PSM Biological Research ISSN(Online): 2517-9586

Research Article 2020 | Volume 5 | Issue 4 | 157-165

Article Info

Open Access

Citation: Edrees, W.H., Anbar, A.A.M., 2020. Prevalence and Antibacterial Susceptibility of Bacterial Uropathogens Isolated from Pregnant Women in Sana'a, Yemen. PSM Biol. Res., 5(4): 157-165.

Received: October 21, 2020

Accepted: October 28, 2020

Published: October 31, 2020

***Corresponding Author:**
Wadhah Hassan Edrees

Email:
edress2020@gmail.com

Prevalence and Antibacterial Susceptibility of Bacterial Uropathogens Isolated from Pregnant Women in Sana'a, Yemen

Wadhah Hassan Edrees[1,2*], Amat Alaleem Mohammed Anbar[3]

[1]Medical Microbiology Department, Faculty of Applied Sciences, Hajjah University, Yemen.
[2]Medical Laboratory Department, Faculty of Medical Sciences, Al-Razi University, Yemen.
[3]Microbiology Department, Faculty of Sciences, Sana'a University, Yemen.

Abstract:
Urinary tract infection is the commonest health problem among pregnant women, particularly in developing countries. The current study was designed to determine the prevalence and antibacterial susceptibility of bacterial uropathogens isolated from pregnant women visiting various hospitals (Al-Sabain, Zaied, and Al-Gmohori) in Sana'a, Yemen. A total of 100 specimens were collected from the midstream urine of pregnant women during the period from March to May 2018. The isolation and identification of bacteria were done following standard microbiological procedures. Antibacterial susceptibility profile was performed according to the Kirby- Bauer disc diffusion technique. The present results revealed that only 18% of urine specimens had a positive growth of bacteriuria, while 82% of specimens were negative. The highest prevalence of bacteriuria was 50% and 44.4% recorded among the age group 23–27 years and pregnant-women had a secondary degree. Also, the high frequency of bacteriuria was (61.1%) found among women with primary and second gravida compared to the low frequency (16.7%) among multigravida (>4). *Escherichia coli* was (50%) the most predominant isolate in this study followed by *Staphylococcus aureus* (27.7%), *Klebsiella* spp. (11.1%), and (5.6%) for each of Coagulase-negative *Staphylococcus* (CoNS) and *Pseudomonas aeruginosa.* The high susceptibility of antibiotics was reported; *E. coli* to ampicillin and cefepime, *S. aureus* to cefepime, ampicillin, and nitrofurantoin, *Klebsiella* spp., to cefepime and nitrofurantoin, *P. aeruginosa* to cefepime, ampicillin, vancomycin. In conclusion, antibacterial resistance profile is required to determine the effective antibiotic before prescription for pregnant women.

Keywords: Antibacterial, Bacteriuria, Pregnant women, Uropathogens, UTI, Sana'a, Yemen.

INTRODUCTION

Urinary tract infection (UTI) considers one of the commonest bacterial infections that widely prevalent with a high rate of morbidity and financial cost in developing countries (Foxman, 2002; Al-Ofairi *et al.,* 2018). It is one of the most frequent hospital-acquired infections and is also known as the second most prevalent cause of bacterial infection in hospitalized cases (Klumpp *et al*., 2006).

UTI affects all age groups particularly in females that are observed more prevalent than in males. It was reported from 50 to 70% of all women during her lifetime particularly pregnant women will acquire one UTI at least. In pregnant women, increasing of UTI infection during the pregnancy stage due to the physiological, immune system, and anatomical changes in UT. Also, old age, diabetes mellitus, sickle cell anemia, parity, UTI history, disorders of the UT, and the increase in the risk of UTI among pregnant women may be resulting from immune deficiency (Giraldo *et al*., 2012; Uddin and Khan, 2016).

However, sexual intercourse, urination after sex, diaphragm use, the indwelling of a urinary catheter, earlier surgical procedure, presence of stones in the kidney, intrauterine device, poor sanitary condition, malnutrition, and low-socioeconomic status especially in the rural areas represent the major risk factors that involved in the UTIs development (Ganeswaran *et al*., 2014; Oladeinde *et al*., 2015).

