of the *intI* and *intII* genes amplified by multiplex PCR

Well 1: DNA ladder, well 2: negative control, well 3: positive control (*intI*-280 bp and *intII*-780 bp), well 4-6: negative strains, well 7-8: *intI* and *intII* positive strains

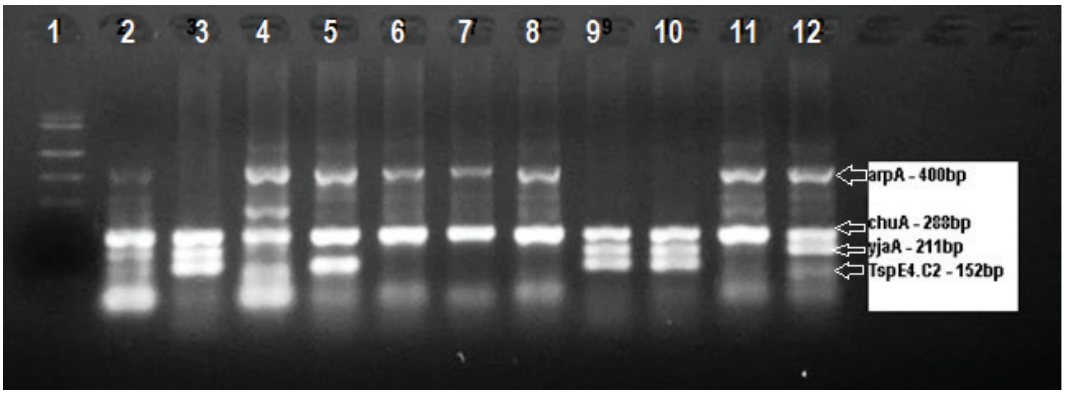

Figure 3: Agarose gel image of the *arpa*, *chuA*, *yjaA* and TspE4.C2 genes amplified by the multiplex PCR.

Well 1: DNA ladder, well 2: positive control, well 3: group B2, well 4-8: group E, well 9-10: group B2, well 11-12: group E.

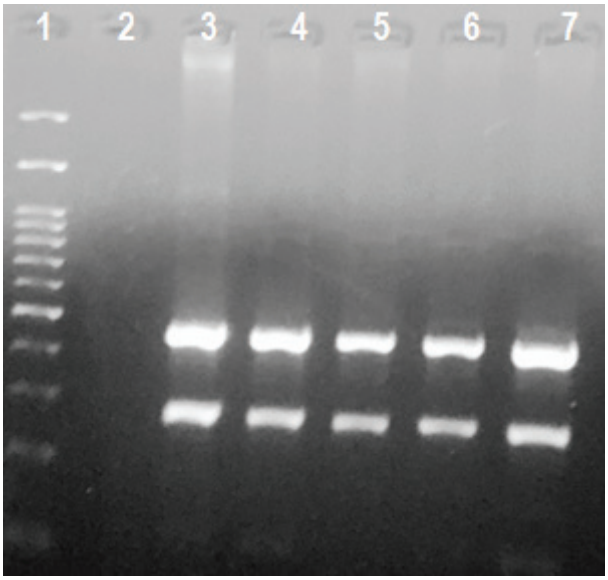

Figure 4: Agarose gel image of the multiplex PCR assays used for detection of E/cladeI groups.

Well 1: DNA ladder, well 2: negative control, well 3-7: *trpBA* (489 bp) and *ArpAgpE* (301 bp) positive strains which are classified as group E.

Statistical analysis

A statistically significant correlation (p=0.014) of the presence of integrons was found only to ampicillin resistance (Table 3).

In integron-bearing strains, the rate of the *usp* gene was shown to be lower than non-integron bearing strains and the difference was statistically significant (p=0.013) (Table 4). There was no statistically significant relationship between the presence of integrons and other virulence genes (p>0.05).

There was no statistically significant (p>0.05) relation between the presence of integrons and phylogroups.

Statistically significant relations between virulence genes and phylogroups were as follows: the presence of *PAI* (p<0.001), *ompT* (p=0.035), and *usp* (p<0.001) genes were higher in group B2. The presence of *sfa/foc*, *PAI* and *usp* genes were found to be lower (p=0.035, p<0.001 and p<0.001, respectively) and the *ompT* (p=0.013) gene was higher in the E phylogroup than the others.

