



Antimicrobial resistance and multidrug resistance patterns of uropathogens isolated from pregnant women with asymptomatic bacteriuria

Aseptomatik bakteriüri olan gebelerden izole edilen üropatojenlerin antimikrobiyal direnç ve çoklu ilaç direnç profilleri

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Abstract

Objective: This study aimed to evaluate the prevalence of asymptomatic bacteriuria (ASB) in the second half of pregnancy, identify causative microorganisms, and assess their antimicrobial resistance and multidrug resistance (MDR) patterns in Kırşehir, Türkiye.

Materials and Methods: Between April-December 2024, 182 pregnant women without urinary tract infection symptoms were screened at Kırşehir Training and Research Hospital. Midstream urine samples were cultured, and bacterial isolates were identified and tested for antimicrobial susceptibility using the BD Phoenix™ automated system. Data were interpreted according to EUCAST 2024 criteria.

Results: ASB prevalence was 37.36%. *Escherichia coli* (51.47%) was the most common pathogen, followed by *Candida* spp. (17.65%), *Klebsiella pneumoniae* (8.82%), and *Streptococcus agalactiae* (7.36%). In Gram-negative isolates, the highest resistance was to ampicillin (72.7%), cefazolin (43.2%), and amoxicillin-clavulanate (40.9%), with universal susceptibility to amikacin, carbapenems, and nitrofurantoin. Gram-positive isolates showed the highest resistance to moxifloxacin and tetracycline (41.7% each). MDR was detected in 20% of *Escherichia coli*, 16.7% of *Klebsiella pneumoniae*, 60% of *Streptococcus agalactiae*, and 66.6% of *Staphylococcus epidermidis* isolates.

Conclusion: ASB prevalence during second half of pregnancy was high, and a significant proportion of pathogens demonstrated MDR. The findings highlight the necessity of culture-based diagnosis and region-specific empirical therapy. High resistance to ampicillin and trimethoprim-sulfamethoxazole suggests that empirical protocols should be updated according to local antibiograms. Strengthening antibiotic stewardship and expanding routine ASB screening are critical to reducing maternal-fetal complications.

Keywords: Asymptomatic bacteriuria, pregnancy, antimicrobial resistance, multidrug resistance

Öz

Amaç: Bu çalışma, gebeliğin ikinci yarısında asemptomatik bakteriüri (ASB) prevalansını değerlendirmek, etken mikroorganizmaları belirlemek ve bunların antimikrobiyal direnç ile çoklu ilaç direnci (MDR) paternlerini incelemek amacıyla Kırşehir, Türkiye’de gerçekleştirilmiştir.

PRECIS: Asymptomatic bacteriuria in pregnancy showed significant prevalence with *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus agalactiae* and *Candida* spp. predominance and high multidrug resistance rates, underscoring the need for culture-based diagnosis and region-specific antibiotic strategies.

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Gereç ve Yöntemler: Nisan-Aralık 2024 tarihleri arasında, Kırşehir Eğitim ve Araştırma Hastanesi'nde üriner sistem enfeksiyonu semptomu olmayan 182 gebe kadın taranmıştır. Orta akım idrar örnekleri kültüre alınmış, bakteri izolatları tanımlanmış ve antimikrobiyal duyarlılık testleri BD Phoenix™ otomatize sistemi ile yapılmıştır. Veriler, EUCAST 2024 kriterlerine göre yorumlanmıştır.

Bulgular: ASB prevalansı %37,36 olarak bulunmuştur. En sık patojen *Escherichia coli* (%51,47) olup, bunu *Candida* spp. (%17,65), *Klebsiella pneumoniae* (%8,82) ve *Streptococcus agalactiae* (%7,36) izlemiştir. Gram-negatif izolatlarda en yüksek direnç ampisilin (%72,7), sefazolin (%43,2) ve amoksisilin-klavulanata (%40,9) karşı belirlenmiş; tüm izolatların amikasin, karbapenemler ve nitrofurantoina duyarlı oldukları saptanmıştır. Gram-pozitif izolatlarda en yüksek direnç moksifloksasin ve tetrasikline (%41,7) karşı görülmüştür. MDR oranları *Escherichia coli* izolatlarında %20, *Klebsiella pneumoniae*'de %16,7, *Streptococcus agalactiae*'de %60 ve *Staphylococcus epidermidis*'de %66,6 olarak belirlenmiştir.

