Original Article

Investigation of Class 1 Integrons and Biofilm Formation in Multi-Drug Resistance Uropathogenic *Escherichia coli* Isolated from Patients with urinary tract infection in Shohadaye Qom Hospital, Iran

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Abstract

Aims: This study aimed to investigate class 1 integrons and biofilm formation in multi-drug resistance (MDR) Uropathogenic *Escherichia coli* (UPEC) isolated from patients with urinary tract infection (UTI). **Materials and Methods:** Three hundred and eighty positive cultures were collected from patients with UTI referred to Shohadaye Qom hospital from 2018 to 2019. Suitable tests were done to diagnose UPEC, and confirmed by *usp* gene polymerase chain reaction (PCR). Antibiotic susceptibility testing was performed using Kirby Bauer disk diffusion. Analysis of biofilm production was conducted using microtiter plate assay. Next, the presence of Class 1 integrons and *dfr-17* gene was surveyed by PCR. Data analyzed using Chi-squared and Fisher's exact tests in SPSS software, $P < 0.05$ was considered statistically significant. **Findings:** In total, 166 isolates of UPEC were retrieved. Among them, 120 isolates were MDR. The highest resistance of MDRs was observed against ampicillin. Among MDRs, 71, 18, 15, and 16 isolates were negative, weak, moderate, and strong biofilm producers, respectively. Meanwhile, 47.5% of the isolates were positive for *int-1* gene and 25.8% of the isolates were positive for *dfr*-17-gene cassette. Out of 57 *int*-1 positive MDRs, 15 isolates (26.3%) showed strong biofilm which indicated a significant correlation ($P < 0.001$). Furthermore, among 31 MDRs with the positive *dfr*-17, 8 isolates (25.8%) had strong biofilm which statistically was significant ($P < 0.001$). **Conclusion:** The present study reported a significant correlation between cassettes genes, Class 1 integrons, and biofilm formation with antibiotic resistance pattern. Hence, continuous screening for antibiotics resistance is vital for infection control and prevention.

Keywords: Antibiotic resistance, biofilm formation, integron, uropathogenic *Escherichia coli*

Introduction

Urinary tract infection (UTI) is a pathogenic invasion to urothelium alongside inflammation that includes lower and upper UTIs.[1] UTI is usually caused by Uropathogenic *Escherichia coli*. Seventy to ninety-nine percent high antibiotic resistance in UPEC (multidrug resistance [MDR]) leads to complications and treatment failures, recurrence, and prolonged UTI which ultimately leads to parenchymal destruction or renal failure.[2,3]

A systematic review and meta-analysis on UTI from Iran in 2020 showed that the pooled prevalence of UTI in women and men was 65.5%, and 30.7%, respectively.[4] In addition, the

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Received: 25-Aug-2021 **Revised:** 10-Jan-2022
Accepted: 10-Jan-2022 **Published:** 29-Mar-2022

Access this article online

Quick Response Code:

Website: http://iahs.kaums.ac.ir

DOI: 10.4103/iahs.iahs_163_21

How to cite this article: Khorshidi A, Zadeh NM, Khaledi A, Moosavi GA, Shakerimoghaddam A, Matinpur A. Investigation of class 1 integrons and biofilm formation in multi-drug resistance uropathogenic *Escherichia coli* isolated from patients with urinary tract infection in shohadaye qom hospital, Iran. Int Arch Health Sci 2022;9:47-52.

same study reported the combined prevalence of MDR strains of uropathogenic *E. coli* (UPEC) recovered from UTI 81.1%.[4]

Integrons and genetic cassettes are key factors and important risk factors for the development of antibiotic resistance in UPEC isolates.[5] Antibiotic resistance results from these genes have raised concerns about the treatment of infections caused by UPEC MDRs.[6] Resistance genes are often spread through mobile genetic elements such as plasmids and integrons. Recent studies on UPEC have shown a strong link between the presence of integrons and antibiotic resistance in various regions of Asia, Europe, and the United States.[7] Genetic cassettes are mobile genetic elements that lack a promoter sequence in their structure; thus, expressing gene cassettes is associated with the presence of promoters in the structure of integrons. The *dfra*1 and *dfra*17, 7 genetic cassettes were recognized in 16% and 70.6% of UPEC isolates, respectively. The dfra17, 7 is highly associated with class 1 integrons.[2] This genetic cassette carries more than 40 resistance genes associated with resistance to aminoglycosides, beta-lactams, chloramphenicol, macrolides, and sulfonamides.[8] Class 1 integron (int1) is recognized in 49% of UPEC species.

