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ESBLs Antibiotic Resistance in *Escherichia coli* Isolated from Vaginas of Pregnant Woman Visiting Al-Zahra Hospital, Tabriz, Iran

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ABSTRACT

Background & Objective: Antibiotic resistance, particularly in bacteria producing extended-spectrum beta-lactamases (ESBLs), is a major challenge in managing bacterial infections. Here we investigated the prevalence of antibiotic resistance and its patterns in *Escherichia coli* strains isolated from pregnant women attending Alzahra Hospital, Tabriz, Iran.

Materials & Methods: This cross-sectional study was conducted on 100 vaginal samples collected from pregnant women. The samples were examined for identifying *Escherichia coli* and antibiotic resistance using microbiological tests and the E-TEST method. Additionally, the prevalence of the CTX-M, SHV, and TEM genes in ESBL-producing strains was determined using PCR.

Results: Out of 100 samples, 45 (45%) were identified as Gram-negative, and 27 (27%) were confirmed to be *Escherichia coli*. Among the 27 *E. coli* strains, 9 (33.33%) were identified as ESBL producers. E-TEST results for the ESBL-producing strains revealed cefotaxime resistance ranging from 1 to 2 mg/mL and ceftazidime resistance ranging from 0.25 to 4 mg/mL. One strain showed resistance to very high concentrations (up to 256 mg/mL). The prevalence of the CTX-M and TEM genes in the ESBL-producing strains was 22.22% and 88.88%, respectively, while the SHV gene was not detected in any strain. Two strains carried both the CTX-M and TEM genes simultaneously. The overall prevalence of CTX-M and TEM genes in all isolated *E. coli* strains was 7.4% and 29.62%, respectively.

Conclusion: These findings highlight the necessity for efficient approaches to manage antibiotic resistance.

Keywords: *Escherichia coli*, ESBL, Antibiotic Resistance, *CTX-M* Genes, *SHV* Genes, *TEM* Genes, Tabriz Al-Zahra Hospital

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1. Introduction

Vaginal infections, also known as vaginitis, are a common condition that can cause discomfort and unusual discharge. Several types of infections can affect the vagina, with bacterial vaginosis, yeast infections, and trichomoniasis being the most prevalent. These infections can lead to a range of symptoms, including itching, burning, unusual discharge, and altered vaginal odor (1).

Vaginitis is common during pregnancy. While vaginitis can be uncomfortable and cause symptoms like itching, burning, and abnormal discharge, it's usually treatable and doesn't always lead to complications. However, certain types of vaginitis, can pose risks to both the pregnant woman and the developing fetus if left untreated (2, 3).

The antibiotic-resistant bacteria, is a major challenge in the treatment of vaginitis during pregnancy. This resistance is caused by inappropriate and excessive use of antibiotics, leading to a reduction in effective treatment options. Treating pregnant women with resistant strains requires drugs that are safe for both the mother and fetus. Therefore, continuous monitoring of antibiotic resistance patterns and the development of evidence-based treatment

guidelines are of great importance to maintain maternal and fetal immunity while combating infection (4).

ESBLs are categorized into three primary groups: Ambler class A ESBL (ESBLA), miscellaneous ESBLs (ESBLM), and carbapenem-degrading ESBLs (ESBLCARBA). The majority of ESBLs globally are classified under the ESBLA group, which encompasses various forms of sulfhydryl-exchange variable beta-lactamases (SHV), Temuneera beta-lactamases (TEM), and cefotaxime-M beta-lactamases (CTX-M). Approximately 90% of Escherichia coli strains that possess TEM-1 exhibit resistance to ampicillin, penicillin, and first-generation cephalosporins, while remaining susceptible to oxyiminocephalosporins. During the 1980s, the evolution of SHV-1 and TEM-1 from non-ESBL to ESBL in Klebsiella pneumoniae and Escherichia coli strains, respectively, through specific amino acid substitutions, enabled them to hydrolyze oxyimino-cephalosporins. Of the 140 TEM and 60 SHV strains identified, some are capable of inactivating third-generation cephalosporins and aztreonam (5).