Bacteria particularly that exist in the intestinal tract, vagina, or nearby the urethra is the most causative agents of UTIs. About 70% of the UTI cases were developed by asymptomatic bacteriuria throughout pregnancy (Ullah *et al*., 2007). Undiagnosed and untreated UTI is associated with complications of maternal and perinatal consequences. The incidence of maternal consequences includes postpartum hypertensive disease, anemia, acute cystitis (40%), and acute pyelonephritis (30%), which might lead to earlier premature and delivery. Also, adverse foetal consequences include low birth weight, prematurity, and increased perinatal mortality (Masinde *et al*., 2009; Celen *et al*., 2011).

The pathogenic bacteria that responsible for pregnant women infections are similar to those present in the general population. Most bacteria infect UT among pregnant women belong to the family of *Enterobacteriaceae* that commonly present normally in the gastrointestinal tract. *Escherichia coli* was (63–85%) the most responsible bacteria for UTI followed by coagulase-negative *Staphylococcus* (>15%), *Klebsiella pneumoniae* (~8%), *S. aureus* (>8%), and group B *streptococci* (2–7%) (Sharma and Thapa, 2007; Verani *et al*., 2010; Ghaima *et al.,* 2018).

The literature on UTI such as the prevalence of bacterial isolates and antimicrobials sensitivity during pregnancy in Sana'a is limited. So, the current work designed to determine the prevalence and antibacterial susceptibility of bacterial uropathogens isolated from pregnant women in different hospitals in Sana'a, Yemen.

MATERIALS AND METHODS

Study design and study area

A cross-sectional study was conducted in various hospitals (Al-Sabain, Zaied, and Al-Gmohori) Sana'a, Yemen, during the period from March to May 2018.

Ethical approval

Approval for this study was obtained from the Ethical Review Committee in the Biology Department, Faculty of Science, Sana'a University.

Inclusion and exclusion criteria

The inclusion criteria were all pregnant mothers attended, registered, consented, and

willing to be included in the study. Also, exclusion criteria were all pregnant mothers who used antibiotics for the past seven days.

Demographic data

The needed demographic data such as age, education level, and gravida were collected using a structured questionnaire.

Sample size and sample collection

One hundred (100) patients were enrolled in this study. The clean-catch of midstream urine specimen was collected from each patient into a sterile test bottle and immediately transferred to the microbiology laboratory for examination process and preparation. A systematic random sampling method was used to select pregnant women.

Isolation and Identification of bacteria

A loop full from each urine sample was inoculated individually into a cysteine lactose electrolyte deficient agar (CLED) and incubated aerobically at 37^{o}C overnight. The result of bacteria growth of ≥ 10^5 CFU/mL of urine sample was considered as significant bacteriuria. The identification of isolated bacteria was primarily performed by colony morphology characterization, Gram staining, selective media, and biochemical reactions according to Bergey's manual of determinative bacteriology (Bergey, 1984; Don *et al.,* 2004; Leboffe and Pierce, 2011; Ali *et al.,* 2016).

Antibiotics susceptibility test

Antibiotics resistance testing for isolated bacteriuria was determined on Mueller-Hinton agar by using Kirby-Bauer disc diffusion according to the CLSI protocol (CLSI, 2016; Iqbal *et al.,* 2016; Saleem *et al.,* 2018a; Saleem *et al.,* 2018b; Shahzad *et al.,* 2017). The bacteria isolates were tested against the following antimicrobials: ampicillin (AMP; 10 µg), erythromycin (E; 15 µg), vancomycin (VA; 30 µg), cefepimee (CPM; 30 µg), and nitrofurantoin (NIT; 300 µg).

Statistical analysis

The finding data were analyzed by using a SPSS program. The Chi-square test (χ^2) and the value of probability $P \leq 0.05$ (significant) were used to compare the variables.

