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Antibiotic Resistance Pattern and Prevalence of Class 1, 2, and 3 Integrons in *Escherichia coli* Strains Isolated from Infected Patients in Zahedan

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ABSTRACT

Background & Objective: *Escherichia coli (E. coli)* is among the most frequently encountered bacterial pathogens in hospital-acquired infections.The only treatment for *E. coli* the use of various antibiotics. In recent years, *E. coli* has demonstrated extensive drug resistance, which is partly to the presence of integrons in its genome. This study aimed to assess the antibiotic resistance profiles and determine the prevalence of class 1, 2, and 3 integrons in *E. coli* strains isolated from patients infected with the hospital in Zahedan.

Materials & Methods: In this cross-sectional study, 200 *E. coli separate* were collected and, then confirmation of the isolates was performed using biochemical tests and ultimately an antibiogram. The presence of class I, II, and III integron genes was then investigated using specific primers and PCR method.

Results: Out of 200 *E. coli separate*,170 strains were MDR, with the highest resistance observed to cefazolin (74.5%), cotrimoxazole (66.5%), ceftriaxone (66.5%),and ampicillin (63.5%). The Int1 and Int2 genes were identified in 40% and 12% of the strains,respectively; however, the Int3 gene was not found in the studied strains.

Conclusion: The findings of this study reveal a relatively high level of antibiotic resistance among the *E. coli* isolates, which may be partly attributed to the increased prevalence of integrons. Consequently, restricting the indiscriminate use of antibiotics could help reducing the spread of resistance genes.

Keywords: Integron, *Escherichia coli*, Antibiotic Resistance

1. Introduction

Escherichia coli (*E. coli*), one of the most common infectious pathogens in hospitals, causes a high number of infections worldwide (1, 2). *E. coli* is a gram-negative, non-sporulating bacillus, facultative anaerobic belonging to the family Enterobacteriaceae (3). While *E. coli* is a natural part of the body's normal flora, it has five pathotypes responsible for intestinal diseases, such as Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), and Enteroaggregative *E. coli* (EAEC). Additionally, strains of this bacterium cause various extraintestinal infections, such as Uropathogenic *E. coli* (UPEC), which leads to urinary tract infections, particularly cystitis (4). Furthermore, *E. coli* is responsible for various infectious diseases, including urinary tract infections (UTIs), pyelonephritis, cystitis, abdominal abscesses, pneumonia, osteomyelitis, soft tissue infections, and bacteremia (5).

According to the World Health Organization, hospital-acquired infections caused by *E. coli* are prevalent in developing countries, with a rate of more than 50 percent, and in developed countries, at 15 to 25 percent (6). Studies conducted between 1997 and 2016 in more than 200 health centers across 45 countries indicated that the prevalence of bloodstream infections caused by *E. coli* was 20.5%, making it the second most common bloodstream infectious agent (7).

The primary treatment for *E. coli* infections involves the use of various antibiotics. Due to the overuse of antibiotics, resistance to antibacterial compounds in *E. coli* has become a common phenomenon (8). According to a 2019 review on the prevalence of antibiotic resistance in *E. coli*, the prevalence rates were as follows: ciprofloxacin 7.7%, trimethoprim 22.2%, sulfamethoxazole 22.5%, chloramphenicol 6.6%, cotrimoxazole 11.5%, tetracycline 37.3%, amoxicillin 53.4%, gentamicin 5%, ampicillin 33.4%, and streptomycin 27.7% (9).

Initially, it was thought that the transfer of resistant genes in bacteria occurred mainly through conjugation and transduction by plasmids, phages, and transposons. However, in 1995, scientists Hall and Colli identified another mechanism for transferring resistance genes through genetic elements called integrons. Integrons are mobile genetic units that, when embedded in plasmids, chromosomes, and transposons, receive and transport resistance genes located within gene cassettes (10). Integrons play a significant role in antibiotic resistance in clinical *E. coli* strains, as they can acquire and express gene sequences that encode antibiotic resistance (10). Horizontal transfer of integrons is recognized as the most effective method of spreading antibiotic-resistant genes and contributing to the emergence of resistant strains (11).

Structurally, all integrons consist of three main components: the 5' conserved end, the 3' conserved end, and a central variable region between the 5' and 3' ends. The central region comprises three genes: integrase (IntI), the attI site, and promoter sequences (Pc and Pint), which are responsible for recombination, cassette attachment, and gene expression, respectively (12). To date, nine classes of integrons have been identified based on differences in the integrase gene, with class 1 integrons being the most prevalent, followed by classes 2 and 3, particularly among clinical isolates (13). Class 1 integrons are the most commonly detected in clinical strains, with a prevalence of 50-70%, whereas class 2 and 3 integrons are less prevalent (10). Identification of different classes of integrons in bacteria is based on the integrase gene (Int) they encode (13).

