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Original article

The relation between class I integrons and multidrug-resistance in extended-spectrum beta lactamase producing *Escherichia coli* isolates

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ABSTRACT

Background: *Escherichia coli* (*E.coli)* is responsible for over 80 percent of urinary tract infections (UTI). The emergence and development of multi-drug resistant (MDR) strains is due to inappropriate use of antibiotics and the horizontal gene transfer between bacteria. The MDR strains of *E. coli* are highly associated with the presence of integrons; also, extended-spectrum beta lactamase (ESBL) producing isolates are usually resistant to various antibiotics. This study aimed to determine the incidence of class 1 integrons and its association with drug resistance in ESBL producing *E. coli* isolated from patients who were suffering from UTI. **Methods:** This study was conducted on 232 hospitalized patients with UTI, from which 160 *E. coli* strains were isolated. Antibiotic susceptibility testing and screening for ESBL production were performed by Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Confirmation for ESBL production was performed by combined disc diffusion test. All the MDR ESBL producing *E. coli* isolates were examined by conventional polymerase chain reaction (PCR) for the presence of *int*I1 gene and related gene cassettes. **Results**: One hundred sixty *E. coli* strains (69%) were isolated from 232 hospitalized patients. The highest percentage of resistance was to aztreonam (92%) followed by ceftazidime and cefotaxime (90%) then ciprofloxacin (79 %). Seventy-two *E. coli* isolates (45%) were found to be ESBL producers and out of them, 61 isolates (84.7%) were MDR. Out of the 61 MDR ESBL-producing isolates, class I integron was identified in 56 isolates (91.8%). **Conclusion:** Our findings indicate a high rate of MDR. Most ESBL-producing isolates are MDR and the high prevalence of class 1 integrons and gene cassettes suggests possible risk for the dissemination of resistance genes and the spread of MDR bacteria.

Introduction

Urinary tract infection (UTI) is one of the most predominant infections in human beings [1]. Gram negative bacteria are the most important causes of UTI; *Escherichia coli* (*E. coli*) is responsible for 80% of infections followed by *Klebsiella spp, Enterobacter spp* and *Acinetobacter spp* [2]. The most important cases of UTI are caused by the Uropathogenic *E. coli* strains [3].

Nowadays, the resistance to the antibiotics used for the treatment of *E. coli* has been increased [4]. The emergence and development of multi-drug resistant (MDR) strains is due to inappropriate use of antibiotics and the horizontal gene transfer between bacteria [5]. Mobile genetic elements such as plasmids, integrons and transposons are important in the development of MDR strains. Moreover, integrons have the ability to be transmitted by transposons or plasmids [6].

Integrons are DNA elements able of

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capturing gene cassettes (including antibiotic resistance genes) and disseminating those using mobile genetic elements. Structurally, integrons consist of two conserved segments (termed 3' conserved segments (3'-CS) and 5' conserved segments (5'-CS) separated by a central variable region that contains the gene cassettes, which encode antimicrobial resistance genes [7]. The 3'-CS is formed by (i) a truncated gene of resistance to quaternary ammonium compounds (ii) a sulfonamide resistance gene (*sul*1); and (iii) an unknown function sequence (*orf*5). The 5'-CS of integrons encode the integrase *(int*I) gene, a primary recombination site (*att*I) and the common promoter (Pc) that ensures the transcription of the cassette genes [8].

In addition to encoding antibiotic resistance genes, the gene cassettes include a recombination site (*att*C). Recombination between the *att*C and *att*l sites leads to insertion of the gene cassette downstream of a resident promoter, and this is mediated by the *int*I gene [7]. Four classes (1-4) of integrons have been identified based on the homology of the *int*I gene; the most prevalent one is class 1 integrons followed by class 2 integrons [9].