Sonuç: Gebeliğin ikinci yarısında ASB prevalansı yüksek olup, patojenlerin önemli bir kısmı MDR göstermiştir. Bulgular, kültür bazlı tanının ve bölgeye özgü ampirik tedavi yaklaşımlarının önemini ortaya koymaktadır. Ampisilin ve trimetoprim-sülfametoksazole karşı yüksek direnç, ampirik tedavi protokollerinin yerel antibiyogramlara göre güncellenmesi gerektiğini göstermektedir. Antibiyotik yönetiminin güçlendirilmesi ve rutin ASB taramalarının yaygınlaştırılması, maternal-fetal komplikasyonların azaltılması açısından kritik öneme sahiptir.

Anahtar Kelimeler: Asemptomatik bakteriüri, gebelik, antimikrobiyal direnç, çoklu ilaç direnci

Introduction

Asymptomatic bacteriuria (ASB) is defined as the growth of a single uropathogen at concentrations typically $\geq 10^5$ colony-forming unit (CFU)/mL in a properly collected midstream urine sample, without the presence of urinary tract infection symptoms⁽¹⁾. During pregnancy, hormonal and anatomical changes, particularly the smooth muscle-relaxing effect of progesterone, cause urinary tract dilatation and decreased bladder tone, thereby increasing urinary stasis and creating a favorable environment for bacterial colonization^(2,3).

In pregnant women, untreated ASB increases the risk of acute pyelonephritis by approximately four to tenfold, potentially leading to serious complications such as premature birth, low birth weight, preeclampsia, and even maternal sepsis⁽⁴⁻⁶⁾. The incidence of pyelonephritis during pregnancy has been observed to rise to as high as 20-40% when ASB is left untreated^(1,7). For this reason, international authorities such as the Infectious Diseases Society of America, the American College of Obstetricians and Gynecologists, the World Health Organization, emphasize the importance of screening with urine culture at least once during early pregnancy and treating positive cases with appropriate antibiotics^(1,8-10).

The prevalence of ASB among pregnant women varies depending on geographic region, socioeconomic status, hygiene practices, access to healthcare services, sampling methods, and diagnostic criteria, with global rates reported between 2% and 15%⁽¹¹⁻¹³⁾. In certain regions such as Africa and South Asia, rates exceeding 20% have been reported⁽¹²⁻¹⁵⁾. Among causative microorganisms, *Escherichia coli* (*E. coli*) is the most isolated and identified, followed by *Klebsiella pneumoniae* (*K. pneumoniae*), *Streptococcus agalactiae* (*S. agalactiae*), *Enterococcus faecalis* and, less commonly, fungal species^(16,17).

Recently, the rise of antimicrobial resistance has emerged as a significant public health concern in the management of ASB during pregnancy. In particular, high resistance rates to ampicillin and amoxicillin suggest that nitrofurantoin, amoxicillin-clavulanate, and cephalosporins may be more reliable empirical treatment options^(1,9,18). However, as resistance patterns exhibit regional variation, regular updates of local antibiogram data are recommended for each healthcare setting^(19,20).

Although several studies on ASB in pregnant women have been conducted in various regions of Türkiye, no published research has specifically addressed the prevalence, causative microorganisms, and antibiotic resistance patterns of ASB in pregnant women in Kırşehir province. This study was therefore designed to determine the prevalence of ASB among pregnant women attending Kırşehir Training and Research Hospital, to identify the causative microorganisms and their associations with maternal age groups, and to evaluate antibiotic resistance rates. The findings are expected to contribute to regional resistance data and provide guidance for clinical practice in managing ASB during pregnancy.

Materials and Methods

Collection and Transportation of Specimens to the Laboratory

Urine samples obtained from pregnant women attending the Obstetrics and Gynecology Outpatient Clinic of Kırşehir Training and Research Hospital for antenatal care were delivered under aseptic conditions to the Medical Microbiology Laboratory. A total of 182 clinical specimens collected between April 2024 and December 2024 were examined. The study was approved by Kırşehir Ahi Evran University Health Sciences Scientific Research Ethics Committee (decision no: 2024-08/55, dated: 02.04.2024), and written informed consent was obtained from all participants.

