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# Microbiological and Antimicrobial Resistance Pattern Among Pregnant Women in The Sultanate of Oman: Comparison Between Symptomatic and Asymptomatic Bacteriuria

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**Bacteriuria is common in pregnancy and is associated with the risk of neonatal morbidity and mortality. In Oman, no studies have been done to determine the percentage of symptomatic and asymptomatic bacteriuria in pregnant Omani patients. This study investigated the prevalence and incidence of antibiotic resistance patterns of symptomatic and asymptomatic bacteriuria among pregnant Omani women. A total of 230 urine samples were collected from symptomatic and asymptomatic pregnant Omani female patients. Clinical patient information was gathered from the Hospital Information System (HIS). Bacterial pathogens were identified in the urine samples using microscopic examination, cultures, and serological techniques. Antibiotic sensitivity tests were performed on the isolated bacterial pathogens. Bacteriuria was found in 14 (6.08%) of the 230 urine samples. Among the 14 bacteriuria-positive samples, 6 were symptomatic (2.06%) and 8 were asymptomatic (3.47%). The most common bacteria were *Escherichia coli* (35.71%) and *Streptococcus agalactiae* (21.43%). Most of the clinical isolates were completely resistant to ampicillin. Asymptomatic bacteriuria was more common than symptomatic bacteriuria in the pregnant Omani population. The detection of antibiotic-resistant pathogenic bacteria, especially in asymptomatic pregnant Omani women, underscores the importance of implementing strict guidelines for the prevention of this public health issue. This includes advising and encouraging pregnant women to follow strict hygiene protocols to avoid Urinary tract infection (UTI) during pregnancy, as this type of infection may have adverse maternal and fetal outcomes.**

**Keywords:** Asymptomatic Bacteriuria; Antibiotic Resistance; Pregnancy; Symptomatic Bacteriuria.

Bacteriuria is the manifestation of bacterial pathogens in the urine with and without causing apparent clinical manifestations[1,2] Biologically, the urinary tract is sterile and designed to prevent infections[3]. The bladder and ureters are structured to stop urine from backing up toward the kidneys. The flow of urine during urination washes bacteria out of the urinary tract.[4] However, infections can still occur because of some influencing factors, such as variations in the host's natural defense mechanisms, premenopausal and menopausal factors and age[2]. Urinary tract infection (UTIs) may result in serious complications such as cystitis, urethritis, and pyelonephritis[5]. Symptomatic and asymptomatic UTIs result in adverse maternal and fetal outcomes[2].

Asymptomatic bacteriuria is a subclinical infection that is existing in cases where the urine culture shows a bacterial pathogen growth of higher than $10^5$ colony-forming units (CFU) per ml of urine and without apparent UTI clinical manifestations in the patient[1] . Proper management of asymptomatic bacteriuria can decrease its incidence by 80%–90% [6,7,8]

The causative agent that accounts for more than 80% of all UTIs during pregnancy is *Escherichia coli*[9]. Other causes include *Proteus mirabilis, Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Klebsiella pneumoniae* [3,8,9].

A previous study in Nigeria on urine samples obtained randomly from 125 pregnant patients, showed that 40% of patients were positive for bacteriuria[10]. Another study in the United Arab Emirates showed that the highest rate of infection among symptomatic pregnant UTI patients was with *E.Coli* followed by Group B *streptococcus* [11]. The percentage of bacteriuria is high in pregnant women due to many factors[12]. One factor is women's immune status during pregnancy. Pregnant women are considered immunocompromised UTI hosts[8]. Other factors include the physical and hormonal changes[1,8,12]. For example, during pregnancy, there is a great increase in fluid discharges, which enhance bacterial growth. In addition, progesterone stimulates urethral smooth muscle resting, causing urinary stasis, which is significant in the etiology of cystitis[9,13]. During pregnancy, the urine pH reaches an appropriate level for *E. coli* growth[13].

Undetected asymptomatic bacteriuria is linked to unfavorable obstetric and fetal outcomes, because of the increased chance of its progression to symptomatic bacteriuria [14,15]. Therefore, screening and management of bacteriuria during pregnancy are essential to avoid UTI adverse effects[3].