Another major factor in the spread of antibiotic resistance is biofilm.[9] Infections induced by biofilm are difficult to treat. *in-vitro* biofilm formation ability is reported in 76.5% of UPECs. The cell proximity in the biofilm can help the exchange of mobile genetic elements and extend antibiotic resistance.[10] When microorganisms disperse in biofilm they will get vulnerable to antibiotic agents. Bacterial resistance is not acquired within the biofilm, but it happens through mutations or mobile genetic elements such as class I integrons and *dfr*-17 gene cassette. This fact suggests that genes associated with antibiotic resistance are involved in biofilm formation.[11] In recent years, the role of integrons as mobile genetic elements in the horizontal transmission of antibiotic resistance has been confirmed, since integrons lead to the broad spread of antibiotic resistance from one strain to another one, therefore, their identification has great importance to implement for infection control programs and prevent the spread of resistant strains.

Hence, this study aimed to investigate class 1 integrons and biofilm formation in MDR UPEC isolated from patients with UTI in Shohadaye Qom hospital from 2018 to 2019.

Materials and Methods

This work was approved by the Ethics Committee of Kashan University of Medical Science; IR. KAUMS. NUHEPM. REC.1397.034.

In this cross-sectional study, 1217 urine samples were collected from patients referred to Shohadaye Qom Hospital, Qom University of Medical Sciences, Qom, Iran, for a year from 2018 to 2019. Among them, 380 (31.2%) were positive for UTI. In total, 166 UPEC isolates (43.7%) were recovered from 380 cultures through phenotypic methods. Then, UPEC isolates were confirmed by polymerase chain reaction (PCR) *usp* house-keeping gene.[12]

Antibiotic susceptibility testing was performed for 120 isolates using Kirby Bauer disk diffusion method according to CLSI 2018 guidelines.[13] The antibiotics used in this study were purchased from Mast Company (UK), which included: Ampicillin (10 µg), Cephalothin (30 µg), Trimethoprim, Sulfamethoxazole (23.75/1.25 µg), cefazolin (30 µg), Ceftriaxone (30 µg), Ceftizoxime (30 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Gentamicin (10 µg) Nitrofurantoin (300 µg), Norfloxacin (10 µg), and Imipenem (10 µg).

Biofilm formation was investigated according to Meshram method.[14] Briefly, isolates were inoculated in a 5 mL BHI medium containing 1% sucrose and incubated at 37°C for 24 h. After that, 1 mL 1% sucrose broth was added to a 2 mL BHI medium to reach 0.5 McFarland turbidity standard (1.5×10^8 colony-forming units [CFU]/mL). Two hundred microliters of dilution 1:1000 McFarland 1.5×10^8 CFU/ml were added to each well and the plates were incubated at 37°C for 24 h. The next day, the wells were washed in PBS three times, and then fixed with ethanol 96% for 15 min, as well stained with crystal violet 2%. Finally, glacial acetic acid 33% was added to each well and the absorbance was read in 492 nm using an ELISA reader; accordingly, the amount of biofilm was calculated.[15]

DNA was extracted through phenol-chloroform-isoamyl alcohol method.[16] At last, 1 mL elution buffer with pH = 7–8 was added to each microtube and put on a shaker for 2 h until the DNA is dissolved and prepared for PCR.

The PCR reaction was performed by the reaction mixture as described before.[17] Following primers were used to amplify virulence genes: F-*int1: 5′*-GACGATGCGTGGAGACC; R-*int1: 3′*-CTTGCTGCTTGGATGCC,[18] F-*dfra: 5′*-GTTA TGGAGCAGCAACGATGT, R-*dfra: 3′*-ACCACTACCG ATTACGCCAT (this study); F-*usp: 5′*-CCGATACGC TGCCAATCAGT; R-*usp3′*-ACGCAGACCGTAGG CCAGAT.[19] PCR Thermocycler program was as follows: 5 min at 94°C and 40 cycles including denaturation at 94°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 60 s and 72°C for 300 s for the final extension in a Thermal Cycler apparatus (Eppendorf master cycler, MA, Germany). The PCR product was electrophoresed on a 1.5% agarose gel in 1x TBE buffer at a voltage of 100 and 50 mA. The gel was then placed in a trans-illuminator and the picture was taken by the gel-documentation system.[17]

Statistical analysis

The analysis was performed using SPSS Statistics (IBMCorp. Released 2012. IBMSPSS Statistics for Windows, Version 16. Armonk, NY: IBM Corp). Chi-square or Fisher's exact tests were used to determine the significance of the differences. A difference was considered statistically significant if the *P* value was < 0.050.