The combination of multiple cassettes in an integron is associated with resistance to several classes of antibiotics and the production of multidrug-resistant (MDR) strains (14). Previous studies have confirmed a strong relationship between the presence of integrons and multidrug resistance, especially among members of the Enterobacteriaceae family (15). Given that drug resistance patterns are influenced by the epidemiology of each region and the importance of identifying molecular mechanisms through which bacteria spread resistance genes, as well as the lack of studies on antibiotic resistance patterns caused by integrons in Zahedan, this study is necessary. Therefore, this research aims to determine the antibiotic resistance patterns and the presence of class 1, 2, and 3 integrons in *E. coli* strains isolated from Imam Ali Hospital, Zahedan, to aid in proper patient treatment and reduce healthcare costs.

2. Materials and Methods

In this study, 200 *E. coli* isolates were collected from infected patients at Imam Ali Hospital, Zahedan University of Medical Sciences, during the year 2024–2025, due to the high number of admitted cases. The isolates were preserved in BHI broth supplemented with 15% glycerol and stored at -15°C to -25°C (16). Standard biochemical tests including oxidase, catalase, methyl red, indole production, Simmons citrate, Voges-Proskauer, urease hydrolysis, ornithine decarboxylase, and sugar fermentation assays were performed for confirmation of *E. coli* isolates (17). After biochemical identification, antibiotic susceptibility testing was conducted using the disc diffusion method.

2.1 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility profiles were determined using the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (18). *E. coli* ATCC 25922 was used as the quality control strain. Multidrug-resistant (MDR) isolates were defined as those exhibiting resistance to more than two classes of antibiotics. MDR isolates were selected for further analyses, and genomic DNA was extracted for subsequent molecular testing (19).

2.2 DNA Extraction

Genomic DNA extracted from the isolates using the boiling method. Briefly, colonies taken from freshly cultured 18-hour nutrient agar plates and inoculated into microtubes containing normal saline to obtain a uniform suspension. This suspension boiled for 20 minutes, then cooled and centrifuged at 12,500 rpm for 15 minutes. 100 µL of the supernatant containing the extracted DNA transferred to 0.5 mL microtubes and stored at -20°C until PCR performed. After extracting DNA from antibiotic-resistant isolates, PCR used to detect the presence and type of integrons.

2.3 Polymerase Chain Reaction (PCR) for Detection of Class 1, 2, and 3 Integrons

To detect integrons in MDR *E. coli* strains, three particular primer sets, as described by Barzegar et al (15), was used. PCR reactions was performed with a final volume of 25 µL, including 7.5 µL of distilled water, 12.5 µL of master mix, 1 µL of each forward and reverse primer, and 3 µL of DNA for each reaction. Thermal conditions for each gene optimized according to the available conditions. Amplified genes run on a 2% agarose gel electrophoresis, and PCR products visualized using a GEL DOCUMENT system. *E. coli* ATCC 25922 used as a positive control for confirming the amplification of class 1, 2, and 3 integron genes (16). For negative control a PCR reaction used without DNA (Table1).

Table 1. Primer sequences and PCR conditions used in the study.

Gene Name	Sequences	Size	Denaturation	Annealing	Extension	References
Int1	F: GGT CAA GGA TCT GGA TTT CG R: ACA TGC GTG TAA ATC ATC GTC	436 bp	94°C for 1 minute	58°C for 35 seconds	72°C for 35 seconds	Barzegar et al (15)
Int2	F: CAC GGA TAT GCG ACA AAA AGG T R: GTA GCA AAC GAG TGA CGA AAT G	788 bp	94°C for 1 minute	60°C for 35 seconds	72°C for 40 seconds	Barzegar et al (15)
Int3	F: AGT GGG TGG CGA ATG AGT G R: TGT TCT TGT ATC GGC AGG TG	600 bp	94°C for 1 minute	59°C for 35 seconds	72°C for 35 seconds	Barzegar et al (15)

3. Result

Based on 200 samples of *E. coli* collected and the results of an antibiogram test, resistance to cefazolin was the highest (74–5%) and to fosfomycin the lowest (4) in the *E. coli* samples. Additionally, the highest sensitivity was observed with amikacin, while the lowest sensitivity was noted with cefazolin. Among the 200 *E. coli* samples tested, 170 samples (85%) were identified as MDR. The results showed a significant relationship ($P<0.05$) between the presence of integrons in *E. coli* strains and antibiotic resistance and the MDR status (Table 2).

In the present study, high levels of resistance to trimethoprim and sulfamethoxazole were observed. The highest percentages of resistance were observed for cefazolin (74.5%), ceftazidime (approximately 68%), trimethoprim-sulfamethoxazole (66.5%), and ceftriaxone (66.5%). Conversely, the lowest resistance was observed for fosfomycin (4%), amikacin (14.5%), and gentamicin (24%) (Figure 1).