The objective of this research was to examine the antibiotic resistance of Extended-Spectrum Beta-Lactamases (ESBLs) in Escherichia coli obtained from pregnant women who were referred to Al-Zahra Hospital in Tabriz, Iran.

2. Materials and Methods

About 100 pregnant women who had been referred to Al-Zahra Hospital in Tabriz were included in the study. The samples were taken from the vagina. Vaginal sampling was performed on pregnant women in their last month of pregnancy and in the delivery ward using a sterile swab. Patients were positioned in the supine position with bent knees, and after hand disinfection, a sterile speculum was used to open the vaginal walls. Then, using a sterile swab, samples were gently collected from the mid-vagina and the cervical opening with a rotating motion. The swabs were placed in Amies transport medium and transferred to the laboratory within two hours. In the laboratory, the samples were processed for culture and identification of the target bacteria. The ethical code IR.IAU.SDJ.REC.1403.061 was adopted for this investigation and all the ethical principles of the research were regarded.

2.1 Culture of samples

The culture was done on 3 culture media: EMB, blood agar, and MacConkey agar to gram-negative, gram-positive, and Escherichia coli. Escherichia coli forms purple colonies on MacConkey medium. On EMB medium, it forms dark purple colonies with a metallic green sheen.

2.2 Bacterial identification using biochemical tests

The tests conducted for bacterial identification included the catalase test, citrate utilization test, motility and indole production test, methyl red (MR) test, Voges-Proskauer (VP) test, Triple Sugar Iron (TSI) Agar test, and the urease test.

2.3 Disk diffusion test

In accordance with the established operational protocols, antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Oxoid, Hampshire, England) utilizing the Kirby-Bauer disk diffusion method (6). In this experiment, different antibiotic discs selected based on the CLSI standard were used to check the antimicrobial sensitivity of enterococcus strains. The selection of antibiotics was based on the study of various articles and the frequency of their use in hospitals, as well as the CLSI standard table. These antibiotics included: Ceftazidime, Cefotaxime, Polymyxin B, Gentamicin, Amikacin, Ciprofloxacin, Trimethoprim, Penicillin, Ampicillin, and Imipenem. The plates were incubated for 16-18 hrs in a 37 ˚C incubator. Then, the diameter of the non-growth zone around the antibiotic discs was measured with a ruler, and the sensitivity and resistance of the strains was determined using the 2017 standard Table (7).

2.4 Isolation of ESBL isolates by phenotypic method

All isolates underwent screening for the production of ESBLs. In this procedure, resistant isolates were cultivated on Mueller Hinton agar medium derived from a 0.5 McFarland equivalent bacterial suspension. Subsequently, two discs each of ceftazidime and cefotaxime, along with two discs of their respective combinations with clavulanic acid (ceftazidime/clavulanic acid and cefotaxime/clavulanic acid), were positioned on the culture medium at a separation of 25 mm. Following a 24-hour incubation period at 37°C, the diameter of the growth inhibition zone surrounding the discs was recorded. An isolate was classified as an ESBL-positive phenotype if the difference in the diameter of the zone surrounding the combination discs (which contained clavulanic acid) compared to that of the single disc (which lacked clavulanic acid) was equal to or exceeded 5 mm.

2.5 Determination of MIC by E-test method

The E-test method was conducted for ceftazidime and cefotaxime on all samples identified as ESBL positive. In this procedure, a bacterial suspension was prepared using the McFarland method, which was subsequently transferred to a Mueller Hinton Agar plate. E-Test strips, each corresponding to a specific antibiotic, were then positioned on the Mueller Hinton Agar, followed by a 24-hour incubation period at 37 ˚C, a triangular growth inhibition zone was formed and then the sensitivity of Escherichia coli bacteria to the said antibiotic was determined by referring to the table provided by the manufacturer of E-Test strips.