The aims of the present study were to investigate the prevalence of symptomatic and asymptomatic bacteriuria Omani among pregnant Omani women and to identify the causative bacterial pathogens, and finally to determine the antibiotic resistance patterns of the bacterial pathogens isolated from this unique group of patients.

## MATERIALS AND METHODS

### Specimens

This prospective study was conducted on pregnant Omani women attending Sultan Qaboos University Hospital (SQUH). The total of 230 urine samples were randomly collected from symptomatic and asymptomatic bacteriuria pregnant Omani patients from September 21 to November 30, 2017. SQUH is a referral hospital for all different regions of Oman.

The inclusion criteria were any pregnant women visited the Antenatal Clinic at SQUH (with and without clinical manifestations of UTI). The exclusion criteria were immunocompramised pregnant women due to HIV infection or diabetes and pregnant women on antimicrobial therapy.

The patients' histories, including age, trimester and symptoms, were obtained from the Hospital Information System (HIS). The Ethical approval was acquired from the Medical Research Ethics Committee, SQU. (Ref.. No. SQU-EC/102/17; MERC# 1497).

### Detection of pathogenic bacteria in urine by routine cultures

Microscopic examination of the urine samples was carried out by loading 60 µL of each urine sample into microtiter plate wells. Then, the urine specimens were examined under an inverted light microscope for the detection of epithelial cells, casts ,crystals, white and red blood cells. A UTI was diagnosed by bacterial culture. All the urine specimens were inoculated aseptically using a bacteriological 3 mm loop on cysteine lactose electrolyte deficient agar (CLED).

Following inoculation of the urine samples on CLED agar, the agar plates were incubated at 37°C for 24 hours. The results were reported depending on the shape and number of colonies. The colonies were counted to determine the degree of significance, which depended on the loop size used. Only plates with more than 11 colonies were reported as significant. When three or more types of bacterial colonies were present, the sample was reported to have urogenital contamination. The CLED agar plates were examined for the presence of yellow or green colonies, which may indicate the presence of lactose-fermenting or non-lactose-fermenting bacteria.

**Pathogenic bacteria identification and antibiotic sensitivity tests**

Biochemical identification of the pathogenic microorganisms in the urine samples and antibiotic sensitivity tests of the isolated bacterial pathogens were carried out for suspected colonies using the BD Phoenix™ 100 (Becton Dickinson, USA). The bacteria isolated from the urine samples were suspended in 0.85% NaCl and diluted to 0.5 McFarland. Then, they were loaded into specific panels and analyzed using the BD Phoenix™ 100.

**Detection of *Streptococcus agalactiae***

*S. agalactiae* detection in the urine samples was conducted using the Alere BinaxNOW® *Streptococcus agalactiae* Antigen Card (Abbott, USA), which is a rapid assay for the qualitative detection of *S. agalactiae (Group B Streptococci)* antigen in a patient's urine sample. The test was carried out by immersing a swab in the urine sample, and then the swab was inserted into the card. The reagent was added to the swab, and the result appeared after 15 minutes. If two lines appeared (control and test), a positive result was indicated. Positive samples by Alere BinaxNOW® *Streptococcus agalactiae* Antigen Card were confirmed by the isolation of *S. agalactiae,* followed by antibiotic sensitivity testing.

**Data analysis**

All patient data were collected and tabulated using the Excel program (Excel 2013). SPSS software.

## RESULTS

**Clinical features**

Of the 230 urine samples analyzed, 216 (93.91%) samples were negative for bacteriuria, while 14 (6.03%) samples were positive. The 14 samples were categorized according to the patients' symptoms into symptomatic and asymptomatic bacteriuria. Table 1 shows the symptoms of the symptomatic bacteriuria patients; some symptomatic patients showed more than one clinical manifestation [Table 1]. The most common

**Table 1.** Symptoms that appeared in symptomatic bacteriuria patients

| UTI Symptoms | Number of cases |
|---|---|
| Dysuria | 5 |
| Lower abdominal pain | 2 |
| Fever | 1 |
| Urinary Frequency | 1 |
| Flank pain | 1 |

