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Examination of broad-spectrum beta-lactamase-producing E. coli vaginal colonization and antibiotic resistance in pregnant women: A pilot study in Iran

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Abstract

Objectives: Urinary tract infection (UTI) is a common complication during pregnancy that can have severe consequences for both the mother and the fetus. ESBL-producing strains of E. coli present a significant challenge in terms of treatment during pregnancy. This study aims to identify pregnant women carrying beta-lactamase-producing E. coli and assess the antibiotic resistance patterns of these isolates.
Methods: This cross-sectional study investigated the presence of broad-spectrum beta-lactamase-producing E. coli isolates in pregnant women attending medical centers affiliated with Kashan University of Medical Sciences. Vaginal colonization, associated complications, and antibiotic resistance profiles were assessed. The antibiogram test using the disc diffusion method was employed to determine colonization and antibiotic susceptibility, while PCR testing was used to detect genes associated with beta-lactamase production.
Results: Among the 175 samples collected, 51 samples tested positive for E. coli, with 15 of these samples containing ESBL-producing isolates. A significant correlation was observed between the frequency distribution of E. coli isolates and factors such as age, history of urinary tract infections, body mass index, and previous abortions in pregnant women. Among the 15 ESBL-producing E. coli isolates, the blaSHV, blaTEM, and blaCTX-M resistance genes were detected in 12 (80%), 9 (60%), and 1 (6.66%) isolates, respectively.
Conclusion: The prevalence of ESBL-producing E. coli in pregnant women underscores the urgent need for public health interventions to combat antibiotic resistance.

Keywords: ESBL-E.coli, Pregnancy, Antibiotic resistance.

Introduction

Premature birth, defined as a pregnancy lasting fewer than 37 weeks, is a leading cause of infant mortality worldwide. Evaluating preterm birth rates is crucial for comparing healthcare systems across different cultures.[1] The prevalence of premature births varies globally, with rates ranging from 9-12% in the United States, 5-7% in Europe, and 5-9% in developing nations.[2] In Iran, research shows varying rates, such as 7.8% in Tehran, 7% in Zanjan, and 4.16% in Mashhad.[3-5]

Pregnancy outcomes are influenced by a complex interplay of maternal, fetal, and placental factors. Risk factors for preterm birth include inadequate prenatal care, multiple births, infertility history, previous premature births, hypertension during pregnancy, smoking, urinary tract infections, anemia, diabetes, and BMI fluctuations. Infections play a significant role in preterm delivery, with over 40% of cases attributed to reproductive system infections.[6,7]

Pregnancy encompasses a range of maternal, fetal, and

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placental factors and influences. Numerous studies have identified risk factors for preeclampsia, including inadequate prenatal care, multiple births, a history of infertility, premature birth history, pregnancy-induced hypertension, male gender, smoking, urinary tract infections, anemia, diabetes, and high or low BMI. Premature birth is a significant concern, with over 40% of preterm deliveries associated with reproductive system infections.[6,7]

Urinary tract infections (UTIs) account for approximately 1-6% of medical visits, affecting the kidneys, bladder, and urinary tract. UTIs occur when the urinary system becomes inflamed in response to bacterial or infectious agents invading the system. E. coli is the most common organism causing UTIs in both outpatients and inpatients. Despite being treatable with various medications, there is a growing concern over rising resistance cases.[8-10]

The risk of UTIs in pregnant women can increase due to factors such as age, delivery, diabetes, sickle cell anemia, previous UTIs, urinary tract disorders, and immunodeficiency. Untreated UTIs during pregnancy can lead to complications like preterm labor, preeclampsia, hypertension, pyelonephritis, anemia, amnionitis, low birth weight, neonatal mortality, bacteremia, and septicemia. However, the risk of complications decreases significantly when UTIs are promptly treated during pregnancy.[11-14]

Neonatal sepsis is a blood infection that occurs within the first month of life after birth and is a major cause of infant mortality and morbidity.[12] Early neonatal sepsis often results from maternal-to-infant transmission during birth and typically presents within the first three days post-delivery. Infections originating from Streptococcus agalactiae and E. coli are common causes of early neonatal sepsis and can lead to severe issues in newborns.[15-17]

To mitigate these risks and complications, it is essential to screen for prompt diagnosis and treat UTIs in pregnant women effectively. Early detection and management of UTIs are crucial in preventing adverse outcomes for both mothers and newborns.