2.6 PCR test

To isolate genomic DNA from cultured bacterial strains, bacteria from 100 ml of liquid media were utilized following the manufacturer's guidelines (DNeasy Tissue Kit, Qiagen, Hilden, Germany). The optical density at 260

nm and 280 nm was measured using the GeneQuant RNA/DNA calculator (Pharmacia Biotech, Freiburg, Germany), and the quality of the DNA was assessed through agarose gel electrophoresis. The sequences of the relevant primers were obtained from the literature, subjected to BLAST analysis, and verified against the sequences available in GenBank. The extracted DNA was utilized for PCR. The GeneAmp® PCR System 9700 (Applied Biosystems, Weiterstadt, Germany) was employed for the amplification of specific DNA sequences. In all PCR procedures, HotStarTaq DNA polymerase (Qiagen) was utilized, which was activated by a 15-minute incubation at 95°C. Various temperature profiles were implemented with the specific primers (Table 1) to identify the antibiotic-resistance genes. The PCR conditions for the target genes are detailed in Tables 2, 3, and 4).

Table 1. Primers used in PCR.

Primer	Sequences
TEM-F	CATTTCCGTGTCGCCCTTATTC
TEM-R	CGTTCATCCATAGTTGCCTGAC
CTX-M-F	TTAGGAAGTGTGCCGCTGTA
CTX-M-R	CGGTTTTATCCCCCACAAC
SHV-F	AGCCGCTTGAGCAAATTAAAC
SHV-R	ATCCCGCAGATAAATCACCAC

Table 2. PCR Conditions for TEM Gene.

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	10 minutes	1
Denaturation	95	1 minute	30
Annealing	57	1 minute	
Extension	72	1 minute	
Final Extension	72	10 minutes	1

Table 3. PCR Conditions for CTX-M Gene.

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	10 minutes	1
Denaturation	95	1 minute	30
Annealing	57	1 minute	
Extension	72	1 minute	
Final Extension	72	10 minutes	1

Table 4. PCR Conditions for SHV Gene.

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	95	10 minutes	1
Denaturation	95	1 minute	30
Annealing	57	1 minute	
Extension	72	1 minute	
Final Extension	72	10 minutes	1

3. Result

3.1 Demographic Information of Patients

Demographic information of patients is listed in Table 5.

3.2 Sampling, isolation and identification of strains

Of the 100 vaginal samples collected, 45 (45%) were identified as gram-negative. Of these 45 samples, 27 (27%) were identified as Escherichia coli by biochemical tests.

3.3 Determination of antibiotic sensitivity

The results of the antibiotic sensitivity assay are shown in Figure 1 and as follows: Highest resistance: Penicillin (63%) and cefotaxime (59%) show that these antibiotics are the most resistant to Escherichia coli. Least resistance: Gentamicin (4%) and trimethoprim-sulfamethoxazole (30%) show the least resistance. Highest sensitivity: Gentamicin and trimethoprim-sulfamethoxazole have the highest sensitivity among antibiotics at 67%. Highest partial sensitivity: Amikacin (37%) and ceftazidime (37%) show the highest percentage of partial sensitivity.

3.4 Phenotypic identification of ESBL producers

Of the 27 Escherichia coli samples collected, 9 (33.33%) were identified as ESBL producers by phenotypic method. Table 6 shows the results of the combined diskette for samples suspected of being ESBL based on resistance results to the antibiotics cefotaxime and ceftazidime.

3.5 E-test results

The results of this test for ESBL-producing samples are given in Table 7. The results indicate that the range of 1 to 2 mg/mL for cefotaxime and 0.25 to 4 mg/mL for ceftazidime was obtained (one complete sample was resistant to band concentrations of up to 256 mg/mL).