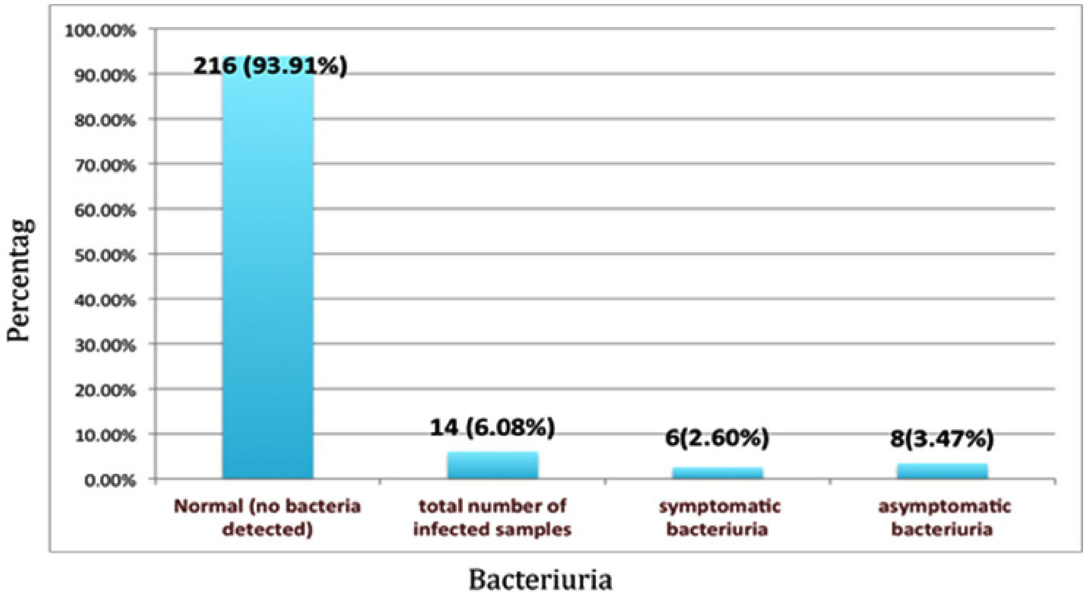


**Fig. 1.** The prevalence of symptomatic and asymptomatic bacteriuria among 230 urine samples

observed clinical manifestations were dysuria followed by lower abdominal pain [Table 1]. Six symptomatic patients (2.06%) were positive for bacteriuria, while eight asymptomatic patients (3.47%) were positive for bacteriuria. [Figure 1], shows the rate of symptomatic and asymptomatic bacteriuria among the 230 isolated urine samples.

**Identity and prevalence of bacterial pathogens in symptomatic and asymptomatic bacteriuria**

The most common bacterial pathogen isolated from the samples of the 14 pregnant patients with bacteriuria was *E. coli.* Figure 2, shows the prevalence of the bacterial pathogens detected in the 14 positive urine samples obtained