Appropriate measures have been developed to prevent the transmission of Streptococcus agalactiae from infected mothers to newborns, thereby reducing subsequent infections. However, E. coli remains a significant bacterium associated with neonatal sepsis in preterm infants, posing a high risk of sudden and severe neonatal infections. In 24% of reported cases involving premature newborns, E. coli infection was identified as the primary cause. This bacterium is commonly found in the digestive systems of both humans and animals. While most strains are harmless, some carry unique pathogenic components that can impact individuals.[17,18]

Gram-negative bacteria, including ESBL-resistant strains of E. coli, play a key role in the global spread of drug-resistant diseases. ESBL strains render all beta-lactam antibiotics ineffective, except for cephamycins and carbapenems.[19,20] The potential for resistance to multiple antibiotic classes presents a significant challenge in treating infections caused by ESBL strains.[21,22] Research has shown that colonization with ESBL-producing E. coli can lead to the transmission of these strains to sexual partners, contributing to the spread of associated illnesses. Moreover, it increases the risk of bacterial transmission in healthcare settings, including during childbirth, and raises the likelihood of urinary infections and neonatal infections in places like the NICU. Women are more prone to recurrent infections compared to men.[23]

Objectives

To effectively allocate resources and implement targeted screening programs, it is crucial to determine the prevalence of these organisms in specific geographic regions. This study aims to identify pregnant women carrying beta-lactamase-producing E. coli and investigate the antibiotic resistance patterns of the isolates.

Methods

This experimental research aimed to investigate the presence of vaginal colonization, complications, and antibiotic resistance in pregnant women carrying broad-spectrum beta-lactamase-producing E. coli isolates who were referred to medical centers affiliated with Kashan University of Medical Sciences between 2022 and 2023.

A total of 175 vaginal swab samples were collected from pregnant women in their 34th to 37th week of pregnancy during routine check-ups by a specialist doctor. The swabs were transported to the laboratory and stored in Stuart's transport medium at room temperature. A gynecologist administered a questionnaire to gather demographic information, clinical history, and disease background from the participants. The questionnaire included inquiries about the participants' age, education level, general health status, pregnancy details such as delivery method, gestational diabetes, anemia, history of previous abortions, urinary infections, and exposure to animals.

To ensure the purification and identification of the samples, each swab was cultured separately on EMB agar and nutrient agar media. Common biochemical tests, including citrate utilization using Simon Citrate Agar

(SCA), CO_2 production in triple sugar iron (TSI) medium, motility, indole production, and sulfur reduction in SIM medium were conducted. Confirmed E. coli cultures were preserved for further analysis using the glycerol stock technique at -20^{oC}.

Antibiogram tests were performed using the disc diffusion method to assess colonization and antibiotic resistance. Additionally, polymerase chain reaction (PCR) was utilized to determine the presence of beta-lactamase-producing genes.

Antibiotic sensitivity testing was conducted following Clinical and Laboratory Standards Institute (CLSI) guidelines using Kirby-Bauer disk diffusion method on Muller-Hinton agar medium.[24] Following CLSI criteria, antibiotic disks for sensitivity testing included ampicillin (10 micrograms), amoxicillin-clavulanate (20.10 micrograms), cefazolin (30 micrograms), cefepime (30 micrograms), cefoxitin (30 micrograms), ceftriaxone (30 micrograms), Aztreonam (30 µg), meropenem (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg).