The inclusion criteria were as follows: pregnant women in the second half of gestation who had no symptoms of urinary infection (such as frequency, dysuria, flank pain, or fever), had not used antibiotics in the previous 14 days, had no history of urological anomalies or chronic kidney disease, and were willing to participate in the study.

All specimens were collected using the midstream clean-catch technique under aseptic conditions⁽²¹⁾. Prior to sampling, participants were instructed on appropriate genital hygiene procedures. The collected samples were placed in sterile screw-capped containers and quickly transported to the Medical Microbiology Laboratory of Kırşehir Training and Research Hospital. Microbiological analyses were performed

within a maximum of two hours after sample arrival at the laboratory.

Microbiological Examination and Antimicrobial Susceptibility

All specimens delivered to the microbiology laboratory under aseptic conditions were inoculated onto 5% sheep blood agar and eosin-methylene blue agar using standard microbiological techniques. The inoculated plates were incubated aerobically at 35-37 °C for 18-24 hours. Growth of a microorganism at a concentration of $\geq 10^5$ CFU/mL was considered indicative of ASB⁽¹⁾. A pure culture was obtained. Preliminary identification of pure culture isolates was performed using Gram staining, catalase, oxidase, and coagulase tests. Bacterial identification and antimicrobial susceptibility testing were subsequently carried out with the BD Phoenix™ automated system. Identification and antibiotic susceptibility tests were performed according to the manufacturer's instructions.

Antimicrobial susceptibility data were interpreted according to the 2024 criteria of the European Committee on Antimicrobial Susceptibility Testing⁽²²⁾.

Regarding Gram-negative bacteria, susceptibility testing was performed for the following antibiotics: amikacin, ciprofloxacin, gentamicin, levofloxacin, trimethoprim-sulfamethoxazole, ampicillin, tigecycline, ceftazidime, ceftriaxone, cefuroxime, cefazolin, imipenem, meropenem, piperacillin-tazobactam, amoxicillin-clavulanate, ertapenem, cefixime, nitrofurantoin, and tobramycin. With respect to Gram-positive bacteria, the tested antibiotics included amikacin, vancomycin, ciprofloxacin, daptomycin, erythromycin, fusidic acid, gentamicin, levofloxacin, linezolid, moxifloxacin, oxacillin, penicillin G, trimethoprim-sulfamethoxazole, tetracycline, clindamycin, and rifampin.

Statistical Analysis

All data were analyzed using SPSS version 26.0. The chi-square test or Fisher's exact test was applied for categorical variables, and statistical significance was reached when $p < 0.05$.

Results

Prevalence of ASB and Age Groups

A total of 182 pregnant women in the second half of pregnancy were included in this research. The mean age of the participants was 29.7 ± 7.0 (minimum: 18 - maximum: 44) years, and for statistical analysis, the age distribution was divided into three groups (Table 1). Significant bacteriuria was detected in 37.36% of the total samples. In the remaining 62.84%, either no bacterial growth was observed or the growth did not meet the diagnostic criteria. No statistically significant relationship was found between age groups and ASB positivity ($p > 0.05$, Table 1).

In the 68 ASB-positive samples, a total of 10 different bacterial species and *Candida* spp. were isolated. The most frequently identified pathogen was *E. coli*, detected in 51.47% ($n=35$) of all

positive samples. This was followed by *Candida* spp. (17.65%), *K. pneumoniae* (8.82%), and *S. agalactiae* (7.36%) (Table 2, Figure 1).

Antimicrobial Susceptibility

When ASB-positive samples were evaluated by species, *E. coli* ($n=35$) isolates showed the highest resistance rates to ampicillin (71%), followed by trimethoprim-sulfamethoxazole (41.2%), amoxicillin-clavulanate (37.1%), ceftazidime (25.7%), and ceftriaxone (20%). *K. pneumoniae* ($n=6$) isolates exhibited intrinsic resistance to ampicillin and showed resistance rates of 83.3% to piperacillin-tazobactam, 66.7% to amoxicillin-clavulanate, and 66.7% to cefazolin. In *S. agalactiae* ($n=5$) isolates, resistance was detected against erythromycin (40%), levofloxacin (40%), moxifloxacin (60%), tetracycline (60%), gentamicin (40%), and chloramphenicol (40%) (Figure 2).