3.6 Prevalence of CTX-M, SHV and TEM genes

The prevalence of CTX-M, SHV and TEM genes was investigated by PCR. The prevalence of CTX-M and TEM genes in ESBL strains was 22.22 and 88.88%, respectively. The SHV gene was not found in any strain. Two strains simultaneously had CTX-M and TEM genes. Also, the prevalence of CTX-M and TEM genes in all isolated Escherichia coli strains was 7.4% and 29.62%, respectively (Figure 2).

3.7 Descriptive statistics of patient characteristics

The highest age was between 30 and 40 years (mostly close to 40 years), which could be because most sexual intercourse and pregnancy occur at this age. The mean age of people infected with Escherichia coli was 34.78 ± 7.516 years. The average age of people carrying ESBL strains was 3 years older than people with ESBL-negative Escherichia coli. Individuals with a diploma or less education were 59.26%, 41.7% had a post-diploma education, and 33.33% had a bachelor's degree or higher. In general, ESBL strains are more common in people with bachelor's degrees and higher. People who had a history of hospitalization during pregnancy had a frequency of 11.11%.

Table 5. Demographic information of patients.

Characteristic	Category	Count (n)	Percentage (%)
Age (Years)	Mean ± Standard Deviation	34.78±7.52	-
	Range (Min-Max)	(19−48)	-
Age Group	19-29 years	6	22.22%
	30-39 years	14	51.85%
	40-49 years	7	25.93%
Education	Diploma or less	16	59.26%
	Post-diploma	2	7.41%
	Bachelor's or higher	9	33.33%
History of previous infection	No	21	77.78%
	Yes	6	22.22%

Table 6. Combination disk results for ESBL suspected samples.

Sample number	Cefotaxime halo diameter	Ceftazidime halo diameter	Cefotaxime + clavulanate halo diameter	Ceftazidime + clavulanate halo diameter	ESBL
81	18	26	20	26	Negative
25	6	9	24	16	Positive
3	40	34	43	38	Positive
14	5/15	11	17	13	Negative
45	8	20	28	26	Positive
82	21	18	22	19	Negative
38	25	24	26	24	Positive
93	31	16	30	22	Negative
18	30	24	28	22	Positive
37	30	20	30	18	Negative
100	25	20	26	24	Positive
28	32	22	31	20	Negative
94	24	22	30	24	Positive
23	22	17	26	20	Negative
37	30	21	30	21	Positive
15	30	24	32	28	Negative
32	33	24	34	28	Positive
28	20	20	31	26	Negative

Table 7. MIC values obtained for cefotaxime and ceftazidime by E-test for ESBL samples.

Sample number	Cefotaxime halo diameter (mg/ml)	Ceftazidime halo diameter (mg/ml)
25	1.5	1.5
3	1	1
45	1.5	4
93	---	0.5
100	---	0.25
94	3	1
23	2	Resistant to concentrations of 0.016-256
15	---	1
32	---	1

