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Original article

Prevalence of urinary tract infections and antibiotic susceptibility among pregnant women

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Abstract: ***Background:*** Urinary tract infection (UTI) is a health problem that must be taken care of during pregnancy. If not treated promptly, UTIs can progress to severe and leave unpredictable complications for both mother and fetus. Currently, in Vietnam, there are few studies on UTIs in pregnancy, especially on pathogens as well as their antibiotic susceptibility characteristics. ***Objectives:*** This study was conducted to determine the bacteria rates among pregnant women with UTI symptoms and antibiotic resistance patterns. ***Methods:*** In a cross-sectional survey, a total of 178 pregnant women at Thuan Kieu General Clinic from 05/2022 to 07/2022 were recruited. Urine from these patients was cultured on BA, MC, and CHROMagarTM Orientation. ***Results:*** The infection ratio was 30.3%. The rate of Gram-positive cocci was 77.8%, and the rate of Gram-negative bacilli was 22.2%. Specifically, the highest proportion was the group of *Staphylococcus coagulase negative* with 26 samples at 46.3%; *Staphylococcus aureus* was isolated in all 8 samples at 14.8%; samples infected with *Streptococci* and *Enterococci* at 14.8% and 1.9%; 6 samples infected with *E. coli* with 11.1%; 4 samples infected by *Acinetobacter* sp with 7.4%; and the remaining were infected with *Klebsiella* sp with 3.7%. In addition, in the study, two samples of *Staphylococci resistant to Methicillin* were isolated at 3.7%. The rate of pathogenic bacteria resistant to the old single antibiotics of the β-lactam is high, while the combined antibiotics give good treatment efficiency with both negative and positive cultures. ***Conclusion:*** The rate of UTI in pregnant women is 30.3%. Pathogens mainly found include *Staphylococcus aureus, E. coli, Klebsiella sp,* and *Acinetobacter sp. E. coli* is no longer the leading cause of UTIs. As a replacement, there was an increase in Gram-positive cocci as *Staphylococci*. Recommended antibiotics for UTIs are nitrofurantoin, and β-lactam antibiotics in combination, such as amoxicillin-clavulanate, and piperacillin-tazobactam.

Keywords: Urinary tract infection, pregnant women, antibiotic susceptibility, UTIs.

1. INTRODUCTION

Urinary tract infection (UTI) is prevalent during pregnancy, detected in as many as 50-60% of all gestation [1]. UTIs include acute cystitis, pyelonephritis, and asymptomatic bacteriuria and all of them have a clinical significance to adverse maternal and fetal outcomes[2,3]. Most UTIs are diagnosed with *Enterobacteriaceae*, including *Escherichia coli* taken for 80 -90% of cases. Besides, we could find another, such as Staphylococcus aureus, Group B *Streptococcus*, or coagulase-negative *Staphylococci* in a lower ratio[4,5].

Pregnancy UTI treatment is essential when antimicrobial resistance is a global health problem, especially in developing countries like Vietnam. Also, UTI suspicion cases without

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ordering urine cultures and antibiotic susceptibility tests have been reported as one of the major causes of irrational antibiotic usage [6]. Besides sexually transmitted infections and respiratory tract infections, UTI is one of the compelling medical conditions that antibiotics should be used in pregnancy.

Therefore, screening for UTIs during pregnancy is extremely important, and antibiotic resistance should be a concern to support physicians in selecting the right antibiotic for each pregnant woman. This study aims to determine the prevalence of urinary tract infections in symptomatic pregnant women and the ratio of bacteria causing infection with antibiotic resistance patterns. In addition, this study data might support the formulation of guidelines for UTI treatment during pregnancy.

2. METHOD

2.1. Study design and participants

We performed a prospective, cross-sectional study at Thuan Kieu General Clinic in Ho Chi Minh City, Vietnam, from May to July 2022. Our study's eligible participants were pregnant women older than 18 with no mental problems and indications for culture tests and antibiograms. The pregnant women who had used antibiotics 48 hours before the screening and participants who had a re-examination were excluded from the list.

The sampling size was calculated according to the one proportion-estimation formula:

$$n \geq \frac{Z^2_{1-\alpha/2}\, p(1-p)}{d^2}$$

n: minimum measure for research

α : error type 1 (α = 0.05)

$Z_{1-\alpha/2}$: value of normal supply (Z =1.96)

p: rate of estimate in the population

d: error of estimate (d=0.05)

with p = 13.4% (according to Da Nang maternity's study[7]). So 178 was chosen as the sample size.

