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# Comparison of Asymptomatic Bacteriuria Agents and Antimicrobial Susceptibility in Term and Preterm Pregnancies

## Term ve Preterm Gebelerde Asemptomatik Bakteriüri Etkenleri ve Antimikrobiyal Duyarlılığın Karşılaştırılması

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**ABSTRACT**

**Aim:** The aim of this study was to compare the resistance to asymptomatic bacteriuria (ABU) between term and preterm pregnancies.
**Material and Methods:** Pregnant women aged 17-41 years who were followed up in the obstetrics department of our hospital and whose urine cultures were sent to the laboratory for analysis and reported as ABU were included in the study. Patients were divided into two groups according to gestational weeks as preterm (<37 weeks) and term (≥37 weeks). The results were compared between the two groups.
**Results:** From among a total of 123 pregnant women, 29 (23.6%) delivered at preterm and 94 (76.4%) at term. The most frequently identified pathogen throughout the study group was 52.0% (n=64) *Escherichia coli* (*E. coli*), followed by 16.3% (n=20) *Streptococcus agalactiae* (*S. agalactiae*). There was no statistically significant difference in terms of the distribution of *E. coli* and *S. agalactiae* between the term and preterm groups (p=0.698 and p=0.930). *E. coli* was resistant to ampicillin 56.3% (n=36), to cefuroxime 40.6% (n=26). While fosfomycin resistance was 1.6% (n=1), nitrofurantoin resistance was not found. Extended-spectrum beta-lactamase positivity was 23.4% (n=15) in *E. coli* strains. No statistically significant difference was found in antibiotic resistance rates of *E. coli* strains between the term and preterm groups.
**Conclusion:** The most commonly isolated pathogen was *E. coli* which was highly resistant to beta-lactams. Screening of pregnant women for ABU and treatment with appropriate antibiotics; is the most effective way to prevent both maternal and fetal complications and antimicrobial resistance.
**Keywords:** Pregnancy; asymptomatic bacteriuria; antibiotic resistance.

**ÖZ**

**Amaç:** Bu çalışmanın amacı, asemptomatik bakteriüri (ASB) etkenlerinin direnç paternlerini term ve preterm gebelikler arasında karşılaştırmaktır.
**Gereç ve Yöntemler:** Hastanemiz kadın doğum bölümünde takip edilen ve idrar kültürleri laboratuvarda incelenmek üzere gönderilen ve ASB olarak rapor edilen 17-41 yaş arası gebeler çalışmaya dahil edildi. Hastalar gebelik haftalarına göre preterm (<37 hafta) ve term (≥37 hafta) olmak üzere iki gruba ayrıldı. Sonuçlar iki grup arasında karşılaştırıldı.
**Bulgular:** Toplam 123 gebeden 29'u (%23,6) preterm, 94'ü (%76,4) term doğum yaptı. Tüm çalışma grubunda en sık izole edilen patojen %52,0 (n=64) ile *Escherichia coli* (*E. coli*) idi ve bunu %16,3 (n=20) ile *Streptococcus agalactiae* (*S.agalactiae*) izlemekteydi. Term ve preterm grupları arasında *E. coli* ve *S. agalactiae* dağılımı açısından istatistiksel olarak anlamlı bir fark yoktu (p=0,698 ve p=0,930). *E. coli* suşları ampisiline %56,3 (n=36) oranında, sefuroksime %40,6 (n=26) oranında dirençliydi. Fosfomisin direnci %1,6 (n=1) olarak belirlenirken, nitrofurantoin direnci saptanmadı. *E. coli* suşlarında genişletilmiş spektrumlu beta-laktamaz pozitifliği %23,4 (n=15) olarak tespit edildi. Term ve preterm grupları arasında *E. coli* suşlarının antibiyotik direnç oranları bakımından istatistiksel olarak anlamlı fark saptanmadı.
**Sonuç:** En sık izole edilen patojen bakteri, beta laktamlara oldukça dirençli olan *E. coli* idi. Gebelerin ASB için taranması ve uygun antibiyoterapi ile tedavisi; maternal ve fetüste gelişecek komplikasyonları ve antimikrobiyal direnci önlemede etkili bir yoldur.
**Anahtar kelimeler:** Gebelik; asemptomatik bakteriüri; antibiyotik direnci.