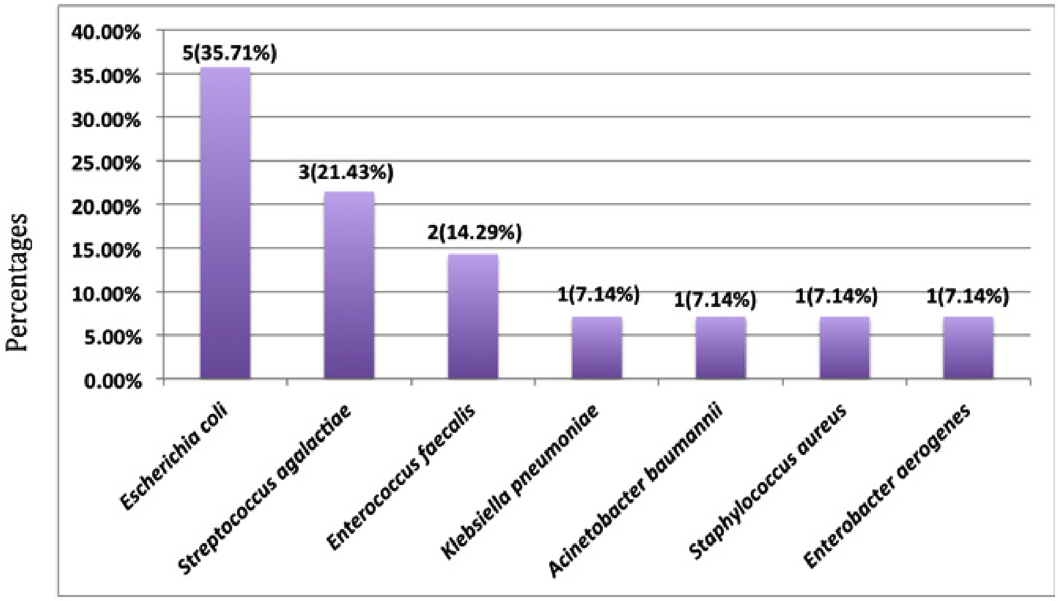


**Fig. 2.** The prevalence of bacterial pathogens isolated from 14 positive urine samples

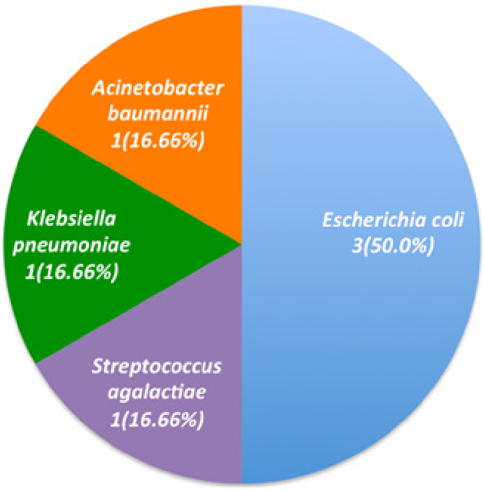


**Fig. 3.** The most common bacteria among symptomatic bacteriuria in pregnancy samples

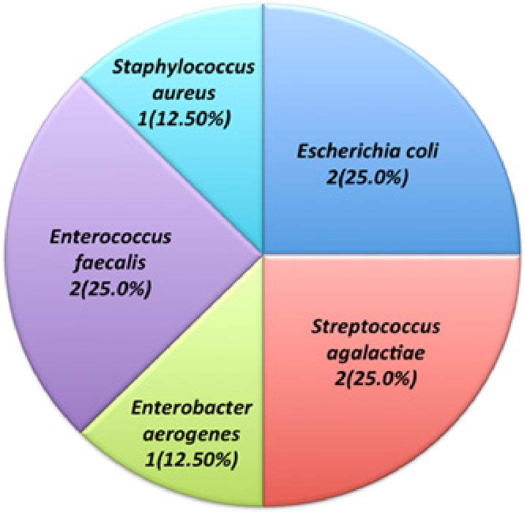


**Fig. 4.** The most common bacteria among asymptomatic bacteriuria in pregnancy samples

from both the symptomatic and the asymptomatic pregnant women. *E. coli* was identified in 5 (35.71%) patients, followed by *S. agalactiae (Group B Streptococci),* in 3 patients (21.43%) and *Enterococcus faecalis* in 2 patients (14.29%). *K. pneumoniae, Acintobacter baumannii, Staphylococcus aureus*, and *Enterobacter aerogenes* were each detected in one patient (7.14%). The most common bacteria identified in the urine samples obtained from the symptomatic and asymptomatic pregnant women are shown in [Figures 3 & 4].

