Prevalence and Antibiogram Pattern of Bacteriuria during Pregnancy

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Abstract

Numerous studies reported that symptomatic and asymptomatic bacteriuria is common in pregnant women. It may be related with serious obstetric complications. Therefore, the current study aimed to finding the prevalence of bacteriuria, bacterial profile and antibiotic resistance pattern of uropathogens. A total of 140 pregnant women were participated in this study from November 2015 to February 2016. The result revealed that the overall prevalence of bacteriuria was 47.14%, while prevalence of bacteriuria with symptomatic and asymptomatic were (43.28%) and (50.68%) respectively, which shows that there was not statistically significant differences between the two studied groups. The pregnant women with age between 15-19 years had the highest rate of bacteriuria (85.71%). The high frequent bacteriuria also was found in third trimester (56.25%) compared with first (30.43%) and second (43.40%). *Escherichia coli* (22.73%), *Staphylococcus aureus* (19.7%), and *Staphylococcus epidermidis* (10.61%) were the predominant isolated uropathogens. Antibacterial susceptibility test was achieved for all isolated strains by the Kirby-Bauer's disk-diffusion method. Our result revealed that more than 90% of the isolates were resistant to cephalexin and amoxicillin/clavulanic acid. However amikacin was the most potent of all studied antibiotic 77.3% of uropathogens were sensitive to it. The resistance rates to ≥3 antimicrobial agents was 98.5% while, only 1.5% were resist to all antimicrobial tested.

Keywords: Asymptomatic bacteriuria, symptomatic bacteriuria, pregnancy, antibiogram

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انتشار واختبار تحسس البيلة الجرثومية للمضادات الحيوية اثناء الحمل

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الخلاصة

عدة دراسات ذكرت ان البيلة الجرثومية ذات الاعراض وبدون اعراض شائعة في النساء الحوامل، قد تترافق هذه مع مضاعفات الولادة. وبالتالي، فإن هذه الدراسة تهدف إلى تحديد مدى انتشار البيلة الجرثومية، لمحة بكتيرية ونمط المقاومة للمضادات الحيوية من البكتيريا الممرض للمجارى البولية. تم تسجيل مجموع 140 امرأة حوامل في هذه الدراسة من نوفمبر 2015 إلى فبراير 2016. وكشفت النتيجة أن معدل انتشار البيلة الجرثومية كان 47.14٪، بينما بلغ معدل انتشار البيلة الجرثومية ذوات أعراض وبدون أعراض (43.28٪) و (50.68٪) على التوالي، مما يدل على أنه لا توجد فروق معنوية بين المجموعتين. وكان للنساء الحوامل مع التقدم في العمر بين 15-19 سنة أعلى معدل للبيلة الجرثومية (85.71٪). و البيلة الجرثومية ذو المتكررات العالية وجد أيضا في الثلث الثالث (56.25٪) مقارنة مع الأولى (30.43٪)، والثانية (43.40٪) للحمل. نسبة بكتيريا *Escherichia coli* (22.73٪)، *Staphylococcus aureus* (19.7٪)، *Staphylococcus epidermidis* (10.61٪) كانوا من العزل السائدة. تم إجراء اختبار الحساسية المضادة للبكتيريا لجميع السلالات المعزولة بطريقة القرص نشر كيربي باور. كشفت لنا النتيجة أن أكثر من 90٪ من العزلات مقاومة للسيفالكسين وأموكسيسيلين / حمض كلافولانيك. ومع ذلك كان أميكاسين الأكثر فعالية من كل المضادات الحيوية المدروسة 77.3٪ من العزلات كانت حساسة لذلك. وكانت معدلات مقاومة لـ ≤ 3 من مضادات الميكروبات 98.5٪ في حين كانت 1.5٪ فقط مقاومة لجميع المضادات الحيوية المستخدمة التي تم اختبارها.

الكلمات المفتاحية : البيلة الجرثومية ذات الاعراض، بدون اعراض، الحوامل، المضادات الحيوية.

Introduction

The physiological and anatomical variations that happen throughout the pregnancy are the predisposing factors for urinary tract infection (UTI) in healthy women (1). As well as, reduction of immunity in pregnant women also enhances the growth of microorganisms (2). Among pregnant women, UTI can be categorized into asymptomatic and symptomatic

bacteriuria (SB) (3). Asymptomatic bacteriuria (ASB) is referred to significant growth of bacteria with no symptoms of an acute UTI (4), whereas symptomatic UTI are divided into cystitis which is a symptomatic significant bacteriuria associated to inflammation of bladder and pyelonephritis which means symptomatic significant bacteriuria with related inflammation of the renal parenchyma, pelvis, and calices (5). The causative agents of UTI are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, *S. saprophyticus*, *S. haemolyticus*, *S. epidermidis*, *Streptococcus agalactiae*, *Proteus mirabilis*, and *Streptococcus faecalis*, *Enterobacter spp* (6, 7), and they are variable according to geographical location (8).