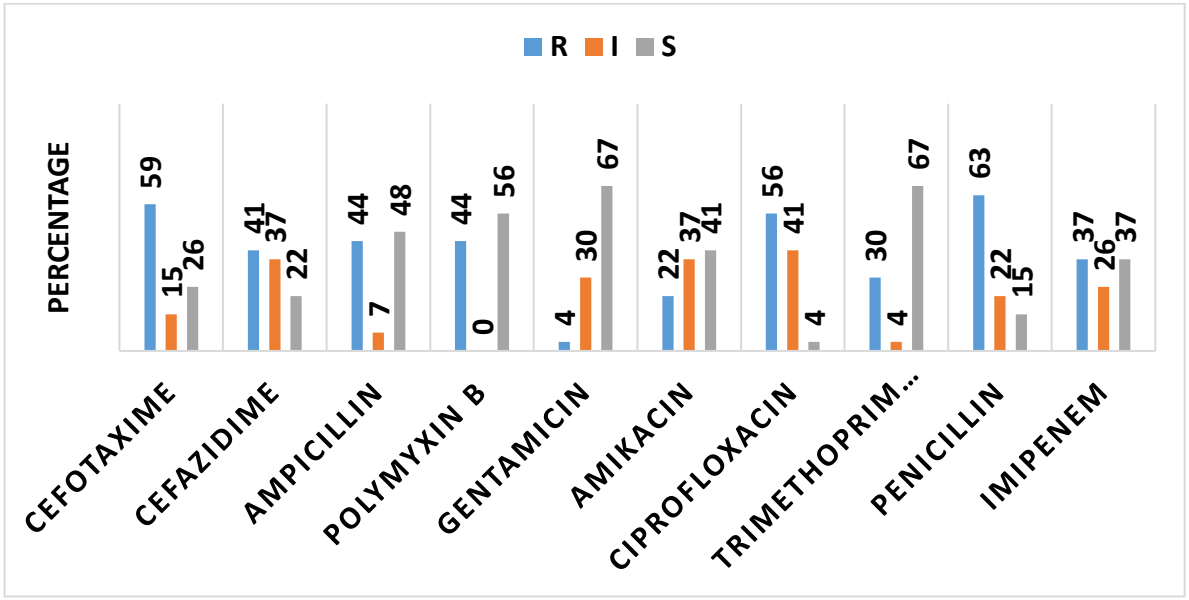
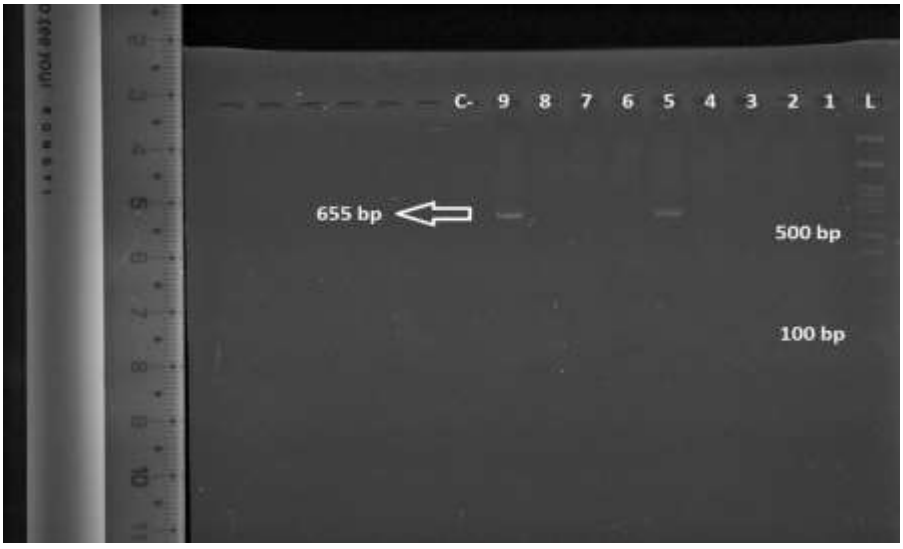
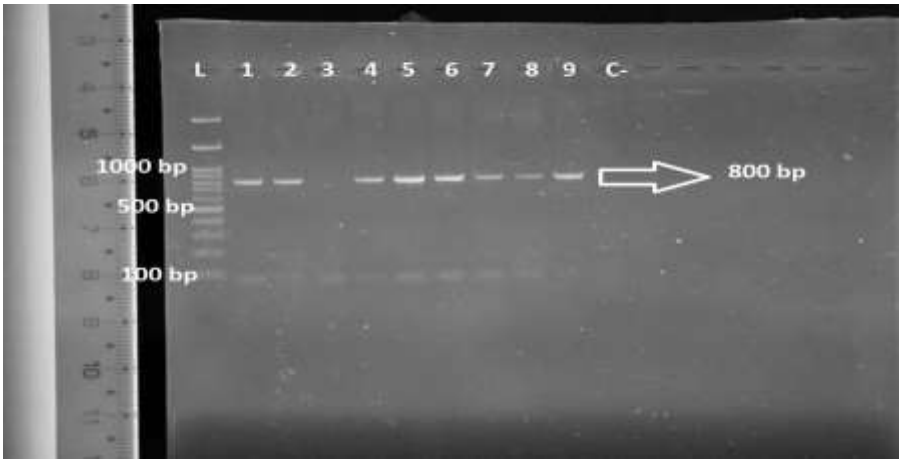


Figure 1. Results of determining antibiotic susceptibility by disc diffusion method (**Prepared by Authors, 2026**).