Only resistance to SXT was found to be statistically significantly (p=0.043) related to group E.

Table 3: Correlation between integrons and antibiotic resistance

	Integron positive (n=38)	Integron negative (n=12)	p-value[a]
AMP			
S	3 (7.89%)	5 (41.67%)	**0.014**[a]
R	35 (92.11%)	7 (58.33%)	
SXT			
S	20 (52.63%)	10 (83.33%)	0.091[a]
R	18 (47.37%)	2 (16.67%)	
CIP			
S	28 (73.68%)	11 (91.67%)	0.257[a]
R	10 (26.32%)	1 (8.33%)	
AMC			
S	30 (78.95%)	9 (75%)	>0.99[a]
R	8 (21.05%)	3 (25%)	
OFX			
S	28 (73.68%)	11 (91.67%)	0.257[a]
R	10 (26.32%)	1 (8.33%)	
CFX			
S	34 (89.47%)	11 (91.67%)	>0.99[a]
R	4 (10.53%)	1 (8.33%)	
ATM			
S	33 (86.84%)	12 (100%)	0.319[a]
R	5 (13.16%)	0 (0%)	
CXM			
S	34 (89.47%)	12 (100%)	0.560[a]
R	4 (10.53%)	0 (0%)	
CRO			
S	34 (89.47%)	12 (100%)	0.560[a]
R	4 (10.53%)	0 (0%)	
NIT			
S	34 (89.47%)	12 (100%)	0.560[a]
R	4 (10.53%)	0 (0%)	
GN			
S	36 (94.74%)	12 (100%)	>0.99[a]
R	2 (5.26%)	0 (0%)	

Data are expressed as n (%), [a]: Fisher's Exact Test, S: susceptible, R: resistant

DISCUSSION

In the present study, 50 UPEC strains isolated from acute uncomplicated cystitis and acute uncomplicated pyelonephritis patients were investigated to determine the relations of integron classes (I-III) with antibiotic resistance, virulence genes and phylogeny. Moreover, the correlation of phylogeny, virulence and antibiotic resistance was also examined.

Table 4: Correlation between integrons and virulence genes

	Integron positive (n=38)	Integron negative (n=12)	p-value[a]
afa			
positive	2 (5.26%)	0 (0%)	>0.99[a]
negative	36 (94.74%)	12 (100%)	
aer			
positive	19 (50%)	5 (41.67%)	0.614[b]
negative	19 (50%)	7 (58.33%)	
cnf1			
positive	8 (21.05%)	4 (33.33%)	0.448[a]
negative	30 (78.95%)	8 (66.67%)	
sfa/foc			
positive	7 (18.42%)	4 (33.33%)	0.424[a]
negative	31 (81.58%)	8 (66.67%)	
pap$^{3/4}$			
positive	1 (2.63%)	1 (8.33%)	0.426[a]
negative	37 (97.37%)	11 (91.67%)	
iroN			
positive	14 (36.84%)	5 (41.67%)	>0.99[a]
negative	24 (63.16%)	7 (58.33%)	
PAI			
positive	17 (44.74%)	8 (66.67%)	0.185[b]
negative	21 (55.26%)	4 (33.33%)	
ompT			
positive	29 (76.32%)	10 (83.33%)	>0.99[a]
negative	9 (23.68%)	2 (16.67%)	
hly			
positive	2 (5.26%)	2 (16.67%)	0.240[a]
negative	36 (94.74%)	10 (83.33%)	
usp			
positive	16 (42.11%)	10 (83.33%)	**0.013**[b]
negative	22 (57.89%)	2 (16.67%)	
pap ½			
Positive	17 (44.74%)	7 (58.33%)	0.411[b]
Negative	21 (55.26%)	5 (41.67%)	

Data were expressed as n (%), [a]: Fisher's Exact Test, [b]: Pearson Chi-Square Test