Results

The prevalence of UTI was 380 (31.2%). The prevalence of UTI in male and female was reported 231 (61%), and 148 (39%), respectively. In total, 166 isolates (43.7%) of UPEC were recovered. Of these, 120 isolates (72.3%) were MDR. One-hundred and fifteen isolates (95.8%) out of 120 MDR strains of *E. coli* were recovered from women and 5 isolates (4.2%) were from men. Most patients were over 19 years old. Five isolates (20.8%) were retrieved from patients aged ≥19 years and 95 isolates (79.2%) were recovered from patients aged >19 years.

The highest resistance of MDRs was observed against ampicillin and no resistance was reported to imipenem. Eighteen isolates showed resistance to 3 classes of antibiotics and 41 isolates showed resistance to four classes of antibiotics. The antibiotic susceptibility pattern is abstracted in [Figure 1].

Among MDRs, 71, 18, 15, and 16 isolates were negative (OD <0.1), weak (OD = 0.1–0.2), moderate (OD = 0.2–0.3), and strong biofilm (OD > 0.3) producers, respectively.

Of the 120 MDR isolates tested, 47.5% of them were positive for the *int*-1 gene [Figures 2 and 3], and 25.8% of the isolates contained the *dfr*-17 gene cassette [Figures 2 and 4], 20% of isolates had both genes, as well 46.7% of the isolates lacked both genes.

As shown in [Table 1], among 57 (47.5%) MDR isolates that were *int*-1 gene-positive, 15 isolates (93.8%) had strong biofilm which showed a statistically significant correlation between *int*-1 gene and biofilm formation ($P < 0.001$). Among 31 (25.8%) MDR isolates that were positive for *dfr*-17 gene, 8 (50%) isolates had strong biofilm which showed a statistically significant relationship between *dfr*-17 gene and biofilm ($P < 0.001$).

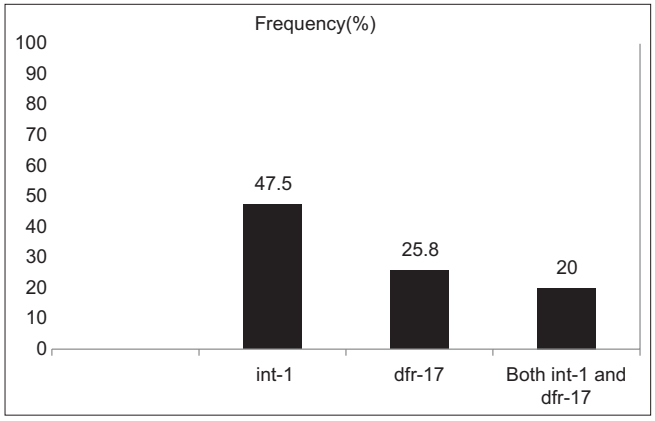

Figure 1: Antibiotic susceptibility pattern of uropathogenic *Escherichia coli* multi-drug resistance isolates. IPM: Imipenem, FM: Nitrofurantoin, NOR: Norofloxacin, CT: Ceftizoxim, AN: Amikacin, GM: Gentamicin, CTX: Cefotaxime, CP: Ciprofloxacin, CAZ: Ceftazidime, CRO: Ceftriaxone, CZ: Cefazolin, SXT: Trimethoprim-Sulfamethoxazole, CF: Cefalotin, AM: Ampicillin

Discussion

As it was mentioned in our study, 72.3% of *E. coli* were MDR which was in line with a study conducted by Fallah *et al*. in Tehran,[20] and a study conducted by Mamani *et al*. in Hamedan, which reported 77.5% and 77%, respectively.[21] Other studies conducted by Iranpour *et al*.,[22] Shams *et al*.,[23] Mirzarazi *et al*.,[24] Babaei-Hemmati *et al*.,[25] and Kazemnia *et al*.,[26] reported the prevalence of 39.3%, 64.1%, 68%, 55%, and 27.7%, respectively. They all reported lower rates of MDR UPEC isolates than our study.