In the *Enterobacteriaceae*, such as *E. coli*, MDR are commonly associated with the presence of integrons [10]. On the other hand, extended-spectrum beta lactamase (ESBL) producing isolates are commonly resistant to various antibiotics [11]. Extended-spectrum beta lactamases are β lactamases that are able of conferring bacterial resistance to the penicillins, cephalosporins, and aztreonam by hydrolysis of these antibiotics and are inhibited by β-lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam [12].

Outbreaks caused by ESBL-producing *E. coli* have been reported from hospitals worldwide [13]. Gene-encoding ESBLs are usually located on plasmids, although many of ESBL genes have frequently been found within integron-like structures [14].

Patterns of antibiotic resistance change continuously in UTI-causing microorganisms such as *E. coli*, therefore appropriate studies about local and national antibiotic resistance will be needed for empirical treatment of UTI [15].

Aim

Due to the importance of integrons and their role in increasing MDR strains of *E. coli*, the current study aimed to determine the incidence of class 1 integrons and its association with drug resistance in ESBL producing *E. coli* isolated from patients who were suffering from UTI and admitted to Suez Canal University Hospitals (SCUHs) in Ismailia.

Methods

This is a cross-sectional descriptive study that was carried out during the period from April 2019 to February 2020 on 232 hospitalized patients with UTIs. Patients from all age groups admitted to different wards in SCUHs were included. Age, sex, ward of patient and presence of urinary catheter were recorded. Informed consent was taken from patients to use their data in the current research work. The ethics committee of Faculty of Medicine, Suez Canal University had reviewed and approved the study.

Sample collection and bacterial identification

Clean catch mid-stream urine specimens were collected from each patient in the early morning under aseptic precautions into sterile, wide mouthed containers and then transferred to the laboratory for processing within one hour of collection.

Urine specimens were inoculated on blood and MacConkey's agar plates (Oxoid, UK) and incubated aerobically at 37°C for 24 hours. After incubation, colony count was performed to confirm significant bacteriuria using a sterile calibrated loop measuring 0.001 ml to ensure the presence of 10^5 or more colony forming unit per milliliter of urine as described by Andersson and Hughes [16]. *E. coli* isolates were identified based on standard protocols such as Gram negative bacilli on Gram staining, culture characteristics, and conventional biochemical tests, including oxidase reaction, tube indole test, Simmons citrate, Lysine decarboxylase, Methyl Red Voges Proskauer (MR-VP) and motility indole ornithine (MIO) test [17].

Antimicrobial susceptibility testing

Antibiotic susceptibility testing of *E. coli* isolates was performed by Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, UK) and the results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020). The tested antibiotics were ceftazidime (30µg), cefotaxime (30µg), aztreonam (30µg), cefepime (30µg), cefoxitin (30 µg), amikacin (30µg), gentamicin (10µg), meropenem (10µg), imipenem (10µg), ciprofloxacin (5µg), levofloxacin (5µg) and co-trimoxazole (1.25/23.75 µg) (Oxoid, Basingstoke, UK) [18]. Isolates resistant to at least three classes of antibacterial agents were considered to be MDR [19].

Phenotypic screening and confirmatory methods for ESBLs production

Screening for ESBLs production in *E. coli* isolates was performed by the disk diffusion test using ceftazidime (30 µg) or cefotaxime (30µg) or ceftriaxone (30µg) or aztreonam (30µg). ESBL production was suspected if the inhibition zone diameter was ≤ 22 mm for ceftazidime, ≤ 27 mm for cefotaxime, ≤ 25 mm for ceftriaxone or ≤ 27 mm for aztreonam [18].

Isolates suspected to be ESBL producers by screening tests was confirmed by using combined disc diffusion test between ceftazidime alone (30 µg) vs. ceftazidime/clavulanic acid (30/10 µg) and cefotaxime alone (30µg) versus cefotaxime/clavulanic acid (30/10µg) (Oxoid, Basingstoke, UK) placed on Mueller-Hinton agar plate lawned with the test organism and incubated at 35°C ± 2°C for 16 h to 18 h. According to the zone diameters, a ≥ 5 mm increase in the inhibition zone diameter for either antibiotic agent tested in combination with clavulanic acid vs. its zone size when tested alone confirmed ESBL production [18]. We used *E. coli* ATCC 25922 as an ESBL quality control strain.