3.1 PCR Results

The outbreak of class 1 and 2 integrons was 40% (n=80) and 12% (n=24). Class 3 integron was not found in any of the samples. PCR products IntI, IntI2, and IntI3 were subjected to DNA sequencing. The DNA sequencing results were analyzed using Vector NTI and BLAST software. Integron 1 showed a 99.9% diagnosis accuracy by sequence in GenBank, while integron 2 had a 99.7% diagnosis accuracy.

Table 2. Results of the Antibiotic Resistance Pattern of *E. coli* Isolates.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	21.5% (n=43)	15% (n=30)	63.5% (n=127)
Gentamicin	56% (n=112)	20% (n=40)	24% (n=48)
Fosfomycin	60% (n=120)	36% (n=72)	4% (n=8)
Amikacin	61.5% (n=123)	24% (n=48)	14.5% (n=29)
Meropenem	46.5% (n=93)	17.5% (n=35)	36% (n=72)
Imipenem	36% (n=72)	21.5% (n=43)	42.5% (n=85)
Piperacillin-Tazobactam	52% (n=104)	16% (n=32)	32% (n=64)
Ceftriaxone	16% (n=32)	0% (n=0)	66.5% (n=133)
Ciprofloxacin	17.5% (n=35)	0% (n=0)	61.5% (n=123)
Trimethoprim-Sulfamethoxazole	14.5% (n=29)	0% (n=0)	66.5% (n=133)
Ceftazidime	17.5% (n=25)	0% (n=0)	68% (n=136)
Cefazolin	13.5% (n=27)	12% (n=24)	74.5% (n=149)

Continuous variables are presented as mean ± standard error (SE) unless otherwise specified. Between-group comparisons: Independent samples t-tests for continuous hemodynamic parameters; Mann-Whitney U tests for ephedrine dose (non-normal distribution); Chi-square tests for adverse event frequencies. Time-point analyses used Bonferroni-adjusted t-tests. SD = standard deviation.

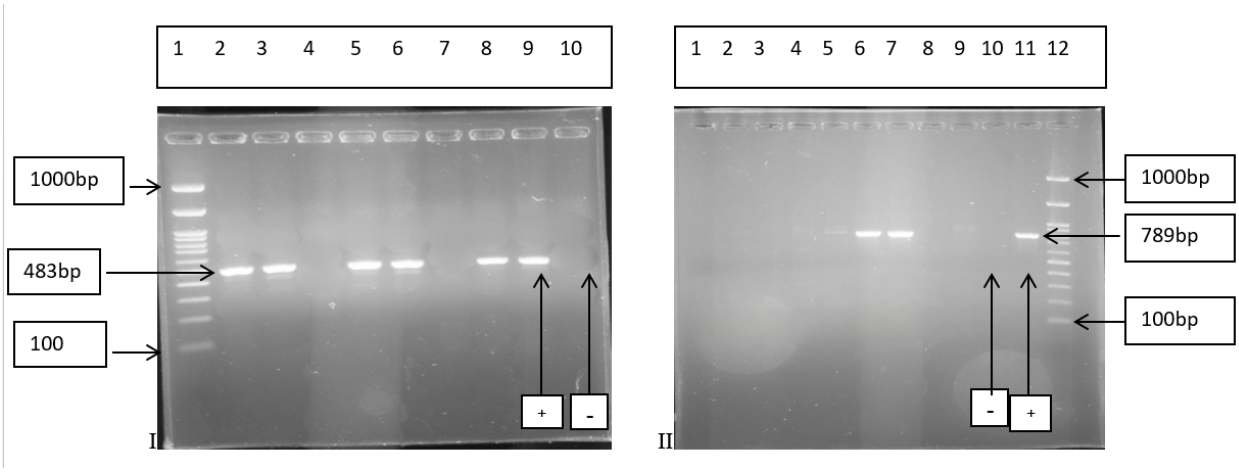

Figure 1. PCR results on integrin class I genes. The DNA ladder (100 bp) is in lane **I:** The positive samples are in lanes 6, 5, 3, 2, and 8. The positive control is in lane 9. The 483 bp is the length of a gene in the I-integration class. **II:** The last lane is a 100-bp DNA ladder; the positive samples are in lanes 6 and 7, the positive control is in lane 11, and the negative control is in lane 10. The 789 bp is the length of an integrin gene in class II (**Prepared by Authors, 2025**).

4. Discussions

Antibiotic resistance is a major problem in the treatment of pathogenic bacteria today is (8). *E. coli* is a prime example of resistance to antibiotics. Antibiotics such as sulfamethoxazole and trimethoprim (cotrimoxazole), which were once the first-line treatment for UTIs, are no longer effective against *E. coli* due to resistance (7).