## INTRODUCTION

Pregnancy is a specific state in which both anatomical and physiological alterations reversibly occur in the urinary tract, giving a conducive environment for bacterial proliferation (1). During pregnancy, several alterations including ureter dilatation, decreased urethral peristalsis and bladder tonus, increased plasma volume and urine estrogen increase the risk of developing bacteriuria (2). Asymptomatic bacteriuria (ABU) is defined as the presence of at least $10^5$ organisms/mL in the urine culture without any systemic or local symptoms in the urinary tract (3,4). The global prevalence of ABU was estimated between 2-15%, although its frequency depends on geographic regions (4).

The most commonly isolated bacteria in ABU are *Escherichia coli* (*E. coli*) and some other bacteria including *Enterobacteriaceae, Streptococcus agalactiae* (*S. agalactiae*), *Pseudomonas aeruginosa*, *Enterococcus* spp. and *Staphylococcus aureus* may cause ABU among pregnant women, while the isolated bacteria widely vary according to geographical areas (1,2,5,6). Studies have reported that ABU during pregnancy increases the risk of developing pyelonephritis and obstetric outcomes that may result in adverse events such as premature delivery and low birth weight (7-9). Possible outcomes of untreated pyelonephritis are increased maternal and fetal morbidity and mortality. In addition, it might lead to acute respiratory distress, acute renal failure, preterm delivery, and maternal fever might as well (10). Some studies have proposed that antimicrobial treatment of ABU improves fetal outcomes and decreases the frequency of low-birth-weight infants and preterm delivery (8,11). Although the adverse effects of antibiotic therapy associated with the treatment of ABU have been described in a limited number of studies, there is sufficient evidence that pyelonephritis in pregnancy is associated with adverse maternal outcomes and that ABU is detected by screening to reduce the incidence of the disease. ABU is recommended to be tested in pregnant women by urine culture (12).

In recent years, rising antimicrobial drug resistance has been observed in ABU cases. As a consequence, the significance of performing antibiotic sensitivity tests and routine urine cultures with the aim of determining the resistance patterns of isolated uropathogens and detecting ABU should be taken into consideration. Therefore, the objective of this study was to compare the resistance to ABU between term and preterm pregnancies.

## MATERIAL AND METHODS

Ethics committee approval for the study was obtained from the clinical research ethics committee of the İstanbul Medeniyet University Göztepe Training and Research Hospital (numbered 154, dated 24.02.2021). Moreover, the study was conducted in accordance with the principles of the Declaration of Helsinki. In this study, urine samples of 1710 pregnant women were analyzed and 123 pregnant women between the ages of 17-41 years were diagnosed with ABU, in our hospital between January 2018 and February 2020. Patients with, dysuria, pollakiuria, complaint of suprapubic pain, vaginal discharge, malodor, those who were using antibiotics, and a history of renal disease or renal calculi were excluded from the study. Patients' demographic such as maternal age, gestational week, mode of delivery, and birth weight of the infant were recorded. Patients were divided into two groups according to gestational weeks, preterm (<37 weeks) and term (≥37 weeks).

Medium-flow urine samples were collected from the patients in a sterile vial after verbal instructions and a culture test was performed within two hours. Urine samples were inoculated on Chrom agar medium (bioMérieux, France). Culture outcomes were read 24 hours following incubation at 37° C. An amount of organism $>10^5$/mL was reported as ABU. Growing microorganisms were defined as matrix-assisted laser desorption ionization time-lapse light mass spectrometry (MALDI-TOF MS). VITEK-2 (bioMérieux, Marcy l'Etoile, France) compact automated systems were used to examine the antimicrobial susceptibility of microorganisms. The results were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (13).

### Statistical Analysis

Categorical variables were presented as numbers and percentages, while continuous variables were expressed as mean±standard deviation and median (minimum-maximum). Categorical variables were compared using the Pearson chi-square or Fisher's exact test. Normal distribution was tested using the Shapiro-Wilk test. Non-normal distributed variables were compared with the Mann-Whitney U test. SPSS v.25.0 (Statistical Package for Social Sciences, IBM Inc., Armonk, NY, USA) was used for statistical analysis. Two-sided p-values of less than 0.05 were considered statistically significant.