The CLSI recommendations were adhered to for the phenotypic identification of ESBL isolates. This involved the use of cefotaxime and cefotaxime-clavulanate (30.10 micrograms) discs, as well as ceftazidime (30 micrograms) and ceftazidime-clavulanate (30.10 micrograms). The isolates were cultured on Mueller-Hinton agar, followed by the placement of ceftazidime discs alone and in combination with ceftazidime-clavulanate, as well as cefotaxime discs alone and in combination with cefotaxime-clavulanate on agar plates. Subsequently, the plates were incubated at 35 to 37 degrees Celsius. After 16 to 18 hours of incubation, the results were interpreted. ESBL isolates were identified if the zone of inhibition around the combined disks was at least 5 mm larger than that around the individual antibiotic disk.

For quality control, ATCC® 35218, an Escherichia coli strain known to produce ESBLs, was used as a positive control, while Escherichia coli strain ATCC® 25922 served as a negative control.

Following the manufacturer's instructions, a DNA extraction kit from Qiagen was used to extract the DNA of ESBL-producing E. coli isolates. All isolates underwent PCR testing for the blaSHV, blaTEM, and blaCTX-M genes. The order of the primers is displayed in Table 1.

The reaction mixture for each PCR amplification included deoxynucleoside triphosphates (dNTPs), extracted DNA samples, primers, and Taq DNA polymerase (Pishgam). The amplification process was carried out using a PCR Eppendorf Mastercycler (Eppendorf, Germany) machine. The cycling settings were as follows: a 4-minute initial denaturation period at 94°C, followed by 30 cycles of 30-second denaturation at 95°C, 30 cycles of 30-second annealing at the appropriate temperature (56°C), 1 minute of extension at 72°C, and a final 10-minute extension time. Amplification products were examined and photographed using a UVP BioDoc System after electrophoresis on a 1% agarose gel.

Data analysis was conducted using SPSS (version 16.0, SPSS Inc, Chicago, IL, USA), employing tests such as the Kolmogorov-Smirnov test, ANOVA, LSD post hoc test, Kruskal-Wallis test, and Mann-Whitney test. The continuous variables were expressed as the mean ± SD, and the categorical variables were presented as a percentage and frequency. A "P-value" less than 0.05 was considered significant.

All participants provided signed written informed consent letters. The study protocol was approved by the Ethics Committee of Kashan University of Medical Sciences with approval number IR.KAUMS.MEDNT.REC.1401.194. All procedures involving human participants adhered to ethical standards set by the institutional and national research committee, following the principles of the 1964 Helsinki Declaration and its subsequent amendments.

Results

During the 34th to the 37th week of pregnancy, a total of 175 vaginal swab samples were collected from pregnant women [Table 2]. These samples were transported to the microbiology lab at Kashan University of Medical Sciences for re-identification using biochemical and microbiological tests. Among the 175 clinical isolates analyzed for this study, E. coli ESBL was isolated from each sample. Out of the 175 samples, 51 E. coli isolates were identified, comprising 15 ESBL and 36 non-ESBL strains, accounting for 29.14% of the total samples.

The frequency distribution of E. coli isolates across different categories revealed a significant association ($p<0.005$) between E. coli isolates and age, particularly with a prevalence rate of 66.66% among pregnant women aged 30 to 45 years. Additionally, a significant relationship ($p=0.001$) was observed between E. coli isolates and urinary tract infections, indicating that 35.29% of pregnant women with E. coli in their vaginal swab samples had urinary tract infections. The correlation between body mass index (BMI) and the occurrence of E. coli isolates showed higher levels in individuals with a BMI greater than 25. Notably, a significant correlation ($p<0.001$) was found between the frequency distribution of E. coli isolates

and a history of abortion.

Factors such as education level, financial status, diabetes, congenital kidney disease, and urinary system surgery did not show any significant associations with E. coli isolates in this study.