RESULTS AND DISCUSSION

The present results revealed that only 18% of urine specimens showed significant growth for uropathogens bacteria, while 82% of urine specimens were negative (Figure 1).

This result is in agreement with the report by Bin Hameed (2020) who showed that the frequency rate of UTI was 18% among pregnant women visiting some hospitals in Al-Mukalla city, Hadhramout/Yemen. The samples that showed negative growth might be referred to as an infection caused by fungi or parasites (Edrees *et al*., 2020).

In contrast, the high rate of bacteriuria among pregnant women in different regions in Yemen was; 24.3% in Sana'a (Alghalibi *et al*., 2007), 30.0% in Al-Mukalla (Al-Haddad, 2005), and 54.5% in Hodeidah (AL-Kadassy *et al*., 2016). Also, Ghaima *et al.* (2018) showed that the prevalence of UTIs was 31.2% reported among pregnant women in Baghdad Hospitals. The high rate in this finding may be referred to poor personal hygiene, inadequate environmental sanitation, low-socioeconomic conditions, and lake awareness about health care.

Table 1 shows that the highest rate of bacterial infection in pregnant women was 50% observed among the age group 23–27 years followed by 27.8% within age 28–32 years. While the age 33-38 years were negative for bacteriuria and there was no statistical significance between the age group and UTIs (P =0.381).

Similar finding reported by Al-Haddad (2005) who illustrated that the age groups of 15 -

24 years, young women, was (53.7%) more infected with UTI than old women. In a study by AL-Kadassy (2016) found the high prevalence of UTIs was 69% among the age 20-29 years. A study by Rohini *et al*. (2017) in India showed that the high frequency of bacteriuria was common among a group of 25- 30 years. Also, Ghaima *et al*. (2018) recorded that the age of 21-30 years was the most group infected bacteriuria with 55%.

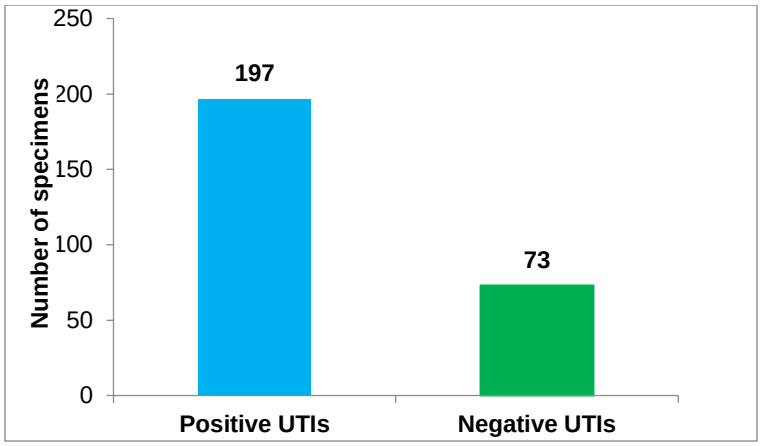

Fig. 1. Distribution of UTI among pregnant women.

Table 1. Frequency of UTIs among pregnant women according to age group.

Age (years)	UTIs	%	Non-UTI	%	χ^2	P value
18-22	4	22.2	13	15.9	3.072	0.381
23-27	9	50.0	32	39.0		
28-32	5	27.8	27	32.9		
33-38	0	0.0	10	12.2		
Total	18	100.0	82	100.0		

χ^2 Chi-square ≥3.84; *P* ≤0.05 (significant)

The results regarding education level, it was found that the highest frequency of bacteriuria was 44.4% among pregnant-women had a secondary degree, while there no recorded among pregnant women with primary education. Also, the statistical analysis showed there no statistically significant association between education level and bacterial UTI (*P* = 0.320) (Table 2).

A similar study by AL-Kadassy (2016) observed that the high prevalence of bacteriuria was 83% among pregnant women with working as a teacher. Also, Fatima *et al*. (2020) found that pregnant women at secondary school education had 61.33% bacteriuria.