Untreated asymptomatic bacteriuria is considered as a factor that induce the symptomatic cystitis (30%) and pyelonephritis (50%) in pregnancy (9). This can lead to retardation of intrauterine growth, infant birth with low weight, premature delivery, and sometimes stillbirth (10, 11). Therefore, periodic checking of pregnant women is very important in order to prevent complications. Thus, this study aimed to evaluate the prevalence of bacteriuria among pregnant women and identify the bacterial profile, as well as their antibiogram patterns.

Materials and Methods

Study population

A study was conducted for a period of four months from November 2015 to February 2016 at both Sheray Naqeeb Maternity Hospital and Sherwana Health Centre, Kalar, Kurdistan region, Iraq. Pregnant women regardless symptoms of UTI between the ages of 15 to 43 years with no gestational age limits were included, while those who received antibiotics therapy within at least 72 hours and non-pregnant women were excluded in this study. Information about age, gestation period and symptoms of UTI was gained from each pregnant woman by using standard questionnaires and saved trustworthy during the research.

Sample collection and bacterial identification

A total of 140 mid-stream urine samples were collected aseptically into sterile not reusable plastic containers. The specimens were labelled and transported to the microbiological laboratory for further management. Using a standard wire loop to inoculate 1µl of

uncentrifuged urine specimen into Nutrient agar, MacConkey agar, Blood agar and. All seeded plates were incubated at 37°C for 18-24 hours. Significant ASB and SB in pregnant women were defined by $\geq 10^5$ colony forming units (CFU) per mL of urine (12). The isolates were identified by cultural characteristics, gram stains, and biochemical testes (13, 14). BIOMÉRIEUX VITEK 2 system was used for confirmation the identification of some isolates. PCR, as gold standard identification, was used for amplification 16S rRNA of one Gram negative bacteria (*E. coli*) and Gram positive bacteria (*S. aureus*) with specific primer (Table, 1).

DNA Extraction

Genomic DNA was extracted from clinical isolates of *S. aureus* and *E. coli* using boil preparation method.

Primers and PCR assay

The used primers (cinagen) in this study, which described in table-1, were designed depending on the complete annotation sequence of *S. aureus* and *Escherichia coli* supplied by the National Centre for Biotechnology Information (Accession No. BA000033.2; Accession No. J01859.1). The PCR assay was performed in 25µl final reaction volume and mixtures were spun down shortly for 5-10 seconds then placed in thermal cycler (TCY, Crealcon, NL) and subjected to the following cycling conditions: initial denaturation at 94°Cfor 4minutes, followed by 35 cycle of denaturation at 94°Cfor 30 seconds, annealing at 55°C (56°C) for 1minute and extension at 72°C for 2minutes and a final extension step at 72°C for 5minutes. The amplified DNA samples were observed in agarose gel electrophoresis.

Table (1): Primer sequences, product size and annealing temperature.

Bacteria	Primers	Sequence primers (5′-3′)	Product size(bp)	Ann. temp.
Escherichia coli	16SrRNA-F 16SrRNA-R	GAAGACTGACGCTCAGGTGCGAA CCGTGGCATTCTGATCCACGATTA	627	55°C
S. aureus	16SrRNA-F 16SrRNA-R	GCATGGTCATCACTTAAGTAATACGGA CTCTCGTATGACCAGCTTCGGTACTACT	216	56°C

Antibiotic susceptibility testing

Antimicrobial susceptibility test was carried out for all the isolated strains by the Kirby-Bauer's disk-diffusion method (15) as suggested by Clinical and Laboratory Standard Institute (16). Mueller-Hinton agar plates were used for assay of 13 different antibiotics (produced by Bioanalyse, Turkey), including vancomycin (10µg), amikacin (30µg), amoxicillin (25µg), amoxicillin/clavulanic acid (30µg) gentamicin (10µg), rifampin (5µg), azithromycin (15µg), cephalexin (30µg), trimethoprim (10µg), nalidixic acid (30µg), nitrofurantoin (100µg), tetracycline (10µg), and penicillin G (10µg). Reference strains of *E. coli* ATCC 25922 and *Staph. aureus* ATCC 25923 were used as quality control measure for both identification criteria and antimicrobial susceptibility tests.