Previous studies investigating the presence of bacterial integrons were mainly reported in various clinical isolates from Iran. Farshad et al. have shown that the prevalence of class I and class II integrons in strains isolated from urine samples of children were 6.25% and 10.4% respectively, and the class III integron was not detected (22). In another study from Iran, Falakian et al. reported that the prevalence of class I integrons was 49% in UPEC strains (10). The frequency of class I and class II integrons in UPEC strains were reported as 52% and 2.5%, respectively, by Khoramrooz et al. (5). Class III integron was not found in any of the isolates. They also showed a strong relationship between the presence of integrons and resistance to co-trimoxazole, ciprofloxacin, ceftazidime, and tetracycline resistance rates (5). Ebrahim-Saraie et al. reported that the prevalence of class I and class II integrons were 59.5% and 7.4%, respectively, in UPEC strains. While in the same study no class III integron was detected, resistance to sulfonamides and the presence of class I integron was found to be statistically related (2). In a study of Mirnezami et al. the prevalence of integrons in 100 UPEC strains were found to be 70% and 3% for class I and class II integrons, respectively. They also reported a strong relationship between the presence of class I integron and resistance to ampicillin, gentamicin, ciprofloxacin, co-trimoxasole, and nalidixic acid (23). Similar to previous studies, our study has shown that there was a high frequency of class I (74%) and class II integrons (44%). Also, the class III integron was detected in one of the UPEC strains.

In a study by El-Najjar et al. in Lebanon, it was shown that 30% of UPEC strains were positive for the class I integron. They also indicated that the prevalence of antibiotic resistance rates was higher in integron harboring strains (4). In Syria, Al-Assil et al. detected that 54.6% of UPEC strains harbored class I integrons and there was a strong correlation between multidrug resistance and class I integrons (24).

In a study from the USA, Solberg et al. reported that the detection rates of class I and class II integrons were 49% and 20%, respectively, in UPEC strains (25). Solberg et al. have declared a high prevalence rate of class II integrons when compared to the studies mentioned above. However, our results have shown a higher detection rate (44%) of class II integrons. In the study of Zeighami et al., 92.5% of UPEC and diarrheagenic *E. coli* strains (DEC) were found to carry integrons; the prevalence of class I and class II integrons were reported to be 85% and 2%, respectively. Class III integrons were not detected. As compared to UPEC, DEC was reported to carry integrons more frequently (94% for class I, 8% for class II) and they emphasized that enteric pathogens could act as a reservoir or donor of several antibiotic resistance genes which can be transferred to other *E. coli* pathogroups (26). Ochoa et al. in Mexico, have shown that class I and class II integron rates in multi-drug resistant (MDR) UPEC strains were 44% and 2%, respectively. They also showed that 48% and 9.5% of extended-drug resistant (XDR) UPECs

harbored class I and class II integrons, respectively, and none of these strains carried class III integron (7).

As seen above, the majority of the studies are mainly from the Middle-East (especially from Iran) and have shown higher frequencies of integrons. However, in a meta-analysis study, Halaji et al. emphasized that there is a strong correlation between class I integrons and high-level antibiotic resistance. In the same study, two issues were emphasized: firstly, the majority of the strains harbored class I integron isolated from hospital-acquired UTIs; and secondly, the frequency of class I integron carrying strains are high in Middle Eastern countries (27). Studies investigating the presence of integrons and their relation with antibiotic resistance in UPEC strains are limited in Turkey. In a multi-centered study, Çopur-Çiçek et al. reported that the prevalence of class I and class II integrons were 26% and 17%, respectively (28).