Amplification of class 1 integron genes by PCR

All the MDR ESBL producing *E. coli* isolates were examined by conventional PCR for the presence of *int*I1 gene and related gene cassettes with a set of primers as described in **table (1)** [20].

The bacterial genome was extracted using DNA extraction kit (QIAGEN, Germany) according to the manufacturer's instructions. The quality and quantity of the extracted DNA was analyzed using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

The amplification of *int*I 1, 5'cs, and 3'cs was performed in a thermal cycler (Eppendorf AG, Hamburg, Germany) in a final volume of 25 µL containing 2 µL of DNA extract mixed with 2.5 µL of 10×buffer, 2 µL of deoxynucleotide triphosphates (dNTP) mix at a final concentration of 2.5 mM, 1.5 µL of MgCl2 50 mM, 2 µL of each primer (**Table 1)** and 1.5 units of DNA polymerase. The conditions of the amplification were as follows: initial denaturation at 94°C for 5 min and 35 cycles of denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, primer extension at 72◦C for 2 min, with a final extension at 72°C for 5 min [21]. Sample free of DNA template was used as a negative control.

After amplification, PCR products were separated by gel electrophoresis on 1.5% agarose gel in 1X Tris-Borate-EDTA (TBE) buffer stained with ethidium bromide and finally visualized with ultraviolet light. A standard DNA ladder (Cleaver scientific, UK) was used as weight markers for determining the PCR product size [22].

Statistical analysis

Statistical analysis was done by SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The association between antibiotic resistance and existence of integrons was estimated by Chi-square test. Proportions were compared using the Chi-square test. The significance level was defined as $p<0.05$.

Table 1. primers used for detection of class I integron in MDR ESBL producing *E. coli*

Primer	Sequences (5' to 3')	Amplicon (bp)	References
5' CS	GGCATCCAAGCAGCAAG	variable	[20]
3' CS	AAGCAGACTTGACCTGA		
*int*I1-F	CAGTGGACATAAGCCTGTTC	160	[20]
*int*I1-R	CCCGAGGCATAGACTGTA		

Results

Identification of *E. coli* isolates

One hundred and sixty isolates of *E. coli* (69%) were collected from 232 hospitalized patients who were suffering from UTIs. The *E. coli* strains were isolated from 67 males (41.9%) and 93 females (58.1%) with a mean age of 46.5 ± 23.1 years.

Antibiotic susceptibility testing

The highest percentage of resistance was to aztreonam (92%) followed by ceftazidime and cefotaxime (90%) then ciprofloxacin (79%). Multi-drug resistance was detected in 41.3 % of the *E. coli* isolates.

Detection of ESBL production

Out of the *E. coli* isolates, 72 (45%) were ESBL producers while 88 (55%) were non ESBL producers. Furthermore, **(Table 2)** illustrates that 84.7% of the ESBL-producers were MDR, while only 5.7 % of the non-ESBL-producers were MDR and this result was statistically significant ($p< 0.05$).

Detection of class 1 integrons genes by PCR

As shown in **figure (1)**, out of the 61 ESBL-producing *E. coli* isolates with MDR, class I integron was detected in 56 isolates (91.8%) and from them, 51 isolates harbored gene cassettes of different sizes **(Figure 2).** These gene cassettes showed two different patterns in gel electrophoresis (45 isolates showed a single band at 160 bp and another 6 isolates showed a single band at 1000 bp). The relationship between the existences of class I integrons and antibiotic resistance in the 61 MDR ESBL-producing *E. coli* isolates was illustrated in **table (3)**. It was found that 88.5%, 83.6%, 83.6% and 81.9% out of 61 MDR ESBL producing *E. coli* isolates that carried class I integrons were significantly resistant to aztreonam, ceftazidime, cefotaxime and ciprofloxacin, respectively ($p < 0.05$).