In Gram-negative isolates, the highest resistance was observed against ampicillin (72.72%), followed by cefazolin (43.18%), amoxicillin-clavulanate (40.9%), and trimethoprim-sulfamethoxazole (34.9%). All isolates were found to be susceptible to amikacin, imipenem, nitrofurantoin, and meropenem (Table 3, Figure 3).

When examining the antibiotic resistance patterns of Gram-positive bacteria, the highest resistance rates were found against moxifloxacin and tetracycline, each at 41.67%. This was followed by levofloxacin at 33.33% and gentamicin at 25%.

Table 1. Distribution of ASB positivity and age groups

Age	Healthy n (%)	ASB n (%)	Test statistic value, p-value
18-25	34 (29.8)	24 (35.3)	$\chi^2=2.023$ p=0.364
26-33	49 (43.0)	22 (32.4)	
34 and above	31 (27.2)	22 (32.4)	
χ^2 : Pearson chi-square, p=0.364, ASB: Asymptomatic bacteriuria			

Table 2. Microorganism distribution at the species level in asymptomatic bacteriuria positive samples

Microorganism	%
<i>Candida</i> spp.	17.65
<i>Enterobacter aerogenes</i>	1.47
<i>Enterococcus faecalis</i>	1.47
<i>Escherichia coli</i>	51.47
<i>Klebsiella pneumoniae</i>	8.82
<i>Lactobacillus</i> spp.	1.47
<i>Proteus mirabilis</i>	2.94
<i>Staphylococcus aureus</i>	1.47
<i>Staphylococcus epidermidis</i>	4.41
<i>Streptococcus agalactiae</i>	7.36
<i>Streptococcus pyogenes</i>	1.47

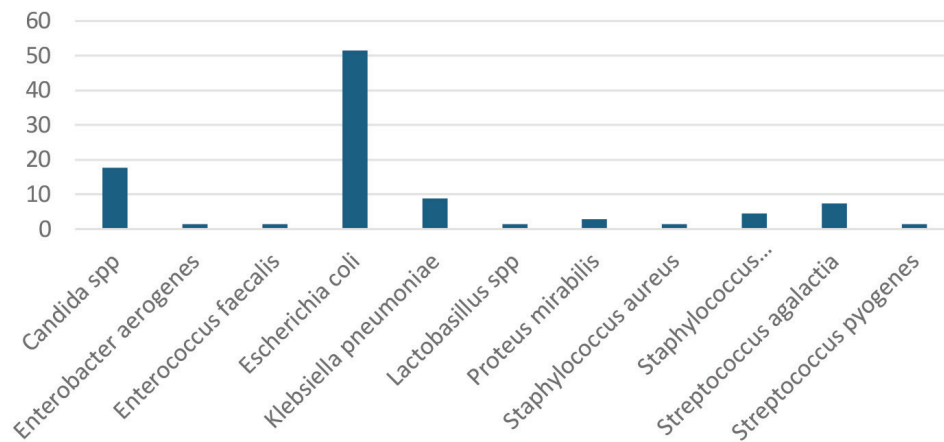


Figure 1. Microorganism distribution at the species level in asymptomatic bacteriuria positive samples