## RESULTS

Significant growth was detected in 123 (7.2%) of a total of 1710 urine samples ($10^5$ organisms/mL). The mean age was found as 28.4±7.2 (range, 17-41) years. No statistically significant difference was found between the term and the preterm groups in terms of age (p=0.671, 28.90±6.87 vs 28.19±7.36, respectively). The mean gestational week was found to be 37.4±2.9 (range, 26-40) weeks. Of the 123 pregnancies, 29 (23.6%) were preterm births.

The most commonly isolated pathogen in entire study group was *E. coli* at 52.0% (n=64) followed by *S. agalactiae* at 16.3% (n=20), *Klebsiella pneumoniae* at 11.4% (n=14), *Enterococcus* spp. at 10.6% (n=13), *Candida* spp. at 3.3% (n=4), *Proteus* spp. at 2.4% (n=3), *Staphylococcus* spp. at 2.4% (n=3), and *Enterobacter cloaca* %1.6 (n=2).

Table 1 and Figure 1 present the distribution of pathogens between term and preterm pregnancies. There was no statistically significant difference in terms of the distribution of *E. coli* (p=0.698) and *S. agalactiae* (p=0.930) between the term and preterm groups.

All *Candida albicans* strains were susceptible to fluconazole. Considering the beta-lactam antibiotic resistance that can be safely used in pregnant women, among the gram-negative causative microorganisms, ampicillin resistance was 51/83 (61.5%), amoxicillin-clavulanate resistance was 32/83 (38.6%), cefuroxime resistance was 28/83 (33.7%), ceftriaxone resistance was 20/83 (24.1%), and ceftazidime was resistance 15/83 (18.1%). While fosfomycin resistance was 6/83 (7.2%), nitrofurantoin resistance was 4/83 (4.8%). *E. coli* strains, which were the

most common causative microorganism, were resistant to ampicillin 36/64 (56.3%), amoxicillin-clavulanate 24/64 (37.5%), cefuroxime 26/64 (40.6%), and ceftriaxone 20/64 (31.3%). While fosfomycin resistance was 1/64 (1.6%), no nitrofurantoin resistance was found. *Klebsiella pneumoniae* was the second most common gram-negative bacterium and resistance was found in 12/14 (85.7%) of the strains to ampicillin, 5/14 (35.7%) to amoxicillin-clavulanate, 4/14 (28.6%) to fosfomycin, 1/14 (7.1%) to nitrofurantoin, while all strains were susceptible to second and third-generation cephalosporins. Carbapenem resistance was not detected in any of the gram-negative agents. Fifteen of the cases had extended-spectrum beta-lactamase (ESBL) positivity and this resistance was only in *E. coli* strains (23.4%, 15/64). ESBL was not positive in *Klebsiella pneumoniae*, *Proteus* spp., or *Enterobacter cloaca* strains.

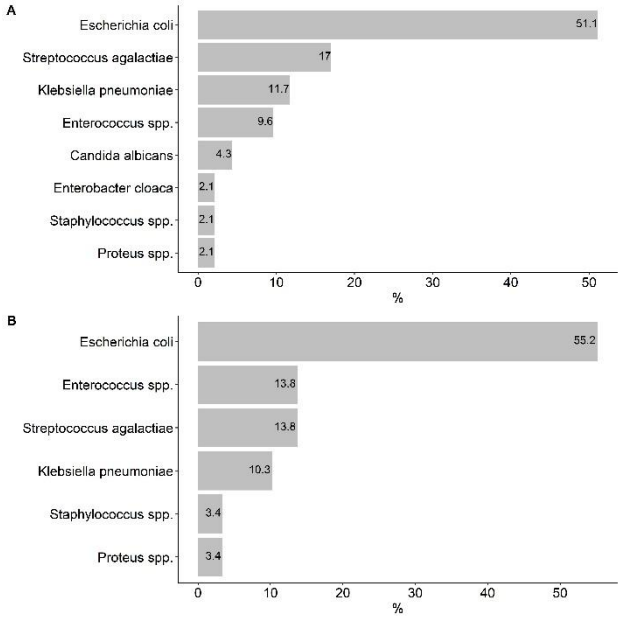


**Figure 1.** Percentages of the isolated microorganisms, **A)** term pregnancies (n=94), **B)** preterm pregnancies (n=29)