A



B



C

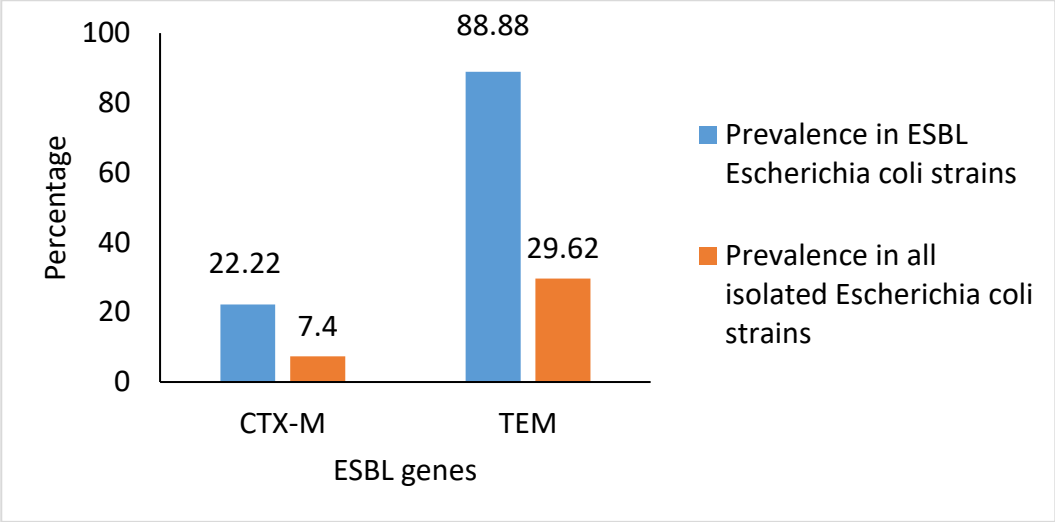


Figure 2. A) Image of the PCR product gel for the detection of the CTX-M gene. The marker used was 100 bp, which detects up to 3000 bp in size, C-: negative control, wells 5 and 9: samples with CTX-M, wells 4-1, 8-6: samples without CTX-M. B) Image of the PCR product gel for the detection of the TEM gene. L: 100 bp, which detects up to 3000 bp, C-: negative control, wells 2-1 and 9-4: samples with TEM, well 3: samples without TEM. C) Prevalence of ESBL genes in Escherichia coli strains (**Prepared by Authors, 2026**).

4. Discussion

The present study reported the highest prevalence of infection among women aged 30–40, potentially due to increased sexual activity and pregnancy in this age group. The mean age of E. coli-infected individuals was 34.78 years, indicating middle-aged individuals are more at risk. High resistance rates to penicillin (63%) and cefotaxime (59%) may result from widespread and prolonged antibiotic use. Conversely, low resistance to gentamicin (4%) could be due to lower usage or higher efficacy.

Peirano et al **(8)** found increasing ESBL production over time, with isolates carrying both CTX-M and TEM genes showing higher resistance **(8)**. This aligns with our findings and suggests a shared resistance mechanism across species. While Peirano's study examined long-term trends, our cross-sectional design highlights the importance of continuous surveillance.

Multiple studies confirm that ESBL-producing bacteria remain significantly susceptible only to carbapenems **(9-13)**. In this study, 33.33% of E. coli isolates were ESBL-positive, and most showed susceptibility to imipenem, consistent with national and international data. Carbapenems remain effective against multidrug-resistant Gram-negative organisms.