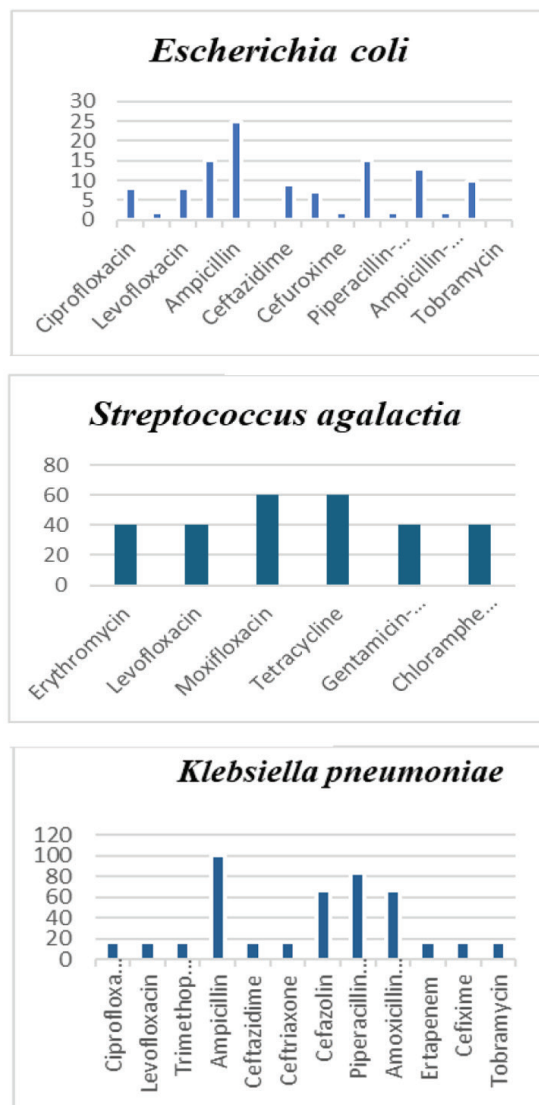


Figure 2. Antimicrobial resistance distribution of pathogens frequently isolated in the diagnosis of asymptomatic bacteriuria during pregnancy

Among Gram-positive bacteria, resistance to ciprofloxacin, fusidic acid, oxacillin, chloramphenicol, and erythromycin was observed at a rate of 16.67%, while rifampin, trimethoprim-sulfamethoxazole, penicillin G, and clindamycin showed lower resistance rates of 8.33%. No resistance was detected to vancomycin, teicoplanin, daptomycin, linezolid, amikacin, or gentamicin (Table 4, Figure 4).

Multidrug Resistance in Bacteria

Examination of the antibiotic classes tested against bacterial isolates from ASB positive samples revealed that some were resistant to three or more classes, qualifying them as multidrug resistance (MDR) organisms. In our study, among Gram-negative bacteria, MDR was detected in 20% of *E. coli* isolates and 16.7% of *K. pneumoniae* isolates. In Gram-positive bacteria, resistance rates were notably higher; 60% of *S. agalactiae* isolates and 66.6% of *S. epidermidis* isolates were resistant to at least three different antibiotic classes (Figure 5).

The chi-square test revealed no statistically significant association between bacterial species and MDR ($\chi^2=5.99$, $p=0.071$), indicating that the variables may be independent. Accordingly, the observed frequencies did not differ significantly from the expected values. The detailed results of the chi-square analysis are presented in Table 5.

Discussion

This study was conducted to reveal the prevalence of ASB in the second half of pregnancy in Kırşehir province, identify the causative microorganisms, and evaluate their antimicrobial resistance patterns. Given the absence of a previous comprehensive investigation at the regional level, our findings aim to contribute both locally and nationally by updating epidemiological data and guiding clinicians toward effective antibiotic therapy.

In our investigation, the prevalence of ASB in the second half of pregnancy was found to be 37.36%. These findings are consistent with certain studies conducted in Türkiye.