2.2. Data collection

After participants were assessed for initial eligibility and invited to participate in this study, pregnant women who were excluded or who withdrew from this study [7]. A mid-stream urine sample was collected from each eligible participant and transported to the microbiology laboratory for culturing. Each sample was coded with an ID, so the information of the participants was secured. Also, bias errors were avoided in collecting data. Diagnosing a urinary tract infection by the detection of the pathogen which causes clinical symptoms is the gold standard. We detected and identified the pathogen by urine culture, which also allows an estimate of the amount of bacteriuria. This study used 10^4 forming units (CFU)/mL urine as the threshold[8].

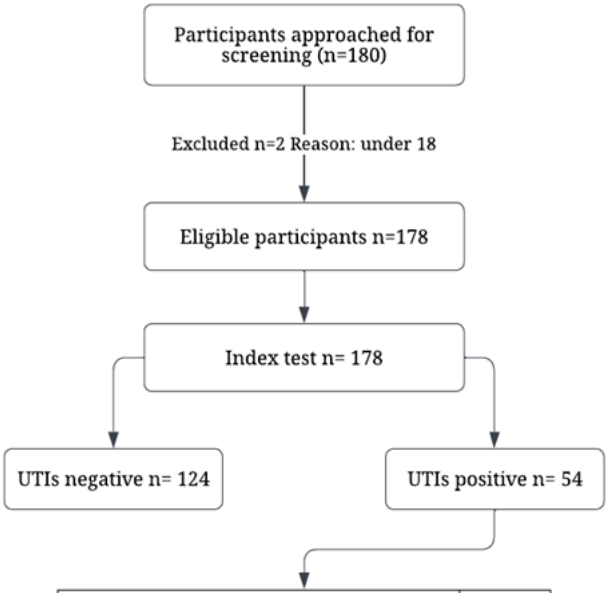
Antimicrobial susceptibility testing (AST) was performed following the guideline of Clinical and Laboratory Standard Institute M100.

2.3. Data analysis

Data were analyzed by using the Student Version of Statistical Package for Social Sciences (SPSS) version 20.0. Categorical variables were presented by frequency and percentages. The $p<0.05$ was considered statistically significant.

3. RESULTS

3.1. The prevalence of urinary tract infection



Coagulase-negative Staphylococcus	46.3%
Staphylococus aureus	14.8%
Enterococci	14.8%
E. coli	11.1%
*Acinetobacteria*sp	7.4%
*Klebsiella*sp	3.7%
β hemolytic Streptococcus	1.9%

Figure 1. Flow chart of participants

In the flow chart of participants, we could see: Between May 2022 and July 2022, a total of 180 pregnant women were assessed for initial eligibility and invited to participate in this study. The figure was shown in the flow chart of participants throughout the study. Participants who were excluded (attached the reason) are noted; there were 178 eligible participants.

The figure in Table 1 shows that of the total 178 participants screened in the study, we found that most were singleton pregnancies, and only 3% were twin pregnancies. Most participants are pregnant women aged between 25 and 34 years old (accounting for 80% of the total sample). Participants in the second trimester, from 14-27 weeks gestation, accounted for the highest rate of 91%.

The number of symptomatic pregnant women having UTIs has shown in Table 2. We can see that there were 54 participants in 178 identified as having UTIs, accounting for 30.3% total sample. Specifically, the ratio of UTI caused by

Gram-positive cocci was 77.8%, while the proportion of UTI caused by Gram-negative bacilli was only 22.2%.

Table 1. Some general characteristics of research subjects

Characteristics		Amount	Percentage
Accommodation	HCM City	160	90%
	Other regions	18	10%
Participants age	18-24	14	8%
	25-34	143	80%
	35-45	21	12%
	Over 45	0	0%
Duration of pregnancy	First trimester	10	6%
	Second trimester	162	91%
	Third trimester	6	3%
Pregnancy feature	Singleton pregnancies	172	97%
	Twin pregnancies	6	3%
Total		178	100%