Table 2. The relation between ESBL production and MDR in 160 *E.coli* isolates.

	ESBL production No. (%)	Non ESBL production No. (%)	Total
MDR	61 (84.7 %)	5 (5.7 %)	66
Non MDR	11 (15.3 %)	83 (94.3%)	94
Total	72	88	160

Table 3. The relationship between class I integrons and antibiotic resistance in 61 MDR ESBL-producing *E. coli* isolates.

Antibiotics	Resistance of isolates containing class I integron (n= 56) No. (%)	Resistance of isolates lacking class I integron (n= 5) No. (%)	Total resistance No. (%)	*p* value
Aztreonam	54 (88.5%)	5 (8.2%)	59 (96.7%)	*0.023**
Ceftazidime	51(83.6%)	5 (8.2%)	56 (91.8%)	*0.028**
Cefotaxime	51(83.6%)	5 (8.2%)	56 (91.8%)	*0.028**
Ciprofloxacin	50 (81.9%)	5 (8.2%)	55 (90.2%)	*0.030**
Co-trimoxazole	50 (81.9%)	4 (6.6%)	54 (88.5%)	*0.157*
Amikacin	40 (65.6%)	3 (4.9%)	43 (70.5%)	*0.137*
Gentamicin	38 (62.3%)	2 (3.3%)	40 (65.6%)	*0.135*
Levofloxacin	37 (60.7%)	2 (3.3%)	39 (63.9%)	*0.133*
Meropenem	37 (60.7%)	1 (1.6%)	38 (62.3%)	*0.119*
Cefepime	22 (36.1%)	1 (1.6%)	23 (37.7%)	*0.110*
Cefoxitin	20 (32.8%)	0 (0%)	20 (32.8%)	*0.051*
Imipenem	19 (31.1%)	0 (0%)	19 (31.1%)	*0.062*

* Statistically significant (*p <0.05*).

Figure 1. Agarose gel electrophoresis of *int*I 1 gene amplicons (160bp). Lane M shows standard DNA ladder with molecular weight size in the range of 100-1500 bp. Lanes from 1-9 show *int*I 1 gene containing samples.