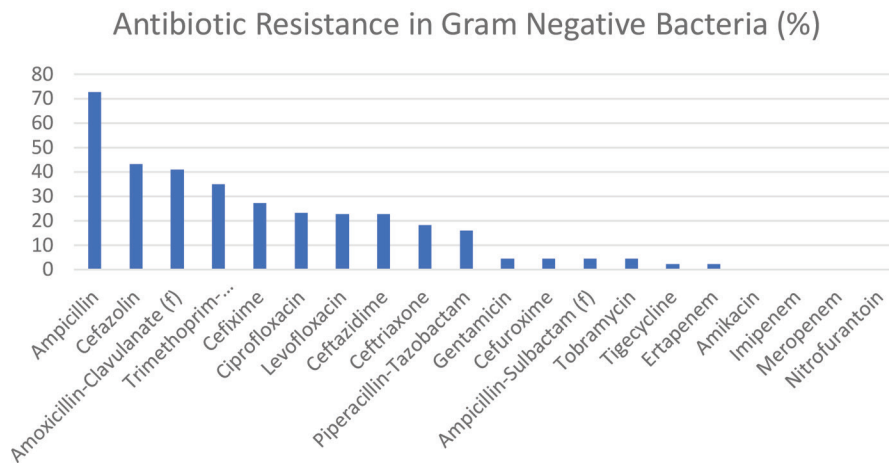


Figure 3. Selected antibiotic susceptibility rates of Gram-negative isolates

Table 3. Antibiotic susceptibility results of Gram-negative isolates

Antibiotics	Resistance	n (%)
Ampicillin	32	72.7
Cefazolin	19	43.2
Amoxicillin-clavulanate	18	40.9
Trimethoprim-sulfamethoxazole	15	34.9
Cefixime	12	27.3
Ciprofloxacin	10	23.3
Levofloxacin	10	22.7
Ceftazidime	10	22.7
Ceftriaxone	8	18.2
Piperacillin-tazobactam	7	15.9
Gentamicin	2	4.5
Cefuroxime	2	4.5
Tobramycin	2	4.5
Tigecycline	1	2.3
Ertapenem	1	2.3
Amikacin	0	0
Imipenem	0	0
Meropenem	0	0
Nitrofurantoin	0	0

Aktün et al.⁽²³⁾ reported a prevalence of 36.5%, while Efe and Kurdoğlu⁽²⁴⁾ reported 35.4%. However, lower prevalence rates (4-20%) have been reported in other regions, a variation likely attributable to geographical differences, socioeconomic conditions, hygiene practices, and discrepancies in laboratory diagnostic methods^(3,25).

In our study, the most frequently isolated pathogen was *E. coli* (51.47%), followed by *Candida* spp. (17.65%), *K. pneumoniae* (8.82%), and *S. agalactiae* (7.36%). This distribution aligns with

previous reports indicating *E. coli* as the predominant etiological agent in ASB during pregnancy^(1,11). The relatively higher proportion of *Candida* spp. in our cohort compared to some earlier studies may reflect local epidemiological characteristics, patient-specific factors such as recent antibiotic exposure or gestational diabetes, or laboratory detection practices.

When analyzed by antimicrobial susceptibility, *E. coli* isolates demonstrated the highest resistance rates to ampicillin (71%), trimethoprim-sulfamethoxazole (41.2%), and amoxicillin-clavulanate (37.1%), with moderate resistance to ceftazidime (25.7%) and ceftriaxone (20%). These results are consistent with national and international data, which report persistently high resistance to ampicillin and increasing resistance to β -lactamase inhibitor combinations^(20,26). Importantly, all *E. coli* isolates in our study retained susceptibility to aminoglycosides (amikacin), carbapenems, and nitrofurantoin, suggesting that these agents remain viable options for empirical therapy in our setting.

The *K. pneumoniae* isolates demonstrated notably high resistance to piperacillin-tazobactam (83.3%) and amoxicillin-clavulanate (66.7%). Among Gram-positive pathogens, *S. agalactiae* exhibited marked resistance to moxifloxacin (60%) and tetracycline (60%), a finding that may have implications for antenatal prophylaxis strategies⁽¹⁰⁾.

When MDR rates were examined, it was determined that 20% of *E. coli* isolates, 16.7% of *K. pneumoniae* isolates, 60% of *S. agalactiae* isolates, and 66.6% of *S. epidermidis* isolates had MDR. The particularly higher MDR rates among Gram-positive bacteria represent a clinically significant finding, as they may limit therapeutic options and increase the risk of maternal-fetal complications^(6,20).

These results underscore the importance of routine ASB screening during pregnancy and the implementation of culture-based antibiotic therapy. The high resistance rates observed for commonly used agents such as ampicillin and trimethoprim-sulfamethoxazole suggest that empirical treatment protocols should be revised in light of local resistance patterns⁽⁵⁾.