Table 2. Incidence of UTI in the study

	Amount	Percentage
Positive	54	30.3%
Negative	125	69.7%
Total	178	100%

3.2. The ratio of bacteria causing UTIs

Table 3. The pattern of bacteria found in pregnant women with UTI

Bacterial	Amount	Percentage
Coagulase-negative Staphylococcus	25	46.3%
Staphylococus aureus	8	14.8%
Enterococci	8	14.8%
E. coli	6	11.1%
Acinetobacteria sp	4	7.4%
Klebsiella sp	2	3.7%
β hemolytic Streptococcus	1	1.9%
Total	54	100%

The prevalence of each bacteria is shown clearly in Table 3. Of 54 pregnancies-related UTIs, *Staphylococci* was observed to be the commonest uropathogens, accounting for 46.3%. The second most frequently detected was *Staphylococcus aureus* and *Enterococci* with both ratio are 14.8%, the next is *E.coli* with 11.1%, *Acinetobacter* accounted for 7%, *Klebsiella* sp.(4%) and the rest were *Streptococci (1.9%)*. There is not any co-infected case in this result.

3.3. The prevalence of antibiotics resistance

According to Table 4, About the antibiotics resistance prevalence of Gram-positive cocci, we can see some essential data like that:

Staphylococci has the highest rate of resistance to penicillin recorded at 72.7%, followed by trimethoprim-sulfamethoxazole at 57.6%, and in third place is erythromycin at 51.5%. On the other hand, the remaining antibiotics also have been noted for a significant rate. Some antibiotics have high sensitivity results above 90% and resistance below 10%: ampicillin, cefepime, ceftriaxone, cefotaxime, nalidixic acid, nitrofurantoin, amoxicillin-clavulanate, vancomycin.

Another Gram-positive coccus, such as *Enterococci*, shows that: there are three antibiotics with all resistance rates of 75%: tetracycline, nalidixic acid, and erythromycin; and β hemolytic *Streptococci* (only 1 sample) gives a result of 100% resistance to most antibiotics used such as cephalosporin 3rd, 4th, nitrofurantoin, nalidixic acid, chloramphenicol; but remain 100% sensitive with erythromycin, tetracycline, vancomycin, linezolid.

There are two *Methicillin-resistant Staphylococcus aureus* strains noted in our study that still show good susceptibility to most antibiotics, including those commonly prescribed for MRSA-induced UTIs in pregnant women, such as nitrofurantoin, and vancomycin.

According to Table 5, About the antibiotics resistance prevalence of Gram-negative bacilli, we can see some essential data like that:

Firstly, *E. coli* is highly resistant to ampicillin (66.7%), tetracycline (50%), and trimethoprim-sulfamethoxazole (50%). This bacterium is also well sensitive to aminoglycosides, carbapenem, nitrofurantoin, and 3,4th generation cephalosporins.

Secondly, *Klebsiella sp* is relatively antibiotic-resistant with cephalosporin 2nd,3rd, ampicillin (100%), ciprofloxacin (100%), Nalidixic acid (100%), but similar to *E. coli*, these bacteria *sensitivity* with aminoglycosides, carbapenem, nitrofurantoin, and cephalosporins 4th generation.

Another Gram-negative bacilli is *Acinetobacter sp* which shows highly resistant to nalidixic acid (80%), ciprofloxacin (60%), chloramphenicol (80%), and trimethoprim-sulfamethoxazole (40%). Penicillin, β-lactam combination, nitrofurantoin, and tetracycline group are still sensitive.

Table 4. The prevalence of Gram-positive cocci resistant to antibiotic

	Antibiotics	Percentage of resistance (%)		
		Staphylococci (n=33)	***Beta hemolytic Streptococcus (n=1)***	***Enterococcus (n=9)***
Pencillins	Ampicillin	6.1		0
	Penicillin	72.7		0
Cephalosporin	Cefepime	6.1	100.0	
	Ceftriaxone	6.1	100.0	
	Cefoxitin	36.4		
	Cefotaxime	6.1	100.0	
Tetracyclines	Tetracycline	24.2	0	75.0
	Doxycycline	15.2		25.0
Quinolones	Levofloxacin	18.2	100.0	12.5
	Ciprofloxacin	24.2		37.5
	Ofloxacin	33.3		
	Nalidixic acid	6.1	100.0	75.0
Other antibiotics	Nitrofurantoin	9.1	100.0	0
	Amoxicillin-clavulanate	6.1		0
	Trimethoprim-sulfamethoxazole	57.6		
	Erythromycin	51.5	0	75.0
	Vancomycin	6.1	0	
	Gentamycin	45.5		
	Novobiocin	3.0		
	Clindamycin	24.2	100.0	
	Chloramphenicol	24.2	100.0	0
	Linezolid	6.1	0	0