**Table 1.** Distribution of pathogens between term and preterm pregnancies

| | Term (n=94) | Preterm (n=29) | p |
|---|---|---|---|
| **Gram-negative**, n (%) | 63 (67.0%) | 20 (69.0%) | 0.845 |
| *Escherichia coli* | 48 (51.1%) | 16 (55.2%) | 0.698 |
| *Klebsiella pneumoniae* | 11 (11.7%) | 3 (10.3%) | >0.999 |
| *Proteus spp.* | 2 (2.1%) | 1 (3.4%) | >0.999 |
| *Enterobacter cloaca* | 2 (2.1%) | 0 (0.0%) | >0.999 |
| **Gram-positive**, n (%) | 27 (28.7%) | 9 (31.0%) | 0.811 |
| *Streptococcus agalactiae* | 16 (17.0%) | 4 (13.8%) | 0.930 |
| *Enterococcus spp.* | 9 (9.6%) | 4 (13.8%) | 0.731 |
| *Staphylococcus spp.* | 2 (2.1%) | 1 (3.4%) | >0.999 |
| **Fungi**, n (%) | | | |
| *Candida albicans* | 4 (4.3%) | 0 (0.0%) | 0.672 |

**Table 2.** Comparison of antimicrobial resistance of isolated *Escherichia coli* strains between term and preterm births

| | Term (n=48) | Preterm (n=16) | p |
|---|---|---|---|
| Ampicillin, n (%) | 27 (56.3%) | 9 (56.3%) | >0.999 |
| Ceftriaxone, n (%) | 14 (29.2%) | 6 (37.5%) | 0.533 |
| Cefuroxime, n (%) | 18 (37.5%) | 8 (50%) | 0.382 |
| Ceftazidime, n (%) | 11 (22.9%) | 4 (25%) | >0.999 |

In Table 2, antibiotic resistance frequencies of *E. coli* strains were compared in the term and preterm groups. In *E. coli* strains, ESBL positivity was 22.9% (11/48) in the term group and 25% (4/16) in the preterm group. However, there was no statistically significant difference between the groups in terms of ESBL positivity (p>0.999).

All strains of *S. agalactiae*, which was the most growing gram-positive agent, were susceptible to benzylpenicillin. *Enterococcus* spp. ampicillin and ampicillin-sulbactam resistance were found in 1/13 (7.7%) and 1/13 (7.7%) of the strains, respectively. Cefoxitin resistance was not detected in any staphylococcal strains.

## DISCUSSION

ABU requires particular attention during pregnancy due to the absence of symptoms and related maternal and fetal consequences (14). In the present study, among 1710 urine samples cultured from asymptomatic pregnant women, 123 samples yielded uropathogens, showing a prevalence of 7.2%. This is consistent with the prevalence of ABU seen in other studies by Aktun et al. (2), Tokak et al. (5), and Aşgın et al. (6), respectively, 7.1%, 7.8%, and 10%. The occurrence of significant bacteria in the lower urinary tract with no symptoms makes the condition more susceptible in pregnant women. Several factors play a role in the development of ABU such as the number of pregnancies, physiological and anatomical changes, and poor genital hygiene. Being isolated from their samples to stop fetal and maternal morbidities, bacteria should be taken into consideration with its antimicrobial sensitivity patterns while treating the positive urine cultures of women (15).

In pregnant women, progesterone relaxes uteretic smooth muscle, which causes dilatation of the uterus caused by the pressure from the enlarging uterus. These factors lead to urinary stasis, dysfunctional urethral valves, and vesicoureteral reflux that assists bacterial colonization (16). Several studies have reported that the geographic variability of pathogens in the case of ABU is limited with the predominance of gram-negative, particularly *E. coli* (2,5,6,17,18). We also detected gram-negative isolates as the causative agent at a higher rate, 67.5%. However, there are studies reporting converse outcomes. Gram-positive isolates were reported as 63.8% by Ali et al. (16) and 64% by Dange et al. (19).

In the present study, the most commonly isolated pathogen was *E. coli* at 52.0% followed by *S. agalactiae* at 16.3%, *Klebsiella pneumoniae* at 11.4%, and *Enterococcus* spp. at 10.6% in all participants. The most common two isolates in the term group were *E. coli* with 51.1% and *S. agalactiae* with 17%. Whereas the most common two isolates in the preterm group were *E. coli* with 55.2% and *S. agalactiae* with 13.8%. There was no

statistically significant difference in terms of the distribution of *E. coli* and *S. agalactiae* between the term and preterm groups.