According to CLSI criteria, the following antibiotic disks were utilized: ampicillin (10 micrograms), amoxicillin-clavulanate (20.10 micrograms), cefazolin (30 micrograms), cefepime (30 micrograms), cefoxitin (30 micrograms), and ceftriaxone (30 micrograms). Ceftazidime (30 µg), ceftazidime-clavulanate (30.10 µg), cefotaxime, and cefotaxime-clavulanate (30.10 µg) discs were employed to phenotypically identify ESBL isolates in accordance with CLSI standards. The microbial isolates were cultured for 18-24 hours, and single colonies were transferred to physiological serum using a sterile swab. After vortexing to homogenize the solution, the turbidity was compared against the turbidity of a McFarland half solution. Once the sample reached half the turbidity of McFarland, it was spread on Mueller-Hinton agar medium using a sterile swab, and antibiotic disks were placed at appropriate distances. The plates were then incubated at 37°C. The diameter of the zones of inhibition was measured with a ruler, and the resistance or sensitivity of each isolate to the antibiotics was determined using a standard table [Figure 1].

Significant differences in antibiotic resistance patterns were observed between ESBL and non-ESBL E. coli strains in pregnant women (p-value 0.001) for all antibiotics. In this study, the highest rate of antibiotic resistance in ESBL E. coli was associated with cefotaxime, with 14 isolates (93.33%), while the highest rate of antibiotic sensitivity was related to cefepime, with 12 cases (80%), and gentamicin, with 11 cases (73.33%) [Figure 2].

Table 1. Primer sequence of *blaSHV, blaTEM, blaCTX-M* genes

Gene of resistance	Primer sequence (5′ to 3′)	Fragment size (bp)
***bla*$_{CTX-M}$**	F: AT GTG CAG YAC CAG TAA RGT KAT GGC R: TG GGT RAA RTA RGT SAC CAG AAY CAG CGG	**593**
***bla*$_{SHV}$**	F: AGC CGC TTG AGC AAA TTA AAC R: ATC CCG CAG ATA AAT CAC CAC	**713**
***bla*$_{TEM}$**	F: C ATT TTC GTG TCG CCC TTA R: C GTT CAT CCA TAG TTG CCT GACTTC	**800**

Table 2. Distribution of E. coli isolates in terms of age, UTI, BMI, education level, history of abortion, congenital kidney disease, surgery of the urinary system, and antibiotic resistance

Criteria	Characteristic	Prevalence
Age	18-30	33.3 %
	30-45	66.6 %
Education	Diploma and college education	37.2 %
	Under high school diploma	62.7 %
Urinary Tract Infection	Yes	35.2 %
	No	64.7 %
Body mass index	18-25	5.8 %
	25 > x	94.1 %
History of abortion	Yes	27.4 %
	No	72.5 %
Diabetes, congenital kidney problem and urinary system surgery	Yes	0 %
	No	100 %
Antibiotic Resistance	ESBL	29.4 %
	Non-ESBL	70.5 %

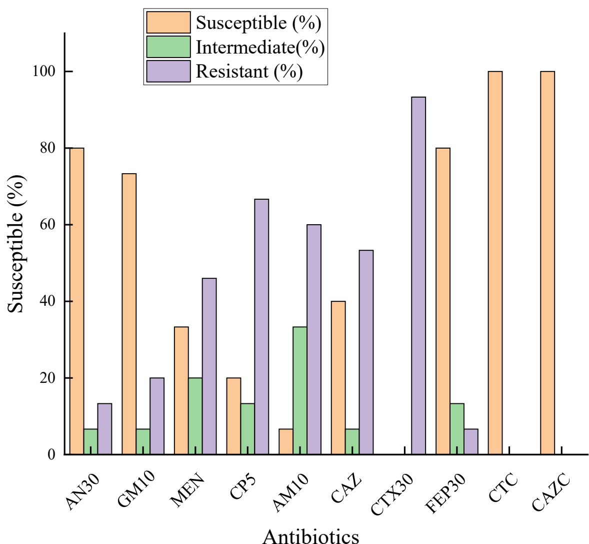

Figure 1. Antibiotic resistance pattern in *E.coli* ESBL

The presence of resistance genes encoding blaSHV, blaTEM, and blaCTX-M in clinical isolates was investigated using the PCR method. Out of 51 E. coli cases, 15 were identified as ESBL clinical isolates. Among these, the prevalence of the studied genes was as follows: blaSHV

in 12 isolates (80%), blaTEM in 9 isolates (60%), and blaCTX-M in 1 isolate (6.66%). The presence of these genes is illustrated in Figure 3.