The results of antibiotic susceptibility testing for 14 antibiotics indicated that 93.3% of isolates showed resistance to ampicillin.[27] Also, Tandogdu *et al*., in 2003–2006 who studied antibiotic susceptibility for UPEC strains, the resistance rate against ampicillin was respectively; 34%, 35%, 40%, 43.3%, 48%, 53.9%, and 56.4 in Germany, France, Poland, Russia, Australia, Italy, Brazil, Spain, and Hungary, respectively.[28] Another antibiotic studied in our study was amikacin with a resistance of 25.7%. According to studies conducted in Iran and other parts of the world, including studies conducted by Hadifar *et al*.,[29] Taheri *et al*.,[30] Tabidehchi *et al*.,[31] Khoramrooz *et al*.,[32] and Mohajeri *et al*.,[33] the resistance rates to Amikacin were reported by 12.1%, 7.5%, 6.6%, 3%, and 0%, respectively. The comparison between our data with the findings of the mentioned studied shows that the level of resistance in the present study is higher.

Table 1: Frequency distribution of uropathogenic multi-drug resistance isolates containing both 1 integron - and dfra-17 gene according to biofilm type

Gen	Strong biofilm isolate (%)	Medium biofilm isolate (%)	Weak biofilm isolate (%)	Lacking biofilm isolate (%)	Total (%)	*P*
Integron-1	15 (93.8)	15 (100)	7 (38.9)	20 (28.2)	57 (47.5)	<0.001
Dfra-17	8 (50)	10 (66.7)	3 (16.7)	10 (14.1)	31 (25.8)	<0.001

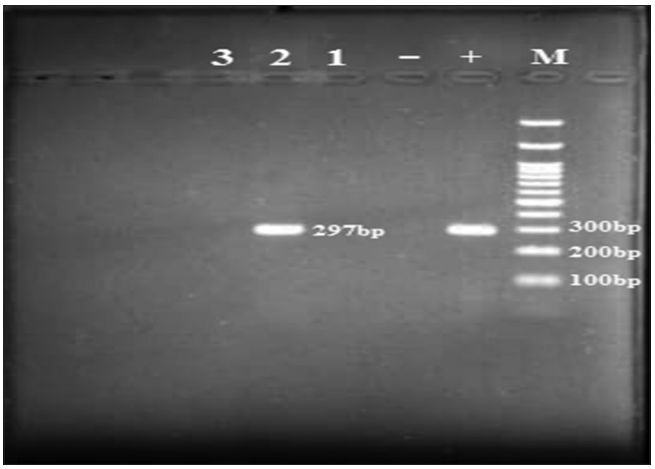

Figure 2: Frequency of uropathogenic *Escherichia coli* multi-drug resistance isolates based on type of genes

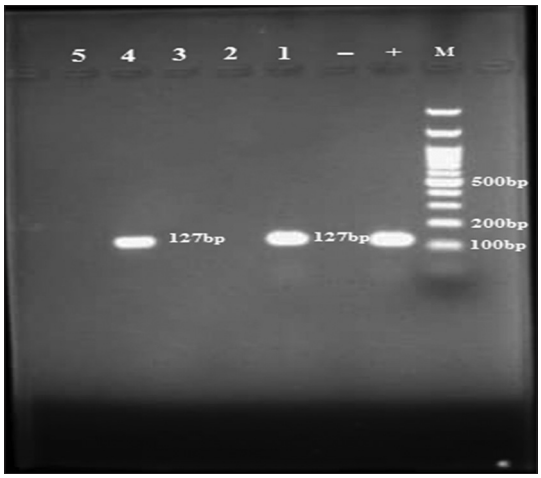

Figure 3: Image of gel electrophoresis related to the *int*-1 gene. (M: 100 bp DNA Ladder, +: Positive control, -: Negative control, No. 1 and 3: Negative isolates for *int*-1 gene, No. 2: Positive isolate for *int*-1 gene

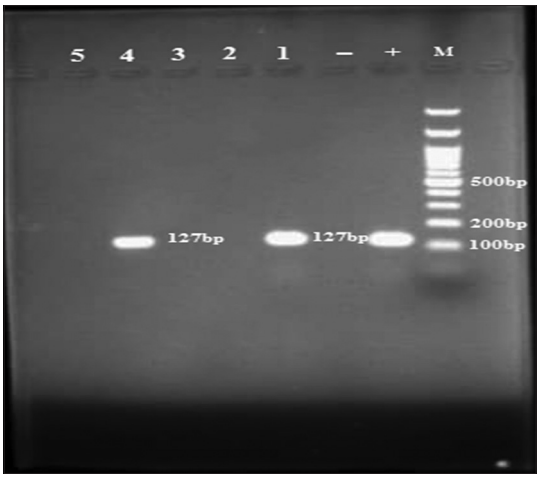

Figure 4: Image of gel electrophoresis related to the *dfr*-17 gene. (M: 100 bp DNA Ladder, +: Positive control, -: Negative control, No. 2, 3 and 5: Negative isolates for *dfr*-17 gene, No. 1 and 4: Positive isolates for *dfr*-17 gene