Andabati et al. (20) and Enayat et al. (21) reported that *E. coli* was the most commonly isolated organism with 51% and 58.96%, respectively. Aktun et al. (2) reported that the organism with the highest incidence was *E. coli* with 69%, while this rate was reported as 61.1% by Sonkar et al. (22) Nteziyaremye et al. (1) reported that the incidence of *E. coli* being 46.4%. Although different rates have been reported in previous studies, *E. coli* was the most commonly isolated pathogen in these studies. While Onu et al. (23) detected *Staphylococcus aureus* as the most common causative microorganism in their study, 45.9%; Ali et al. (16) found *E. coli* and *Staphylococcus aureus* strains to be equal in etiology at a percent of 31%. The variation between the rates of organisms might have resulted from the differences in geographic locations, socioeconomic state, and time of the study.

Therapeutic options are very limited and challenging due to different bacterial agents and their different resistance patterns in ABU (24). A concerning point, which limits therapeutic options in ABU is the emergence of significantly high drug resistance and over or misuse of antibacterial agents (25). What is more effective than treatment is ABU with some points of view such as monitoring, prevention, health condition, and cost. Antimicrobial drugs are frequently prescribed in pregnant women, and the increasing drug resistance must be taken into account (7). In this study considering the beta-lactam antibiotic resistance that can be safely used in pregnant women, *E. coli* is resistant to ampicillin 56.3%, amoxicillin-clavulanate 37.5%, cefuroxime 40.6%, ceftriaxone 31.2%. While fosfomycin resistance was 1.6%, nitrofurantoin resistance was not found. In the study of Aktun et al. (2), ampicillin resistance was 46%, cefuroxime resistance was 15%, ceftriaxone resistance was 10%, and nitrofurantoin resistance was 6.3%, while fosfomycin resistance was found below 1% in *E. coli* strains. In the study of Aşgın et al. (6), ampicillin resistance of *E. coli* isolates was 53%, and cefuroxime resistance was 18%, while fosfomycin resistance was 3%. In the same study, ESBL rates were found to be 8% in *E. coli* strains and 13% in *Klebsiella pneumoniae* strains (6). While we detected ESBL positivity in 23.4% of *E. coli* strains, we did not detect ESBL positivity in *Klebsiella pneumoniae*, *Proteus* spp., or *Enterobacter cloaca* strains. In the present study, pregnant women with ABU were treated with appropriate antibiotics and resistance rates in *E. coli* strains were compared between women with term and preterm deliveries. However, there was no statistically significant difference between the groups.

However, it is well known that the resistance patterns of these organisms vary significantly between countries and even among healthcare centers (26). It has been reported that in the prevention of ABU, the gold standard for treatment options and prevention of serious complications is screening the patients with urine culture and prescribing antibiotics correlated to the urine culture (7). Preterm birth incidence is indicated to be approximately 11% (27). A study by Uncu et al. (28) showed culture-negative group has 9.3% preterm birth, compared to the ABU group has 26% preterm birth. In addition, Lallar et al. (29) provided data that the preterm birth rate is 30% in the ABU group. The present study detected 23.6% preterm birth among pregnant women with ABU. Although mechanisms behind ABU causing preterm labor and ABU's independency as a risk factor are unclear (30), the statistical relationship between preterm birth and ABU distinct. Therefore, ABU should be diagnosed and treated early in pregnant women. In this way, the incidence of acute symptomatic urinary tract infections can be reduced by up to 70% (7,17).

The major limitations of this study are its retrospective design, relatively small sample size, and being conducted in a single center. We believe that our results would be encouraging for further extensive studies to be performed in the future.

## CONCLUSION

The most commonly isolated pathogen was *E. coli* and no statistically significant difference was found between the term and preterm pregnancies in terms of the resistance in pregnant women with ABU. Considering undesired complications of ABU for the mother and fetus, screening at certain intervals for bacteria seems a better strategy than treating with antibiotics most of which are resistant to multiple therapeutic agents.

**Ethics Committee Approval:** The study was approved by the ethics committee of İstanbul Medeniyet University Göztepe Training and Research Hospital (24.02.2021, 154).

**Conflict of Interest:** None declared by the authors.

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