Frequently UTI-associated cases belong to poor communities that lack safe water supply, lake of personal hygiene, the low literacy rate in population, and poor sanitary conditions (Fatima *et al*., 2020).

The current work found that the high frequency of bacterial UTI according to gravidity status was 61.1%, among women had from 1-2 gravid compared to the low frequency (16.7%) among multigravida (>4). There was no statistical association between the gravidity and bacterial UTI (*P* = 0.360) (Table 3).

Similar findings have been reported by Mohammad (2013) found that most pregnant women with UTI were primigravidas with non-

significant differences between infected and non-infected women. Similarly, Ranjan *et al*. (2017) observed that the highest incidence was recorded in primigravida (60%) and the lowest incidence is in multigravida (19%).

According to various studies, multigravida has an increased risk factor of developing bacteriuria among pregnant women (Mohamed *et al*., 2017; Ranjan *et al*., 2017).

Table 2. Prevalence of UTI among pregnant women in relation to education level

Level of Education	No. of examined (%)	UTIs No. (%)	χ2	P value
Illiterate	33 (33)	4 (22.2)	3.503	0.320
Primary	6 (6)	0(0)		
Secondary	39 (39)	8 (44.4)		
University	22 (22)	6 (33.3)		
Total	100 (100)	18 (100)		

χ2 Chi-square ≥3.84; P ≤0.05 (significant)

Table 3. Prevalence of UTIs among pregnant women according to gravidity

Gravidity	UTIs	%	Non-UTIs	%	χ2	P value
1-2	11	61.1	38	46.3	2.044	0.360
3-4	4	22.2	33	40.3		
More than 4	3	16.7	11	13.4		
Total	18	100.0	82	100.0		

χ2 Chi-square ≥3.84; P ≤0.05 (significant)

In the present study, it was found that the frequency of gram-negative bacteria 12(66.7%) causing UTI was higher than gram-positive bacteria 6(33.3%). *E. coli* was 9(50%) the most isolated bacteria from UTI followed by *Staphylococcus aureus* 5(27.7%), *Klebsiella* spp. 2(11.1%), and 1(5.6%) for each of Coagulase-negative *Staphylococcus* (CoNS), and *Pseudomonas aeruginosa* (Figure 2).

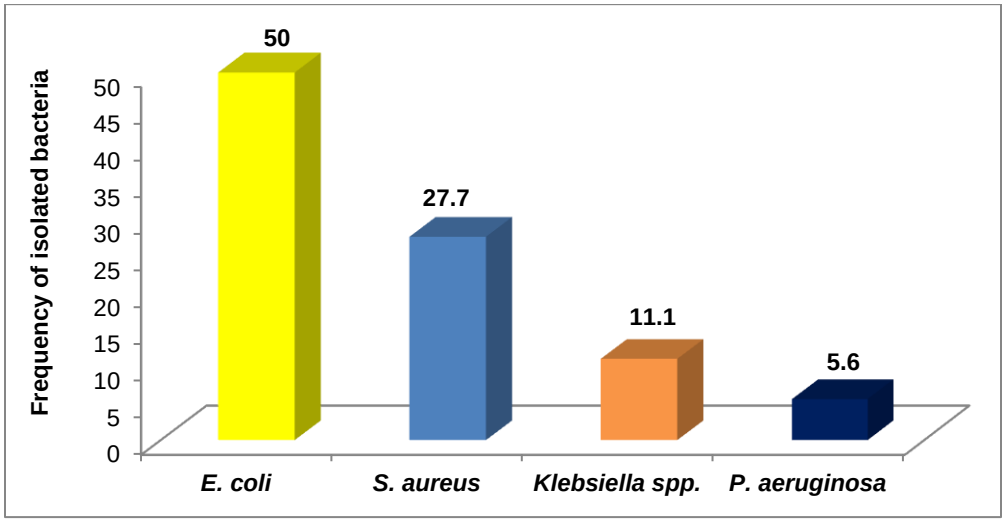

Fig. 2. Frequency of isolated bacteria from pregnant women with UTIs.