Numerous studies have examined the relationship between the presence of integrons, antibiotic resistance and phylogeny in *E. coli*. In a study by Poey and Lavina from Uruguay, UPEC strains isolated from pregnant women and children with urinary tract abnormalities were examined. The prevalence rates of class I and class II integrons were reported to be 22% and 8%, respectively. They have shown that the proportion of integron bearing strains were higher in children with urinary tract abnormalities due to recurrent antibiotic treatments. Resistance to ampicillin, cephalotin, and co-trimoxazole was found to be significantly associated with the presence of integrons. They also found a strong correlation between class I integron and phylogroup D (6). A similar approach was carried out by Oliveira-Pinto et al. from Brazil. They compared the presence of class I integrons in strains isolated from feces of healthy individuals who did not use any antibiotics and urine of women with community-acquired UTIs. They showed a higher incidence of class I integron in *E. coli* strains isolated from urine (65%) than commensal *E. coli* strains (12%); only urine strains were shown to be multi-resistant to all antibiotics tested. Most of the urine strains were grouped in phylogroup B2, however, 43% of commensal strains were shown to be assigned in phylogroup A (9). Gündoğdu et al. reported that the prevalence of class I and class II integrons were found to be 34.3% and 5.1%, respectively, and class III integrons were not detected in UPEC strains isolated from hospitalized patients in Austria. The prevalence of multi-drug resistance was shown to be significantly higher among integron positive strains and the majority (78%) of the strains were assigned to phylogroup B2 (29). Yekani et al., in Azerbaijan, showed that class I and class II integrons were found to be harbored in 64% and 4.5% of UPECs, respectively, and class III integrons were not found. The authors suggested that multi-drug resistance was significantly associated with the presence of class I integrons. Although strains were mostly shown to be assigned to phylogenetic group B2 (61%), a high prevalence of class I and class II integrons were detected among group B1 strains (3). In another study from Mexico examining multi-drug resistant UPECs, it was reported that class I integrons were distributed in phylogenetic groups A, B2, and D (40%, 44%, and 44%, respectively); class 2 integrons were distributed in phylogenetic groups B2 and D (3% and 2%, respectively) (7). Olivera-Pinto et al. from Brazil showed that 55% of UPEC isolates belonged to phylogroup B2, and to a lesser extent to group D, whereas most commensal *E. coli* isolates were grouped in phylogroup A (43%) (9). It seems that the presence of integrons is shown to be statistically significantly related to phylogroups B1, B2 and D in UPECs (3, 6, 9, 29).

In our study, 74% of the UPECs were shown to carry class I and/or class II. Furthermore, we found that the integrons were encoded mostly on plasmids. Previous studies have clearly shown that the presence of integrons is associated with particular multi-drug resistance patterns; in our study, the presence of integrons and ampicillin resistance were found to be correlated. According to our results, it was shown that the majority of the UPECs were distributed equally in groups B2 (38%) and E (38%). However, we did not find any statistically significant relationship between the presence of integrons and phylogroups. The phylogroup E and resistance to SXT were shown to be statistically correlated.

The limited number of studies showed the possible relations between the presence of integron and virulence factors. Poey and Lavina showed that class I integrons were found to take part in strains that carry P fimbria, yersiniabactin, and aerobactin systems (6). Düzgün et al. reported that there was a statistically significant relationship between the presence of class I integrons, *CTX-M* and *fim* genes in UPECs (30). In our study, there was no significant correlation between the presence of integrons and virulence genes.

Another issue about UPEC strains is to clarify the relationship of their virulence factors with phylogenetic distributions. Ochoa et al. concluded that most of the MDR-UPEC strains were grouped in D (55%) and B2 (39%) and the most frequent virulence genes in these groups were several fimbrial genes (*ecpA*, *fimH*, *csgA*, and *papGII*), an iron uptake gene (*chuA)* and a toxin gene (*hlyA)* (7). Similarly, in South Korea, Lee et al. indicated that most of the virulence genes (*fimH, sfa, pap, sfa, hly, feoB, irp2, iroN*) were found to be significantly higher in the strains grouped as B2 and D (31). Yılmaz and Aslantaş from Turkey showed that *E. coli* strains isolated from urine were mostly shown to be defined as group $B2_3$ (36%) and group A1 (21%); most strains were reported to carry at least one virulence gene (32). In line with other research,

we found that *PAI* (p<0.001), *ompT* (p=0.035), and *usp* (p<0.001) genes were significantly related to group B2 while only the *ompT* gene was significantly related to group E (p=0.013).

In conclusion, unlike previous studies, we investigated the prevalence of integrons on genomic and plasmid DNAs separately. We have shown that they were more prevalent on plasmids (class I integron 68%, class II integron 38%) which highlights the common occurrence of horizontally transferring. To our knowledge, this is the first report detecting the presence of class III integron in UPEC strains isolated in Turkey and other countries from Europe. According to our results, the presence of integrons was found to be related neither to virulence genes nor phylogroups; however, the presence of *PAI*, *ompT,* and *usp* genes was found to be significantly related to phylogroups B2 and E.

Informed Consent: Written consent was obtained from the participants.

Ethics Committee Approval: This study was approved by the Istanbul Yeni Yüzyıl University Medical Faculty Research Ethics Committee (Date: 01.10.2018, No: 8).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- D.G., F.K.Y., M.K.; Data Acquisition- D.G., F.K.Y., D.C., M.O.; Data Analysis/Interpretation- D.G., F.K.Y., D.C., C.M.; Drafting Manuscript- D.G., F.K.Y., M.K.; Critical Revision of Manuscript- D.G., F.K.Y., M.K.; Approval and Accountability- D.G., F.K.Y., F.D.C., M.O., A.C.M., M.A.K.