Table 5. The prevalence of Gram-negative bacilli resistant to antibiotic

	Antibiotics	Percentage of resistance(%)		
		E. coli (n=6)	***Klebsiella sp (n=2)***	***Acinetobacter sp (n=4)***
Aminoglycosid	Amikacin	0	0	20.0
	Gentamycin	33.3	0	0
	Kanamycin	0	0	20.0
	Neltimycin	0	0	0
	Tobramycin	16.7	0	20.0
Beta-lactam	Cefaclor	16.7	50.0	0
	Cefoxitin	0	0	0
	Cefuroxime	33.3	50.0	20.0
	Cefoperazone	0	0	0
	Cefotaxim	33.3	50.0	20.0
	Ceftazidime	0	0	20.0

	Antibiotics	Percentage of resistance(%)		
		E. coli (n=6)	*Klebsiella sp* (n=2)	*Acinetobacter sp* (n=4)
	Ceftriaxone	0	50.0	20.0
	Cefepime	0	0	20.0
	Ampicillin	66.7	100.0	0
	Amoxicillin-clavulanate	0	0	0
	Piperacillin-tazobactam	0	0	0
Carbapenem	Ertapenem	0	0	20.0
	Imipenem	0	0	0
Fluoroquinolone	Ciprofloxacin	0	100.0	60.0
	Levofloxacin	0	50.0	0
	Nalidixic acid	33.3	100.0	80.0
	Ofloxacin	0	50.0	0
Other antibiotics	Nitrofurantoin	0	0	0
	Doxycycline	33.3	50.0	0
	Tetracycline	50.0	50.0	0
	Trimethoprim-sulfamethoxale	50.0	50.0	40.0
	Cloramphenicol	33.3	0	80.0

4. DISCUSSION

These days, multidrug resistance bacteria are highly increasing due to over usage of antibiotics. It is very dangerous for health, especially during pregnancy. Currently, in Vietnam, studies on the epidemiology of UTIs in pregnancy are still limited despite this topic being one of the most common problems in reproductive health care. In our study, about 30% of UTI cases were recorded in symptomatic pregnant women. Specifically, pregnant women with UTIs caused by Gram-positive cocci account for a significant proportion at 77.8%, whereas the others infected by Gram-negative bacilli account for only 22.2%. It has a difference compared with two studies conducted in Indonesia and Pakistan. They had a low rate of pregnant women infected by Gram-positive cocci (about 28% and 13.5%) and the rest were directly related to the prevalence of Gram-negative bacteria at higher numbers of 77% and 86.5%, respectively[9,10]. Besides, my figure is in contrast with the research of Tu Du Hospital and Nguyen Tri Phuong Hospital in 2009. Both pieces of research showed that the ratio of symptomatic women whose UTIs were caused by Gram-positive and Gram-negative is fifty-fifty[11,12]. But then, our results were similar to the ones at Da Nang in 2022. They recorded the incidence of Gram-positive cocci at 85% and Gram-negative bacilli at only 15%[7]. In addition, the pathogenic bacteria we collected have many similarities with the results in the study in Da Nang when the ratio of CoNS *Staphylococci* and *Staphylococcus aureus* predominated in the total. That contrasts with other studies on the same topics in Indonesia and India when they reported a high prevalence of Gram-negative bacilli, specifically *E. coli* being frequently mentioned as a factor cause of urinary tract infections[7,9,13]. Parallel to that, the percentage of *E. coli* in my study is just 10%, minor compared to the result in one study by Nubia L Ramos et al. on isolating pregnant women with UTI caused by *E. coli* when their report got 42% of cases out of the total number of participants in Vietnam[14]. These differences and disparities between many countries may stem from periods, climate, and antibiotic usage. Particularly, the main reason why my result is not the same as the proportion of studies at Tu Du Hospital and Nguyen Tri Phuong Hospital in our country may be the distance of time when they started their study more than 10 years ago. Through that, our results indicate a shift in the spectrum of bacteria causing UTIs in pregnancy and need more studies with a larger scale to verify.

There is not any co-infected case in this study, which can be explained by the study's samples coming from a non-multi-research study. Moreover the participants are not in severe conditions so they choose a general clinic instead of an obstetrics hospital to take care of health.