Similar studies found the *E. coli* and *Staphylococci* being the main pathogens causing UTI have been reported in other parts of Yemen. A study by Al-Haddad (2005) found that 41.5% of cases were infected *E. coli* and 19.5% by *S. aureus*. AL-Kadassy *et al*. (2016) also found that the most predominant bacteria was *E. coli* with 50.0%, followed by *S. aureus* 13.3%.

A study by Bin Hameed *et al*. (2020) reported that the most isolated pathogen bacteria was *E. coli* followed by CoNS, *Citrobacter freundii, S. aureus, P. aeruginosa, S. saprophyticus, Klebsiella pneumoniae,* and *Enterobacter* species.

However, *E. coli* is the major responsible for TUI due to several specific of virulence factors for colonization and invasion of the epithelium tissue in the urinary tract, including P and S fimbrial adhesins (Moyo *et al.*, 2010).

In the current study, the antimicrobial susceptibility results showed that the *E. coli* was found to be sensitive to ampicillin and cefepime (87.5% each), vancomycin, and (75%), and nitrofurantoin (62.5%). Similarly, *S. aureus* was sensitive to cefepime (80%), ampicillin and nitrofurantoin (60% each). Also, *Klebsiella* spp. was sensitive to cefepime and nitrofurantoin (100% each) and (50%) to ampicillin. Whereas, *S. aureus* and *Klebsiella* spp. were resistant to vancomycin and erythromycin. In addition, *P. aeruginosa* was found to be sensitive (100%) to cefepime, ampicillin, vancomycin, and nitrofurantoin, and resistant to erythromycin (Table 4).

In a similar result by AL-Kadassy *et al.* (2006) found that all bacteria isolates were susceptibility to ampicillin, gentamicin, and chloramphenicol antibiotics.

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Table 4. Antibiotics resistance pattern for isolated bacteria from pregnant women.

Bacterial isolates	CPM		Amp		VA		E		NIT	
	S	R	S	R	S	R	S	R	S	R
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
E. coli	7 (87.5)	1 (12.5)	7 (87.5)	1 (12.5)	6 (75)	2 (25)	3 (37.5)	5 (62.5)	5 (62.5)	3 (37.5)
S. aureus	4 (80)	1 (20)	3 (60)	2 (40)	1 (20)	4 (80)	1 (20)	4 (80)	3 (60)	2 (40)
Klebsiella spp.	2 (100)	0(0)	2 (100)	0(0)	0 (0)	2 (100)	1 (50)	1 (50)	2 (100)	0(0)
P. aeruginosa	1 (100)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)	1 (100)	1 (100)	0(0)
Coagulase-negative Staphylococcus	0 (0)	1(100)	0	1 (100)	0	1 (100)	0(0)	1 (100)	1 (100)	0(0)

AMP: Ampicillin, VA: Vancomycin, E: Erythromycin, CPM: Cefepime, NIT: Nitrofurantoin, S: sensitive, M: Moderate, R: Resistant

In this study, nitrofurantoin is a promise in the management of urinary tract infections during pregnancy. The antibiotic activity of nitrofurantoin corresponds to literature claims (Sheiner *et al.*, 2009). In Yemen, the misuse of antibiotics without description by physician and laboratory sensitivity test consider one of the factors that lead to an increase in bacteria resistant to common antibiotics (ALhlale *et al*., 2020).

CONCLUSION

In conclusion, the high frequency of bacteriuria during the pregnancy stage lead to life-threatening for pregnant women and their fetus. It is important to control using empiric or

non-prescribed antibiotics. Also, antibiotic susceptibility testing is very necessary to determine the effective antibiotic for bacteriuria among pregnant women without complications on both maternal and fetal health.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Medical Microbiology Department, Faculty of Applied Sciences, Hajjah University for supporting scientific research and thanks to the Medical Laboratory Department, Faculty of Medical Sciences, Al-Razi University for facilitating work in the labs.

CONFLICT OF INTEREST

There is no conflict of interest.

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