In the present study, high levels of resistance to trimethoprim and sulfamethoxazole were observed. The highest percentages of resistance rates were observed for cefazolin (74.5%), ceftazidime (approximately 68%), trimethoprim-sulfamethoxazole (66.5%), and ceftriaxone (66.5%). The lowest resistance was observed for fosfomycin (4%), amikacin (14.5%), and gentamicin (24%). According to a 2019 review conducted in Denmark on the prevalence of antibiotic resistance in *E. coli*, the resistance rates were as follow: ciprofloxacin 7.7%, trimethoprim 22.2%, sulfamethoxazole 22.5%, chloramphenicol 6.6%, cotrimoxazole 11.5%, tetracycline 37.3%, amoxicillin 53.4%, gentamicin 5%, ampicillin 33.4%, and streptomycin 27.7% (12). In another study on *E. coli* strains, the highest resistance was reported for tetracycline (92.86%), followed by sulfonamides (71.43%) and ampicillin (52.38%). Only 26.19% and 11.91% of strains were resistant to gentamicin and ciprofloxacin, respectively (18).

Arzanlou et al (15) in a similar study reported that 84.7% of 163 *E. coli* isolates were multidrug-resistant (MDR). The highest levels of resistance were observed against ampicillin (89.6%), trimethoprim-sulfamethoxazole (73.6%), and cefazolin (60.7%), whereas nitrofurantoin showed the lowest resistance rate at 1.2%. Resistance to imipenem was noted in 36.2% of the isolates. Additionally, the prevalence of class 1 and class 2 integrons was 39.9% and 14.1%, respectively, the class 3 integrons was not detected in any of the samples (15).

Integrons are mobile genetic elements that, when embedded in plasmids, chromosomes, or transposons, receive and transport resistance genes carried by gene cassettes. They can acquire and express gene sequences that encode antibiotic resistance, known as gene cassettes (10). Horizontal transfer of integrons is recognized as the most effective method of spreading antibiotic-resistant genes and contributing to the emergence of resistant strains (11).

Previous research conducted in Iran has reported the combined prevalence of class 1 and class 2 integrons to be 41.7% and 17.4%, respectively. Notably, no class 3 integrons have been detected in *E. coli* isolates except for a study by Kargar et al (19), which reported the first identification of class 3 integrons in *E. coli* in Iran. Our results revealed that 40% of *E. coli* isolates carried class 1 integron genes, while 12% harbored class 2 integrons, and no class 3 integrons were detected, aligning partially with prior studies. Given these findings, the use of certain antibiotics should be restricted. Specifically, antibiotics such as trimethoprim-sulfamethoxazole, cefazolin, ceftazidime, and ceftriaxone appear to be less effective due to elevated levels of resistance. Conversely, antibiotics such as fosfomycin, amikacin, and gentamicin, which exhibit lower resistance rates, may offer more promising treatment options.

Strengths of the Study

This study's strengths include the largest sample size analyzed to date compared to previous research, as well as providing comprehensive data on the regional antibiotic resistance patterns and the prevalence of three major integron classes in *E. coli* strains

Limitations of the Study

After some staff refused to cooperate, higher-level management in the treatment unit was consulted or persuaded to allow the research. Lack of access to

advanced equipment, misclassification, unavailability, and incomplete information in the samples were addressed by either tracking complete records or replacing the samples.

5. Conclusion

Identifying antibiotic resistance patterns in local strains and the molecular mechanisms by which bacteria spread resistance genes is essential. This study was necessary because epidemiology in each region affects drug resistance patterns and because little research has been done on the evolution of antibiotic resistance caused by integrons in the city of Zahedan. This study was conducted to determine the antibiotic resistance pattern and the presence of integrons 1, 2, and 3 in *Escherichia coli* strains isolated from Imam Ali (AIS) Hospital in Zahedan. Given the high levels of resistance to various antibiotics in *Escherichia coli* and the high prevalence of integrons in this, area, the results of the analysis of *Escherichia coli* strains and prevalence of integrons showed a direct correlation.

6. Declarations

6.1 Acknowledgments

The authors thank the staff of Ali ibn Abi Talib Hospital's laboratory for their cooperation in collecting the data.

6.2 Ethical Considerations

This study was approved under the ethics committee of Zahedan University of Medical Sciences, Zahedan, Iran (IR.ZAUMC.REC.1403.236).

6.3 Authors' Contributions

Amirhossein Vahid**:** conceptualization, investigation, methodology, project administration, writing, review and editing. Mohammad Bakaeian, Zakaria Bamari: reviewed and approved the final manuscript. Alireza Ansari Moghaddam: statistical analysis. All authors reviewed, and approved the final version of the manuscript

6.4 Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article.

6.5 Fund or Financial Support

This research was derived from a master's thesis in medical microbiology, approved by Zahedan University of Medical Sciences, under research project number 11377.

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

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