**Table 2.** Antibiotics sensitivity test results for *Escherichia coli, Klebsiella pneumoniae, Acintobacter baumannii* and *Enterobacter aerogenes*

| Bacterial Strains | N (%) | | | | AMP | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *E.coli* | 5 (35.71%) | 0 | 75% | 80% | 60% | 100% | 80% | 60% | 75% |
| *K.pneumoniae* | 1(7.14%) | 0 | 100% | 100% | 100% | 100% | 0 | 0 | 100% |
| *A.baumannii* | 1(7.14%) | 0 | 100% | 100% | 0 | 100% | 100% | 100% | 100% |
| *E.aerogenes* | 1(7.14%) | 100% | 0 | 100% | 0 | 100% | 100% | 100% | 100% |

[0: Resistant, 100% : Sensitive]
AMX = Ampicillin; CIP = Ciprofloxacin; ERY = Erythromycin; LZD = Linezolid; TNC = Tetracycline; TEI = Teicoplanin; VAN = Vancomycin; CRO = Ceftriaxone; CXM = Cefuroxime; SXT = trimethoprim/sulfamethoxazole; AMK = amikacin; IMP = Imipenem; MEM = Metropenem

**Table 3.** Antibiotics sensitivity test results for *Streptococcus agalactiae*

| Bacterial Strains | N(%) | CLI | ERY | LZD | PNC | TNC | VAN |
|---|---|---|---|---|---|---|---|
| *S. agalactiae* | 3(21.43%) | 33.3% | 33.3% | 100% | 100% | 0 | 100% |

[0: Resistant, 100% : Sensitive]
AMX = Ampicillin; CIP = Ciprofloxacin; ERY = Erythromycin; LZD = Linezolid; TNC = Tetracycline; TEI = Teicoplanin; VAN = Vancomycin; CRO = Ceftriaxone; CXM = Cefuroxime; SXT = trimethoprim/sulfamethoxazole; AMK = amikacin; IMP = Imipenem; MEM = Metropenem

**Table 4.** Antibiotics sensitivity test results for *Staphylococcus aureus*

| Bacterial Strains | N(%) | AMP | PNC | CIP | ERY | TNC | TMP/SMX | LZD | IMP |
|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* | 1(7.14%) | 0 | 0 | 100% | 100% | 100% | 100% | 100% | 0 |

[0: Resistant, 100% : Sensitive]
AMX = Ampicillin; CIP = Ciprofloxacin; ERY = Erythromycin; LZD = Linezolid; TNC = Tetracycline; TEI = Teicoplanin; VAN = Vancomycin; CRO = Ceftriaxone; CXM = Cefuroxime; SXT = trimethoprim/sulfamethoxazole; AMK = amikacin; IMP = Imipenem; MEM = Metropenem

**Table 5.** Antibiotics sensitivity test results for *Enterococcus faecalis*

| Bacterial Strains | N (%) | AMP | CIP | ERY | LZD | TNC | TEI | VAN |
|---|---|---|---|---|---|---|---|---|
| E.faecalis | 2(14.29%) | 100% | 50% | 0 | 100% | 0 | 100% | 100% |

0: Resistant, 100% : Sensitive]
AMX = Ampicillin; CIP = Ciprofloxacin; ERY = Erythromycin; LZD = Linezolid; TNC = Tetracycline; TEI = Teicoplanin; VAN = Vancomycin; CRO = Ceftriaxone; CXM = Cefuroxime; SXT = trimethoprim/sulfamethoxazole; AMK = amikacin; IMP = Imipenem; MEM = Metropenem.

**Antibiotic susceptibility**

The results of the antibiotic sensitivity tests for *E. coli, K. pneumoniae, A. baumannii*, and *E. aerogenes* are shown in [Table 2]. These bacteria were all sensitive to meropenem, while *E. coli*, *K. pneumoniae*, and *A. baumannii* were resistant to ampicillin [Table 2]. Table 3 presents the antibiotic sensitivity test results for *Group B streptococci (S. agalactiae)*, which was fully sensitive to linezolid, vancomycin, and penicillin [Table 3]. Table 4 shows the *S. aureus* antibiotic sensitivity test results, indicating complete sensitivity to ciprofloxacin, erythromycin, tetracycline, linezolid, and trimethoprim/sulfamethoxazole. Table 5 demonstrates the antibiotic sensitivity of the two samples with *E. faecalis*, which were completely sensitive to ampicillin, linezolid, and vancomycin and completely resistant to tetracycline and erythromycin.