After being diagnosed with UTI, pregnant women will be prescribed antibiotics for the treatment process. However, the selection range is already restricted due to side effects, risk of congenital abnormality, and increasing multi-resistance pathogenic bacteria strains.

Antibiotics of the β-lactam family are considered first-line antibiotics in the treatment regimen. In our study, out of 33

samples isolated with *Staphylococci*, the number of bacterial strains resistant to penicillin was up to 73.5%, indicating similar results recorded in Indonesia and India[9,13]. In contrast, the combined antibiotic amoxicillin-clavulanate gave a better effect with only about 5.9% resistance. For Gram-negative pathogens such as *E.coli*, *Klebsiella sp* has recorded high resistance to ampicillin, consistent with reports in India and South Africa[13,15]. Similarly, the study about *E. coli* in UTIs by Nubia L Ramos in 2009 also showed that multidrug resistance is more common in Viet Nam and Uganda than in other countries[14]. On the other hand, combination β-lactam antibiotics such as amoxicillin-clavulanate and piperacillin-tazobactam are sensitive to these two bacteria. *Acinetobacter* sp is a rare cause of UTI in pregnant women but has now tended to develop and increase antibiotic resistance, especially those belonging to the family of chloramphenicol. However, similarly to *E. coli* and *Klebsiella* sp., *Acinetobacter* sp in my study is still sensitive to β-lactam antibiotics in combination with amoxicillin-clavulanate and piperacillin-tazobactam. Therefore, in cases of the urgent need for treatment or the initial treatment with β-lactam antibiotics alone is not efficacious, doctors should choose combination antibiotics with high efficiency but still ensure the level of function and safety for pregnant women. On the other hand, for mothers with more severe symptoms, third and fourth-generation cephalosporins, gentamycin, and nitrofurantoin are recommended[16,17]. However, in Vietnam, the indiscriminate use of antibiotics has led to resistance to the above antibiotic groups. In our study, antibiotics belonging to the cephalosporin like cefotaxime, ceftazidime or other antibiotics such as gentamycin have recorded resistant strains of Gram-negative and Gram-positive bacteria; nitrofurantoin still records good treatment ability when there is a resistance rate of 0%. This point of the figure shows relevance to the results of other studies in Indonesia (a country in Southeast Asia that shares the same economic and climatic characteristics as Vietnam)[9]. We did not isolate ESBL strains and found two samples infected with *MRSA*. Fortunately, *MRSA* strains in our study are sensitive to most antibiotics recommended for pregnancy, including vancomycin. Nevertheless, when using the drug, we should stop doing the empirical treatment but pay attention to the antibiogram to avoid increasing other multiantibiotic-resistant strains of bacteria, such as *ESBL*, *Carbapenemase*, *VRSA*, or *Vancomycin-resistant Enterococci*.

Our study also has limitations: Due to limited time and cost constraints, the collected samples were still small compared to other studies with the same topic, and samples used in our study were not from multi-center. Therefore, our results can not be representative of all pregnant women in Vietnam. We hope to improve it in the future with a multicenter study.

Conclusion

The rate of UTI in pregnant women is 30.3%. Pathogens mainly found include *Staphylococcus aureus*, *E. coli*, *Klebsiella sp*, and *Acinetobacter sp. E. coli* is no longer the leading cause of UTIs. As a replacement, there was an increase in Gram-positive cocci as *Staphylococci*. Recommended antibiotics for UTIs are nitrofurantoin, and β-lactam antibiotics in combination, such as amoxicillin-clavulanate, and piperacillin-tazobactam.

The antibiotic resistance of bacterial strains is increasing, posing many worrying problems, especially multidrug-resistant bacterial strains. Therefore, the current treatment of UTIs requires careful attention in the selection of antibiotics to avoid increasing the incidence of resistance.

ETHICAL STATEMENT

Before being recruited into this study, participants have clearly explained the purposes, benefits, and disadvantages of pregnant women in this study and can opt out of the study. Also, we have permission from Thuận Kiều Clinic which is the only unit that provided the samples for this study. This research was approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City (approval number: 437-438 /HĐĐĐ-ĐHYD).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTION

Anh-Thu Nguyen and Van-Thanh Thi Nguyen performed the study's experiments, collected the data, and wrote the report. Bich-Ngoc Tran and Truc-Anh Thi Nguyen participated in the development of the study design, the analysis and interpretation of the data, and the writing of the report. All authors read and approved the manuscript.

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