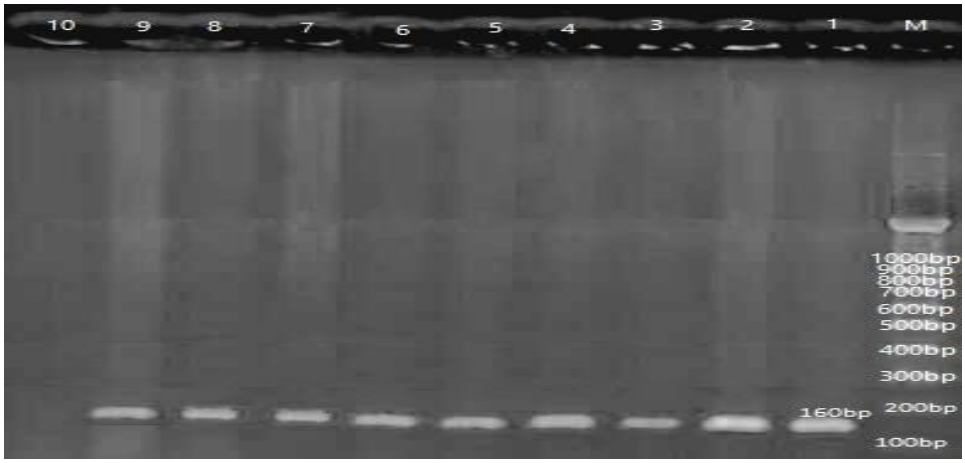
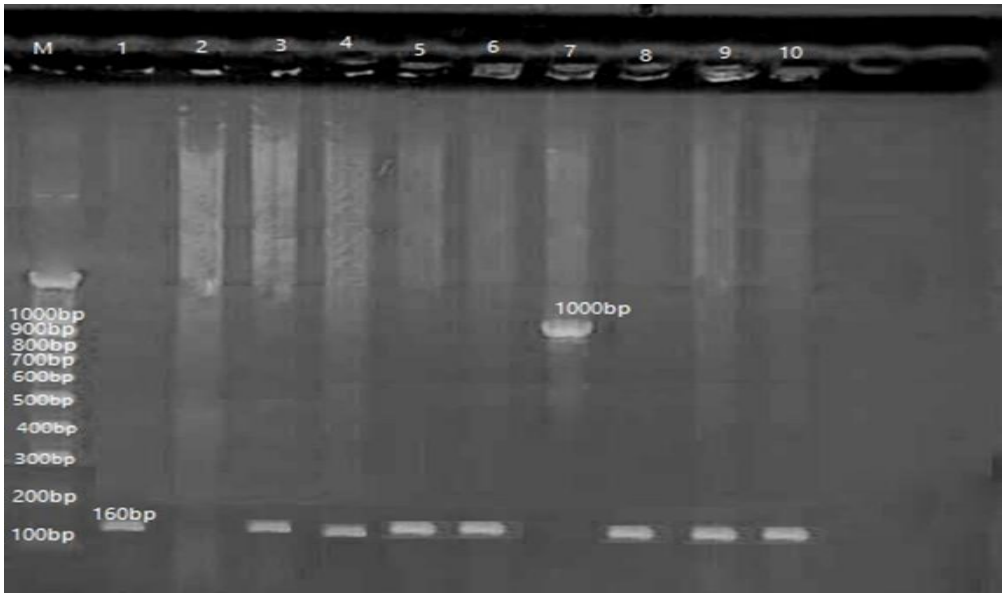


Figure 2. Agarose gel electrophoresis of class I integron gene cassettes. Lane M shows standard DNA ladder with molecular weight size in the range of 100-1500 bp. Lanes 1, 3-6 and 8-10 show clinical isolates containing gene cassettes with a length of 160 bp, lane 7 indicates clinical isolates containing gene cassettes with a length of 1000 bp; lane 2 does not have class I integron gene cassettes.



Discussion

Antimicrobial resistance in the *Enterobacteriaceae* family has become a major health problem, reaching alarming levels all over the world. In particular, the dissemination of ESBLs has recently increased and became a serious health threat worldwide [22].

In the current study, the highest percentage of resistance was to aztreonam (92%) followed by ceftazidime and cefotaxime (90%) then ciprofloxacin (79%). Multi-drug resistance was detected in 41.3 % of the *E. coli* isolates. For all the tested antibiotics, ESBL-producing isolates showed significantly higher antibiotic resistance than non-ESBL producing isolates ($p<0.05$). The spread of mobile genetic elements among different strains may have resulted in the antimicrobial-resistant phenotypes observed in this study. **Akya et al.** in Iran found that the highest rate of resistance was noticed for ceftriaxone, cefotaxime, and cotrimoxazole [23] which agrees with a previous study in Iran [24]. A higher percentage of MDR (87.9%) among *E. coli* isolates was reported by **Al-Hammadi** and colleagues in Yemen [25].

In contrast**, El-Hendawy et al.** in Egypt demonstrated that all of *E. coli* strains causing nosocomial infections were MDR and only 40–50% of this E. coli showed resistant to aztreonam, imipenem, ceftriaxone, and ceftazidime [26].

The current study revealed that 45% of the isolated *E. coli* was found to be ESBL producers. Furthermore, 84.7 % out of ESBL-producing isolates were MDR. **Akya et al.** found that 27.5 % of isolated *E. coli* strains from UTIs were ESBL-producers and among them, 98.5% were MDR [23]. Moreover, **El-Hendawy et al.** in Egypt demonstrated that ESBL genes were positive among 24 out of 32 *E. coli* isolates studied (75%) and all of them were MDR [26]. This emphasizes the importance of ESBL as a good indicator for MDR in *E. coli* isolates.