## DISCUSSION

In this study, among the 230 urine samples collected from pregnant Omani female patients admitted to Sultan Qaboos University Hospital and screened for bacteriuria, only 14 (6.08%) cases were positive for the presence of bacteriuria. Among the 14 positive samples, 6 (2.06%) of the symptomatic pregnant patients were positive for bacteriuria, while 8 (3.47%) of the asymptomatic pregnant patients were positive for bacteriuria. These findings are comparable to a study conducted in Saudi Arabia, where 419 (15.8%) out of 2642 cases were positive for the presence of bacteriuria[15]. Of the 419 positive subjects, 188 (7.1%) subjects were asymptomatic for bacteriuria, and 231 (8.7%) were symptomatic[15].

In the present study the most common symptoms seen in symptomatic patients were dysuria followed lower abdominal pain. These findings were in contrast to previously published study in United Arab Emirates were lower abdominal pain was the most common followed by dysuria[11].

In the existing study, *E. coli* (35.71%) was the most frequent cause of bacteriuria in both the asymptomatic and symptomatic pregnant patients. This finding is consistent with studies carried out in Saudi Arabia and UAE respectively, where *E. coli* was the most common contributing agent to bacteriuria[11,15]. The detection of *E. coli* can be explained by the difficulty in maintaining hygiene during pregnancy[9]. In addition, in pregnancy, urine pH reaches a level appropriate for *E. coli* growth[9]. Conversely, these results are inconsistent with a study conducted in Brunei, where the leading cause of bacteriuria in pregnancy was *Klebsiella spp.* (2.94%), followed by *E. coli*[16]. In our study, the most common bacterial pathogens present in the symptomatic bacteriuria patients after *E. coli* (35.71%) were *S. agalactiae*, *K. pneumoniae*, and *A. baumannii* (all 16.66%). The most common bacterial pathogens present in the asymptomatic bacteriuia patients after *E. coli* (25.00%) were *S. agalactiae* (25.00%), *E. faecalis* (25.00%), *S. aureus* (12.50%), and *E. aerogenes* (12.50%).

In the present study the isolated *E. coli, S. aureus, K. pneumoniae*, and *A. baumannii* were all completely resistant to ampicillin. This finding is coherent with a study performed in India, where most of their isolates were highly resistant to ampicillin[17]. This suggests that ampicillin should not be used as a first line of defense against UTIs in pregnancy in Oman. In the present study, Imipenem and meropenem were sensitive for most of the clinical isolates, so they might provide effective alternatives for treatment. In the present study *Group B streptococci (S. agalactiae),* was fully sensitive to linezolid, vancomycin, and penicillin. This finding is constant with previously published studies [11,18,19,20]. Antibiotic over Prescribing for UTI results in the emerging of antibiotic resistant bacterial pathogens.[21] Asymptomatic bacteriuria caused by antibiotic resistant organisms is alarming and screening for asymptomatic bacteriuria during pregnancy is of great importance to avoid adverse maternal and fetal outcomes[22].

## CONCLUSION

Our data suggest that asymptomatic bacteriuria is more predominant than symptomatic bacteriuria in pregnant patients in Muscat, Oman. *E. coli, S. agalactiae*, and *E. faecalis* were the most common contributors to bacteriuria in pregnant women in Muscat, Oman. The outcomes of this investigation provide valuable understanding into the dynamics between symptomatic and asymptomatic bacteriuria in pregnancy. In addition, the present study shows the importance of frequent urine testing during pregnancy for early detection and treatment of UTI. Also, the present study shows the need for implementing new guidelines to prevent UTIs during pregnancy, with special reference to educating and encouraging pregnant women on the importance of personal hygiene in preventing UTIs during pregnancy and ultimately, preventing any adverse effect on the developing fetus.

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**Conflict of Interest**

The author(s) do not have any conflict of interest.

**Data Availability Statement**

This statement does not apply to this article.

**Informed Consent Statement**

The ethical approval for this study was obtained from Medical Research Committee, SQU.

**Clinical Trial Registration**

This research does not involve any clinical trials

**Author contributions**

Thikra Hilal Hamed Al dhuhli; Methodology, Analysis, Review; Ruwaida Nasser Abdulla AL-Lamki: Supervised-Methodology, Review; Mohamed Mabruk: Conceptualization, Design Supervision , Review-Editing.

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