The presence of TEM, SHV, and CTX-M genes, combined with membrane permeability changes, may cause carbapenem resistance **(14)**. ESBL-positive strains showed higher resistance (71.4%) than ESBL-negative ones (50%). The TEM gene appears especially impactful in promoting resistance. Co-transfer of resistance genes (e.g., aminoglycoside and TEM) via plasmids is likely, as is chromosomal resistance to fluoroquinolones in ESBL-producing Enterobacteriaceae **(15)**.

Jena et al **(16)** found that 93.47% of ESBL-positive isolates carried TEM. Similarly, our study found TEM in 88.88% of ESBL-positive isolates, confirming its prevalence **(16)**. Farzi et al **(17)** reported 21% ESBL positivity and lower resistance to cefotaxime and ceftazidime compared to our study (59% and 41%, respectively). Our results showed a higher TEM gene prevalence (88.88%) than theirs (20%) **(17)**.

In Sah et al **(18)** study, 27.3% of E. coli isolates were ESBL producers, with higher CTX-M (58.4%) and lower TEM (41.6%) gene prevalence than our study, which showed the reverse (22.22% CTX-M, 88.88% TEM) **(18)**.

Ghenea et al (19) found blaCTX-M-15 in 92.85% and blaTEM-1 in 78.57% of E. coli strains, while our findings showed a lower CTX-M (22.22%) but higher TEM (88.88%) prevalence. Resistance to quinolones was moderate in their study but higher in ours (56%) (19). Afsharikhah et al **(20)** reported similar TEM prevalence (89.6%) and higher CTX-M prevalence (44.3%) compared to our study (22.22%). Both studies showed comparable ciprofloxacin resistance (~56%) **(20)**. Lesani et al **(21)** in Qom found higher resistance to ampicillin (96%) and higher SHV prevalence (45%) compared to our findings (44% resistance, 0% SHV). Differences likely reflect regional antibiotic usage and infection control.

Moradi et al **(22)** observed a 38% ESBL prevalence and higher SHV rates (12.1%). Our study, which focused on TEM and CTX-M, found no SHV-positive isolates. Despite some differences, both studies underline the diversity and persistence of ESBL-related genes.

Differences across studies may stem from regional antibiotic usage, sample type, patient populations, and laboratory methods. Geography, for instance, can influence bacterial resistance patterns due to different prescribing habits and environmental pressures. Additionally, patient demographics (e.g., heart patients vs. pregnant women) and diagnostic methods (e.g., PCR sensitivity) affect results.

5. Conclusion

Overall, the results of this study highlight the need to develop and implement effective strategies to reduce the indiscriminate use of antibiotics and improve resistance surveillance systems. In addition, improving awareness about the management and treatment of bacterial infections, especially in vulnerable groups such as pregnant women, is essential to reduce the prevalence and impact of antibiotic resistance. The study also recommends that further studies on the diversity of resistance genes and their mechanisms be conducted at national and local levels to help improve treatment and prevention protocols.

6. Declarations

6.1 Acknowledgments

This research project was approved by Islamic azad university Sanandaj branch. The authors of this article express their utmost gratitude to the University Research and Technology Vice-Chancellor.

6.2 Ethical Considerations

This research project was approved by Sanandaj branch, Islamic Azad University, Sanandaj, Iran (IR.IAU.SDJ.REC.1403.061).

6.3 Authors' Contributions

Conceptualization, K.D. and S.B.M.; methodology, S.B.M.; software, S.M.; validation, K.D.; writing—original draft preparation, S.M.; editing, S.B.M.; supervision, K.D. All authors reviewed, edited, and approved the final version of the manuscript.

6.4 Conflict of Interest

The authors declare that they have no conflict of interest.

6.5 Fund or Financial Support

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6.6 Using Artificial Intelligence Tools (AI Tools)

The authors confirm that they were not utilized AI Tools.

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