In our study, it was found that class I integron gene was detected in 56 (91.8%) out of the 61 MDR ESBL-producing *E. coli* isolates and from them, 51 isolates harbored gene cassettes of different sizes. These findings indicates the important role of integrons in the development of antibiotic resistance among different strains due to acquiring various resistance genes. Moreover, the presence of these gene cassettes in integrons plays a very important role in horizontal gene transmission and emergence of resistant strains.

On the other hand, other studies have shown that class 1 integron values were nearly closer to the value of class 1 integrons in our study. For example, **Akya et al.** found that class I integron was detected in 92.3% of MDR ESBL-producing *E. coli* isolates and among them, 57 isolates harbored gene cassettes of different sizes [23]. Also, **Pérez-Etayo et al.** demonstrated that class 1 integron was present in 92% of the ESBL-producing *E. coli* isolates [22].

In addition, **Salimizand et al.** found that *int*I1 was detected in all (100%) of the MDR *Klebsiella* spp. Moreover, they found that the gene cassettes integrated into the class 1 integrons were identified in different sizes from 100 to 2300 bp. All of the isolates (100%) had 800 bp bands, 87% had 400 bp bands and 65% isolates had 2300 bp bands [27].

In contrast, the class 1 integron value in our study was higher than that observed in other studies. In Egypt, **Salem et al.** revealed that 105 out of 188 (56%) MDR *E. coli* isolates harbored *int*1 gene [28]. **El-Hendawy et al.** revealed that 63.8% of *E. coli* isolated from nosocomial infections had the *int*I1 gene [26].

In Yemen, **Al-Hammadi et al.** found that class 1 integron was found in 67% of the MDR *E. coli* isolates [25]. In Jazan area Kingdom of Saudi Arabia, **Abdelhaleem et al**. reported that class 1 integrons value was 54.4 % [29]. The higher percentage of class I integron in this study comparing with these studies may be related to the fact that we only examined ESBL producing isolates for integrons.

The relationship between the carriage of class I integrons and antibiotic resistance in the 61 MDR ESBL-producing *E. coli* isolates was statistically analyzed in our study. It was found that 88.5%, 83.6%, 83.6% and 81.9% out of 61 MDR ESBL producing *E. coli* isolates that harbored class I integrons were significantly resistant to aztreonam, ceftazidime, cefotaxime and ciprofloxacin, respectively ($p<0.05$). Similarly, **Akya et al.** observed a significant correlation between antibiotic resistance to co-trimoxazole ($P = 0.01$), streptomycin ($P= 0.018$) and ceftazidime ($P=0.032$) with class I integrons, which emphasizes the association between resistant genes and this class of integron [23].

A study conducted in Sudan by **Ibrahim et al.** reported that the percentage of class 1 integrons was 40.6% and the MDR *E. coli* isolates carrying this class of integrons showed high level resistance to trimethoprim-sulfamethoxazole (98.1%), tetracycline (88.9%), ciprofloxacin (70.4%), amoxicillin-clavulanic acid (66.7%), ceftazidime (46.3 %) and chloramphenicol (29.6%) [30]. **Al-Hammadi et al.** showed that the presence of class 1 integron was observed to be significantly higher in ciprofloxacin ($p=0.0002$), amoxicillin-clavulanic acid ($p=0.003$), aztreonam ($p=0.006$), cefepime ($p=0.01$), cefotaxime ($p=0.0003$), ceftazidime ($p=0.002$), ceftriaxone (p = 0.03), norfloxacin ($p=0.0002$) and trimethoprim-sulfamethoxazole ($p<0.0001$) [25].