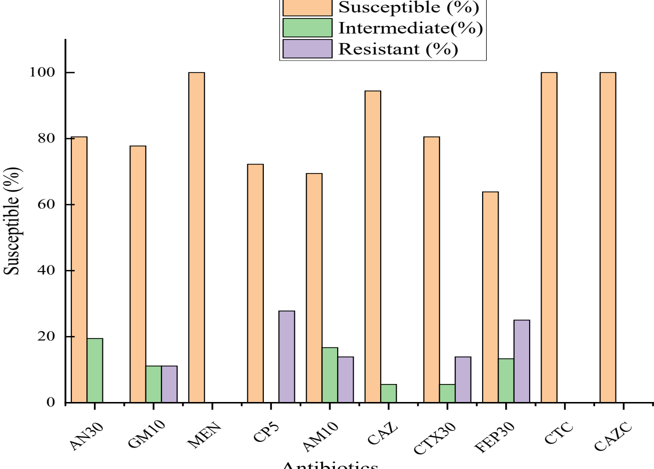

Figure 2. Antibiotic resistance pattern in *E. coli* Non- ESBL

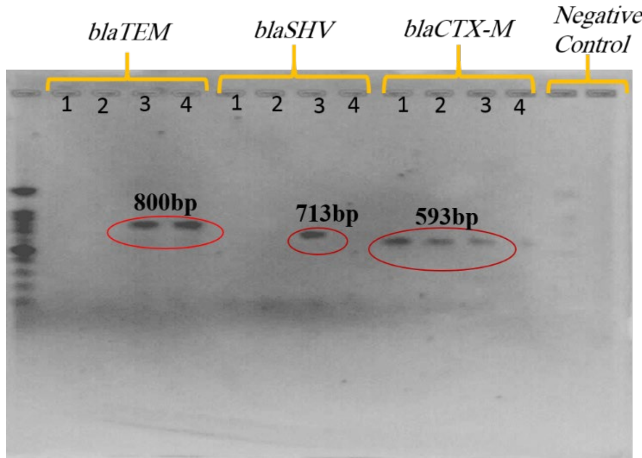

Figure 3. PCR products on agarose gel electrophoresis

Discussion

In recent times, gram-negative bacteria have been increasingly exhibiting resistance to cephalosporins and carbapenems.[13] Broad-spectrum beta-lactamase (ESBL)-producing E. coli strains are now recognized as significant contributors to hospital-acquired infections. It is crucial to promptly identify metallo-beta-lactamases and beta-lactamase-producing bacteria resistant to carbapenems and cephalosporins for both epidemiological purposes and to aid physicians in selecting the most effective medications for successful patient treatment and management of drug-resistant strains.

In our study, out of 175 samples, 51 E. coli isolates (29.14%) were identified, with 15 (29.41%) being ESBL cases and 36 (70.58%) non-ESBL cases. A previous study in Lebanon reported that 32% of E. coli strains recovered from patients were ESBL producers,[25] while another investigation in 2016 found third-generation cephalosporin resistance in 59% of E. coli strains.[26] In a study from Iraq in 2010 focusing on pregnant women's vaginal samples, it was revealed that 52% of E. coli strains were ESBL producers.[27] The varying prevalence rates of ESBL-producing E. coli across different regions and countries may account for the differences observed.