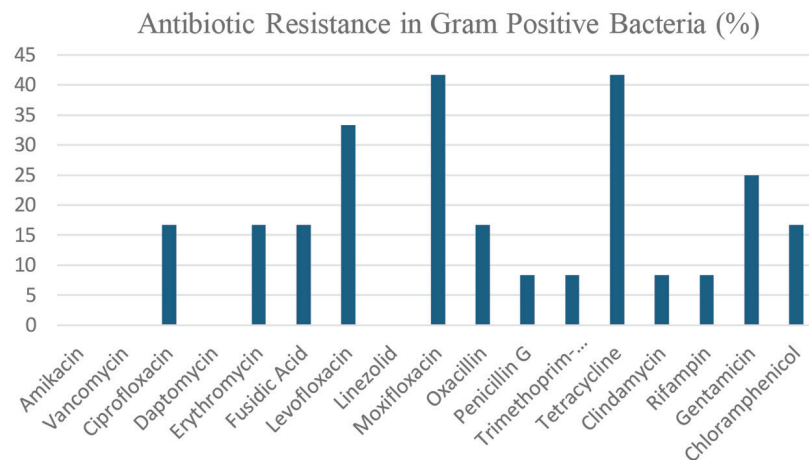


Figure 4. Susceptibility rates of Gram-positive isolates to selected antibiotics

Table 4. Antibiotic susceptibility results of Gram-positive isolates

Antibiotics	Number of isolate (n)	Resistance rate (%)
Rifampin	1	8.3
Moxifloxacin	5	41.7
Tetracycline	5	41.7
Ciprofloxacin	2	16.7
Fusidic acid	2	16.7
Oxacillin	2	16.7
Chloramphenicol	2	16.7
Gentamicin	3	25.0
Levofloxacin	4	33.3
Erythromycin	2	16.7
Trimethoprim-sulfamethoxazole	1	8.3
Penicillin G	1	8.3
Clindamycin	1	8.3
Vancomycin	0	0
Teicoplanin	0	0
Daptomycin	0	0
Linezolid	0	0
Amikacin	0	0

Study Limitations

Expanding the sample size, extending the study period, and performing multicenter comparative investigations would provide more comprehensive insights into the prevalence and antimicrobial resistance patterns of ASB in second half of pregnancy.

Table 5. The relationship between bacterial species and multidrug resistance

	MDR + n (%)	MDR – n (%)	Test statistic value, p-value
<i>E. coli</i>	7 (20.0%)	28 (80.0%)	$\chi^2=5.99^*$; $p=0.071$
<i>K. pneumoniae</i>	1 (16.7%)	5 (83.3%)	
<i>S. agalactiae</i>	3 (60.0%)	2 (40.0%)	
<i>S. epidermidis</i>	2 (66.7%)	1 (33.3%)	

*: Fisher's exact test, MDR: Multidrug resistance

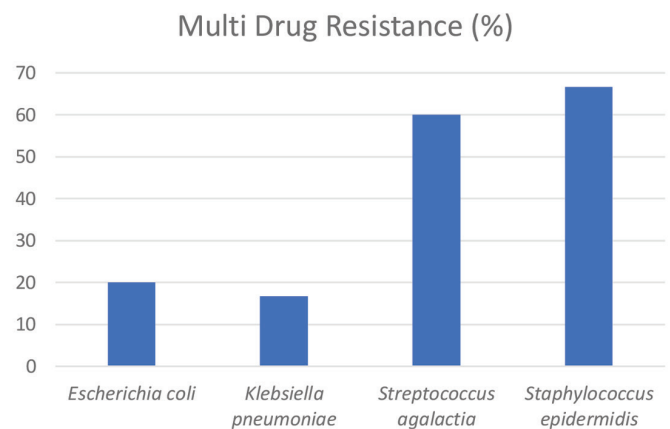


Figure 5. Distribution of multidrug-resistant bacterial isolates

Conclusion

In the study, the prevalence of ASB in the second half of pregnancy is high, and a substantial proportion of the causative agents exhibit multidrug resistance. In particular, *E. coli* and *K. pneumoniae* isolates demonstrated high resistance to ampicillin, amoxicillin-clavulanate, and cephalosporins, whereas *S. agalactiae* showed significant resistance to fluoroquinolones and tetracycline. Therefore, diagnosis of ASB during pregnancy

should always be guided by urine, and antimicrobial susceptibility testing, and empirical treatment should be based on local resistance profiles.

Moreover, the high MDR rates highlight the need to reassess antibiotic use policies in pregnant populations and to strengthen strategies aimed at combating antimicrobial resistance. In this context, expanding routine ASB screening and tailoring treatment protocols according to updated regional resistance data are of critical importance for the health of both mothers and their infants.

Ethics

Ethics Committee Approval: The study was approved by Kırşehir Ahi Evran University Health Sciences Scientific Research Ethics Committee (decision no: 2024-08/55, dated: 02.04.2024).

Informed Consent: Written informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Concept: C.Ö., M.K., Design: C.Ö., M.K., Data Collection or Processing: M.K., R.A., Analysis or Interpretation: C.Ö., R.A., M.B., Literature Search: C.Ö., M.K., R.A., M.B., Writing: C.Ö., M.B.

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