In conclusion, our findings indicate that *E. coli* isolates confer high rate of antibacterial resistance to the drugs commonly used for the treatment of UTIs. Most ESBL-producing isolates are MDR and the high prevalence of class 1 integrons with its integrated gene cassettes suggests possible risk for the dissemination of resistance genes and plays an important role in the spread of MDR bacteria.

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References

1-**Su J, Shi L, Yang L, Xiao Z, Li X, Yamasaki S.** Analysis of integrons in clinical isolates of *Escherichia coli* in China during the last six years. *FEMS* Microbiology Letters 2006; 254(1):75–80.

2-**Akram M, Shahid M, Khan A.** Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Annals of Clinical Microbiology and Antimicrobials 2007; 6(4): 1-7.

3-**Dormanesh B, Safarpoor Dehkordi F, Hosseini S, Momtaz H, Mirnejad R, Hoseini M, *et al.*** Virulence factors and o-serogroups profiles of uropathogenic *Escherichia coli* isolated from Iranian pediatric patients. Iranian Red Crescent Medical Journal 2014; 16(2): e14627.

4-**Ranjbaran M, Zolfaghari M, Japoni-Nejad A, Amouzandeh-Nobaveh A, Abtahi H, Tabib Nejad M, et al.** Molecular investigation of integrons in *Escherichia coli* and *Klebsiella pneumoniae* isolated from urinary tract infections. Journal of Mazandaran University of Medical Sciences 2013; 23(105): 20–27.

5-**Haddadi A, Mikaili Ghezeljeh S, Shavandi**

M. Prevalence of class 1 and 2 integrons among the multidrug resistant uropathogenic strains of *Escherichia coli.* Asian Biomedicine 2017; 9(1): 49–54.

6- **Zeighami H, Haghi F, Hajiahmadi F.** Integrons and their role in antibiotic resistance. Zanjan University of Medical Sciences 2014; 5(22): 61–71.

7-**Collis C and Hall R.** Site-specific deletion and rearrangement of integron insert genes catalyzed by the integron DNA integrase. Journal of Bacteriology 1992; 174(5): 1574–1585.

8- **Ahangarzadeh Rezaee M, Langarizadeh N, Aghazadeh M.** First report of class 1 and class 2 integrons in multidrug-resistant *Klebsiella pneumoniae* isolates from northwest Iran. Japanese Journal of Infectious Diseases 2012; 65(3): 256–259.

9-**Levesque C, Piche L, Larose C, Roy P.** PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrobial Agents and Chemotherapy 1995; 39(1): 185–191.

10-**Ibrahim N, Wajidi M, Yusof M, Tay S**. The integron prevalence of extended-spectrum beta-lactamase producing enterobacterial isolates in a Malaysian teaching hospital. Tropical Biomedicine 2011; 28(3): 668–671.

11- **Machado E, Canton R, Baquero F, Galan J, Rollan A, Peixe L, et al.** Integron content of extended-spectrum-beta-lactamase-producing *Escherichia coli* strains over 12 years in a single hospital in Madrid, Spain. Antimicrobial Agents and Chemotherapy 2005; 49(5): 1823–1829.

12-**Bush K and Fisher J.** Epidemiological expansion, structural studies, and clinical challenges of new ß-lactamases from gram negative bacteria. Annual Review of Microbiology 2011; 65: 455-478.

13-**Phongpaichit S, Tunyapamit W, Pruekprasert P.** Antimicrobial resistance, class 1 integrons and extended spectrum beta-lactamases in *Escherichia coli* clinical isolates from patients in South Thailand. Journal of Health Sciences 2011;57: 281–288.

14-**Bonnet R, Sampaio J, Chanal C, Sirot D, De Champs C, Viallard JL, *et al.*** A novel class A extended-spectrum beta-lactamase (BES-1) in *Serratia marcescens* isolated in Brazil. Antimicrobial Agents and Chemotherapy 2000; 44(11): 3061–3068.

15- **Hryniewicz K, Szczypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W**. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. Journal of Antimicrobial Chemotherapy 2001; 47(6): 773–780.