Contrary to findings in Morocco, Poland, and Spain where no pregnant women were colonized by ESBL isolates,[28,29] African nations have reported ESBL prevalence rates between 8 and 15%.[30] In 2020, Nahed Ghaddar et al.,[31] evaluated the phenotypic and genotypic characteristics of broad-spectrum beta-lactamases produced by E. coli colonized in pregnant women. The results revealed that 59 out of 308 women (19.1%) harbored ESBL-producing gram-negative bacteria. All E. coli isolates underwent PCR analysis to identify the most prevalent ESBL-producing genes. The predominant gene detected was blaCTX-M (90.7%), followed by blaTEM (88.4%), and blaSHV (44.2%). The isolates exhibited sensitivity to gentamicin (91.5%), ciprofloxacin (49.2%), cotrimoxazole (39%), aztreonam (18.6%), and cefepime (35.6%).

Despite the study being conducted during a different time period and in a different geographical location, the findings closely mirrored those of the current study, with similar proportions of sensitivity observed. The prevalence of E.coli ESBL was lower in these investigations compared to our analysis, likely due to variations in the geographic distribution of the affected population.

The analysis of antibiotic resistance patterns between ESBL and non-ESBL E.coli strains in pregnant women showed significant differences ($p \leq 0.001$) for all antibiotics tested. In our study, the highest antibiotic sensitivity among ESBL E. coli isolates was observed with Cefotaxime (93.33%), while the highest resistance rates were seen with Cefepime (80%) and Gentamycin (73.33%). Overall, our findings highlight the importance of monitoring antibiotic resistance patterns in E. coli isolates, particularly ESBL-producing strains, to guide effective treatment strategies and combat the rise of drug-resistant infections in clinical settings.

According to a study conducted by Enaiat et al.,[32] urinary tract infections in pregnant women are linked to high levels of E. coli resistance to nalidixic acid, tetracycline, and cotrimoxazole, along with low susceptibility to ampicillin, gentamicin, and amikacin. These results align with our study's findings, indicating that E. coli has limited sensitivity to ampicillin, gentamicin, and amikacin. Among the 51 identified E. coli cases, 15 were ESBL isolates, showing the presence of

blaSHV, blaTEM, and blaCTX-M genes at frequencies of 80%, 60%, and 6.66%, respectively.

In a study by Shams et al.,[33] focusing on the detection of broad-spectrum beta-lactamase genes (blaTEM, blaSHV, and blaCTX-M) in E. coli strains isolated from clinical samples, it was revealed that out of 161 E. coli isolates, 31 (19%) were resistant to cefotaxime based on disc diffusion and MIC results. Among these resistant isolates, 24 (77%) were identified as ESBL producers. The study found that 90%, 42%, and 3% of the strains carried the blaCTX-M, blaTEM, and blaSHV genes, respectively. These findings are in line with our research, confirming that blaCTX-M was the predominant gene identified.

Overall, the results from both studies support the notion of increasing resistance in E. coli strains, particularly towards cephalosporins, emphasizing the importance of continued surveillance and appropriate antibiotic management strategies in clinical settings.

Conclusions

E. coli, typically a commensal organism, can cause UTIs and may possess antibiotic resistance genes that contribute to treatment failure. Our findings demonstrate a significant correlation between the frequency distribution of E. coli isolates and age, UTIs, body mass index, and history of abortion in pregnant women. Among the 15 E. coli ESBL isolates, the blaSHV, blaTEM, and blaCTX-M resistance genes were present in 80%, 60%, and 6.66% of isolates, respectively.

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

Urinary tract infection: UTI; Simon Citrate Agar: SCA;
Triple sugar iron: TSI; Polymerase chain reaction: PCR;
Clinical and Laboratory Standards Institute: CLSI;
Deoxynucleoside triphosphates: dNTPs;
Body mass index: BMI.

Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

All participants provided signed written informed consent letters. The study protocol was approved by the Ethics Committee of Kashan University of Medical Sciences with approval number IR.KAUMS.MEDNT.REC.1401.194. All procedures involving human participants adhered to ethical standards set by the institutional and national research committee, following the principles of the 1964 Helsinki Declaration and its subsequent amendments.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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