Here, nitrofurantoin has the lowest resistance after imipenem with a resistance rate of 5% which was in line with other studies conducted in our country by Sahebnasagh *et al*.,[34] and Ranjbaran *et al*.[35] Furthermore, in our study, no antibiotic resistance was observed against imipenem. like that a study conducted by Tabidehchi *et al*. in Tehran in 2015,[31] Mohajeri *et al*. in Kermanshah in 2011,[33] and Ranjbaran *et al*. in Sanandaj in 2012[35] reported a low-level resistance against it, too. Therefore, imipenem still is a therapeutic choice in the treatment of UTI caused by UPEC strains. In our study among all UPEC MDRs 47.5% isolates were positive for *int*-1 gene and 25.8% isolates were positive for *dfr*-17 gene cassette. Overall, different studies worldwide showed that the prevalence of integrons in isolates cause nosocomial UTIs and even community-acquired infection was different in various geographical areas and different populations.[5] A study conducted by Bodour Al-Assil *et al*. in Syrian in 2013,[36] reported the prevalence rate of 54%, in contrary, we reported a higher rate.

Other studies conducted by Muhammad *et al*.,[7] and Ochoa *et al*.,[37] from 2007 to 2015 reported the prevalence of class 1 integrons between 20% and 50%. These findings indicate that antibiotic resistance by integrons is expanding and play an important role in the development of antibiotic resistance, especially through horizontal transmission.[6] The results showed that in isolates containing *int*-1 and a *dfr-17* gene, the rate of biofilm formation was higher, which indicates a significant relationship between the presence of these genes and biofilm formation. This phenomenon is because, within the biofilm, the exchange and uptake of gene cassettes of integrons are enhanced, and also the inhibitory effect of the biofilm reduces the severity of drug penetration.[38]

In the current study, 15 (93.8%) out of 16 *E. coli* MDR isolates that had strong biofilm contained *int*-1 gene showing a statistically significant correlation between biofilm formation and int-1 gene presence ($P < 0.001$). Antibiotic resistance mechanisms such as efflux pumps, modifying enzymes, and target mutations do not appear to be responsible for protecting bacteria within the biofilm. On the other hand, most studies have shown that there is a relationship between efflux pumps and biofilm formation in *E. coli*. It has also been shown that *E. coli* mutants without genes encoding efflux pump formed less biofilm.[39] The current study showed the correlation between resistance genes and biofilm formation. When bacteria inside the biofilm disperse, they get quickly vulnerable to antibiotics because the bacterial resistance within the biofilm is probably due to mutations or mobile genetic elements such as class I integrons and the *dfr-17* gene cassette and is not acquired. This suggests that genes associated with antibiotic resistance are involved in biofilm formation.[40] The biofilm formation has closely related to the antibiotic susceptibility pattern which is routinely used to treat UTI. The antibiotic resistance in biofilm-forming *E. coli* might cause chronic and recurrent UTI. In the present study, imipenem, amikacin, and Nitrofurantoin were effective against UPEC MDR isolates that were able to form biofilm. To achieve more accurate results, it is suggested to study other classes of integrons as well as their relationship with biofilm and their role in hospital infections.

Conclusion

The result of this study indicated that the antibiotic resistance rate is increasing which is associated with the development of multidrug-resistant strains. Furthremore, we reported a significant correlation between cassettes genes, class 1 integrons, and biofilm formation with antibiotic resistance pattern. Thus, continuous screening for antibiotics resistance and the application of effective methods and proper therapeutic approaches is vital for infection control and prevention of its spread.

Acknowledgments

We are grateful to the laboratory personnel of Shohadaye Qom hospital for the invaluable help with gifting some isolates.

Financial support and sponsorship

This publication is based on the data achieved from an MSc degree thesis which was financially supported by Vice-Chancellor of Research, Kashan University of Medical Sciences, Kashan, Iran (Grant number: 97104).

Conflicts of interest

There are no conflicts of interest.

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