16- **Andersson D and Hughes D.** Antibiotic resistance and its cost: is it possible to reverse resistance? Nature Reviews Microbiology 2010; 8 (4): 260–271.

17-**Tille P.** *Enterobacteriaceae*. In: **Tille P,** editor. *Bailey & Scott's Diagnostic Microbiology*, 14th ed. Canada: Mosby Elsevier; 2018: part III, section 7, ch.19.

18-**Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing.** 30th ed. CLSI Supplement M100. USA: Wayne, PA; 2020.

19-**Magiorakos A, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al.** Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical*

Microbiology and Infection 2012; 18 (3):268-281.

20-**Mirnejad R, Mostofi S, Masjedian F**. Antibiotic resistance and carriage class 1 and 2 integrons in clinical isolates of *Acinetobacter baumannii* from Tehran, Iran. Asian Pacific Journal of Tropical Biomedicine 2013; 3(2): 140–145.

21-**Shahcheraghi F, Badmasti F, Feizabadi M.** Molecular characterization of class 1 integrons in MDR *Pseudomonas aeruginosa* isolated from clinical settings in Iran, Tehran. FEMS Immunology and Medical Microbiology 2010; 58(3): 421-425.

22-**Pérez-Etayo L, Berzosa M, González D Vitas A**. Prevalence of integrons and insertion sequences in ESBL-Producing *E. coli* isolated from different sources in Navarra, Spain. International Journal of Environmental Research and Public Health 2018; 15(2308): 1-12.

23-**Akya A, Lorestani R, Rostamian M, Elahi A, Baakhshii S, Aliabadi M, et al.** The relationship of class I integron gene cassettes and the multidrug-resistance in extended-spectrum β-lactamase producing isolates of *Escherichia coli*. Archives of Pediatric Infectious Diseases 2019; 7(3): e87961.

24-**Haddadi A, Mikaili Ghezeljeh S, Shavandi M**. Prevalence of class1and 2 integrons among the multidrug resistant uropathogenic strains of *Escherichia coli.* Asian Biomedicine 2017; 9(1): 49–54.

25-**Al-Hammadi M, Al-Shamahy H, Ali A, Abdulghani M, Hassan Pyar H, AL-Suboal I**. Class 1 integrons in clinical multi drug resistance *E. coli*, Sana'a Hospitals, Yemen. Pakistan Journal of Biological Sciences 2020; 23(3): 231-239.

26-**El-Hendawy G, Melake N, Salama A, Eissa N, Zahran W, Elaskary S**. Distribution of class 1 integrons among multidrug-resistant *Escherichia coli* in Menoufia University Hospitals and commensal *Escherichia coli* isolates. Menoufia Medical Journal 2017; 29: 772–782.

27-**Salimizand H, Shahcheraghi F, Kalantar E, Badmasti F.** Molecular characterization of class 1 integrons and gene cassettes in multidrug resistant (MDR) *Klebsiella spp*. isolated from hospitalized and outpatients in Iran, 2009. Iranian Journal of Microbiology 2013; 5 (1): 48-55.

28-**Salem M, Muharram M, Alhosiny I.** Distribution of classes 1 and 2 integrons among multi drug resistant *E. coli* isolated from hospitalized patients with urinary tract infection in Cairo, Egypt. Australian Journal of Basic and Applied Sciences 2010; 4(3): 398-407.

29-**Abdelhaleem A, Homeda H, Hershan A, Makeen A, Alsanosy R.** Class 1 integrons gene in drug resistant *E. coli* and isolated from different clinical specimens in Jazan area K.S.A. International Journal of Current Research 2014; 6: 9283-9286.

30-**Ibrahim M, Magzoub M, Bilal N, Hamid M.** Distribution of class I integrons and their effect on the prevalence of multi-drug resistant *Escherichia coli* clinical isolates from Sudan. Saudi Medical Journal 2013; 34 (3): 240-247.