Conflict of Interest: Authors declared no conflict of interest

Financial Disclosure: This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (Project No: 2209-a/2019).

REFERENCES

1. Azam H, Ghezeljeh SM, Mahmoud S. Prevalence of class 1 and 2 integrons among the multidrug resistant uropathogenic strains of *Escherichia coli*. Asian Biomed 2017;9(1):49-54. [CrossRef]
2. Ebrahim-Saraie HS, Nezhad NZ, Heidari H, Motamedifar A, Motamedifar M. Detection of antimicrobial susceptibility and integrons among extended-spectrum ß-lactamase producing uropathogenic *Escherichia coli* isolates in Southwestern Iran. Oman Medical J 2018;33(3):218-23. [CrossRef]
3. Yekani M, Memar MY, Baghi HB, Sefidan FY, Alizadeh N, Ghotaslou R. Association of integrons with multidrug-resistant isolates among phylogenic groups of uropathogenic *Escherichia coli*. Microbiol Res 2018;9(1):10-3. [CrossRef]
4. El-Najjar N, Farah MJ, Hashwa FA, Tokajian ST. Antibiotic resistance patterns and sequencing of class I integron from uropathogenic *Escherichia coli* in Lebanon. Lett App Microbiol 2010;51(4):456-61. [CrossRef]
5. Khoramrooz SS, Sharifi A, Yazdanpanah M, Hosseini SAAM, Emaneini M, Gharibpour F et al. High frequency of class 1 integrons in *Escherichia* coli isolated from patients with urinary tract infections in Yasuj, Iran. Iranian Red Crescent Med Journal 2016;18(1):1-6. [CrossRef]
6. Poey ME, Laviña M. Integrons in uropathogenic *Escherichia coli* and their relationship with phylogeny and virulence. Microbiol Patho 2014;77:73-7. [CrossRef]
7. Ochoa SA, Cruz-Córdova A, Luna-Pineda VM, Reyes-Grajeda JP, Cázares-Domínguez V, Escalona G, et al. Multidrug-and extensively drug-resistant uropathogenic *Escherichia coli* clinical strains: phylogenetic groups widely associated with integrons maintain high genetic diversity. Front in Microbiol 2016;7(2042):1-12. [CrossRef]
8. Partridge SR, Tsafnat G, Coiera E, Iredell JR. Gene cassettes and cassette arrays in mobile resistance integrons. FEMS Microbiol Rev 2009;33(4):757-84. [CrossRef]
9. Oliveira-Pinto C, Diamantino C, Oliveira PL, Reis MP, Costa PS, Paiva MC et al. Occurrence and characterization of class 1 integrons in *Escherichia coli* from healthy individuals and those with urinary infection. J Med Microbiol 2017;66(5):577-83. [CrossRef]
10. Falakian Z, Nikokar I, Nafisi MR, Karimi A, Validi M. Frequency of class 1 integrons among *Escherichia coli* isolates of patients with urinary tract infection. Arch of Clin Infect Dis 2012;6(4):1-4.
11. Mandal P, Kapil A, Goswami K, Das B, Dwivedi SN. Uropathogenic *Escherichia coli* causing urinary tract infections. Indian J Med Res 2001;114:207-11.
12. Puente JL, Brettfinlay B. Pathogenic *E. coli*. In Groisman EA (ed) Principles of Bacterial Pathogenesis. California: Academic Press, 2001;388-428.
13. Yamamoto S. Molecular epidemiology of uropathogenic *Escherichia coli*. J Infect Chemother 2007;13:68-73. [CrossRef]
14. Miyazaki J, Ba-Thein W, Kumao T, Obata Yasuoka M, Akaza H, Hayshi H. Type 1, P and S fimbriae, and afimbrial adhesin I are not essential for uropathogenic *Escherichia coli* to adhere to and invade bladder epithelial cells. FEMS Immunol Med Microbiol 2002;25:23-6. [CrossRef]
15. Uzun C, Oncül O, Gümüş D, Alan S, Dayioğlu N, Küçüker MA. Virulence genes and antibiotic susceptibilities of uropathogenic *E. coli* strains. Clin Lab 2015;61(8):941-50. [CrossRef]
16. Nougayrède JP, Fernandes PJ, Donnenberg MS. Adhesion of enteropathogenic *Escherichia coli* to host cells. Cell Microbiol 2003;5:359-72. [CrossRef]
17. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Applied Environ Microbiol 2000;66(10):4555-8. [CrossRef]
18. Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Reports 2013;5(1):58-65. [CrossRef]
19. Khairy RM, Mohamed ES, Abdel Ghany HM, Abdelrahim SS. Phylogenic classification and virulence genes profiles of uropathogenic *E. coli* and diarrheagenic *E. coli* strains isolated from community acquired infections. PLoS One 2019;14(9):1-10. [CrossRef]
20. Ren C, Zhao Y, Shen Y. Analysis of the effect of integrons on drug-resistant *Staphylococcus aureus* by multiplex PCR detection. Molecular Med Reports 2013;7(3):719-24. [CrossRef]

21. Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, et al. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob Agents Chemother 2001;45(3):723-6. [CrossRef]
22. Farshad S, Japoni A, Hosseini M. Low distribution of integrons among multidrug resistant *E. coli* strains isolated from children with community-acquired urinary tract infections in Shiraz, Iran. Pol J Microbiol 2008;57(3):193-8.
23. Mirnezami M, Ranjbar R, Niakan M, Ahmadi MH. Frequency of antimicrobial resistance and class 1 and 2 integrons in *Escherichia coli* strains isolated from urinary tract infections. Iran J Pharma Res 2020;19(3):282-7.
24. Al-Assil B, Mahfoud M, Hamzeh AR. First report on class 1 integrons and Trimethoprim-resistance genes from dfrA group in uropathogenic *E. coli* (UPEC) from the Aleppo area in Syria. Mob Gen Elements 2013;3(3):1-6. [CrossRef]
25. Solberg OD, Ajiboye RM, Riley LW. Origin of class 1 and 2 integrons and gene cassettes in a population-based sample of uropathogenic *Escherichia coli*. J Clin Microbiol 2006;44(4):1347-51. [CrossRef]
26. Zeighami H, Haghi F, Masumian N, Hemmati F, Samei A, Naderi G. Distribution of integrons and gene cassettes among uropathogenic and diarrheagenic *Escherichia coli* isolates in Iran. Microb Drug Resist 2015;21(4):435-40. [CrossRef]
27. Halaji M, Feizi A, Mirzaei A, Sedigh Ebrahim-Saraie H, Fayyazi A, Ashraf A, et al. The Global Prevalence of Class 1 Integron and associated antibiotic resistance in *Escherichia coli* from patients with urinary tract infections, a systematic review and Meta-Analysis. Microb Drug Resist 2020;26(10):1208-18. [CrossRef]
28. Çopur ÇA, Sandallı C, Budak EE, Yağmur G, Cizmeci Z, Ak S, et al. Characterization of class 1 and class 2 integron gene cassettes in *Escherichia coli* strains isolated from urine cultures: a multicenter study. Mikrobiyol Bult 2016;50(2):175-85. [CrossRef]
29. Gündoğdu A, Long YB, Vollmerhausen TL, Katouli M. Antimicrobial resistance and distribution of sul genes and integron-associated intl genes among uropathogenic *Escherichia coli* in Queensland, Australia. J Med Microbiol 2011;60(11):1633-42. [CrossRef]
30. Düzgün AÖ, Okumuş F, Saral A, Çiçek AÇ, Cinemre S. Determination of antibiotic resistance genes and virulence factors in *Escherichia coli* isolated from Turkish patients with urinary tract infection. Revista da Sociedade Brasileira de Medi Tropic 2019;52:1-5. [CrossRef]
31. Lee JH, Subhadra B, Son YJ, Kim DH, Park HS, Kim JM, et al. Phylogenetic group distributions, virulence factors and antimicrobial resistance properties of uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infections in South Korea. Lett App Microbiol 2016;62(1):84-90. [CrossRef]
32. Yılmaz EŞ, Aslantaş Ö. Phylogenetic group/subgroups distributions, virulence factors, and antimicrobial susceptibility of *Escherichia coli* strains from urinary tract infections in Hatay. Revista da Sociedade Brasileira de Medi Tropic 2020;53:1-6. [CrossRef]