Statistical analysis

All outcome data were analyzed using Statistical Package for Social Sciences (SPSS; Version18.0). Probability values of ($P < 0.05$) were considered as statistically significant.

Results and Discussion

Prevalence of bacteriuria

During the period of study, a total of 140 pregnant women regardless symptoms of UTI were investigated. Sixty six (66) had significant bacteriuria, the overall prevalence of UTI was 47.14 % (Table, 2). The high prevalence of bacteriuria may be due to increasing the level of progesterone during pregnancy, and mechanical pressure of the gravid uterus to the distal ureters causes urinary stasis so enhance bacterial colonization (17). Although about (70%) of those developed glycosuria which is induce the bacterial growth (18).

Our results is confirmed by Okonko *et al.* (2009) in which the UTI prevalence (47.5%) in between pregnant women in Nigeria (19), while higher than the prevalence rate (14.0%, 14.6 % and 16.4%) that reported by Hamdan *et al.*, (2011) (20) , Masinde *et al.*, (2009) (21) and Olsen *et al.*, (2000) (22) respectively, but less than (49.4%, 54.0% and 58%) were reported by Manjula *et al.(*2013), Obiogbolu *et al*., (2009) and Onifade *et al*. (2005) (23, 7, 24).

Prevalence of bacteriuria according to symptoms

The prevalence of bacteriuria with symptomatic and asymptomatic in pregnant women were (43.28%) and (50.68%) sequentially, which shows that there was not statistically significant between the two studied groups, because of the high prevalence ASB, a routine screening for pregnant women should be done in order to avoid complications which has been reported by Mc Isaac *et al.* (2005) (25). This result was in contrast with those observed by Masinde *et al*. (2009) (21) in which the prevalence of SB and ASB among pregnant women were 17.9% and 13.0% respectively, while Amadi *et al*. (2007) (26) who reported 78.7% had ASB. This variation may be attributed to socio-economic status, quality of medical care, age, the standard of personal hygiene and education (27, 28) In addition anaemia, sexual activity and catheterization are also related with increased probability of bacteriuria (29).

Table 2: Prevalence of SB and ASB in pregnant women

Types of bacteriuria	Significant bacteriuria		Total No. (%)
	Positives No. (%)	Negative No. (%)	
Symptomatic bacteriuria	29(43.28%)	38(56.72%)	67(47.86%)
Asymptomatic bacteriuria	37(50.68%)	36(49.32%)	73(52.14%)
Total	66(47.14%)	74(52.86%)	140(100%)
***P* value**	**0.381** ^{NS}		

Prevalence of bacteriuria according to age group

Table (2) declared the prevalence of bacteriuria associated with age group of participated, pregnant women of the age group15-19 years had highest rate of bacteriuria (85.71%).

Followed by age group 40-44 years (57.14 %), 25-29 years (50%) and 30 -34 years (44.44%), 20-24 years (42.11%). While the age group 35- 39 years had the lowest incidence of infection (30.77%). In general, there was not statistically significant between the age groups of pregnant women and prevalence of bacteriuria (*P* value > 0.05), while the highest incidence of bacteriuria within age groups 15-19 years may be due to sexual activity which rise the possibilities of bacterial infection of female urethra, Thakre *et al*., (2005) (30) also observed that pregnant women who had recent sexual intercourse ≥3 times/week were more prone to UTI than those who had less than < 3 times/week.

Table 3: Prevalence of bacteriuria associated with age group in pregnant women

Age group	Total Number	Significant bacteriuria	
		Positives No. (%)	Negatives No. (%)
15-19 years	7	6(85.71)	1(14.29)
20-24 years	38	16(42.11)	22(57.89)
25-29 years	48	24(50)	24(50)
30-34 years	27	12(44.44)	15(55.56)
35-39 years	13	4(30.77)	9(69.23)
40-44 years	7	4(57.14)	3(42.86)
Total	**140**	**66(47.14)**	**74(52.86)**

Prevalence of bacteriuria according to the gestational age

The result in Table (4) shows the prevalence of bacteriuria according gestational age, pregnant women in 3rd trimester had highest prevalence rate (56.25%) followed by 2nd trimester (43.40%), while women in 1st trimester had the lowest prevalence (30.43%). The statistical analyses show significant differences between 1st trimester and 3rd trimester. Our finding in accord with those of Nworie and Eze (2010) (31) and Okonko *et al.*, (2009) (19), they found an increased incidence rate of UTI in third trimester compared to the first and

second trimester, this difference may be as a result of urethral dilatation which begins as early as 6 weeks and up to the maximum during 22-24 weeks (18).

Table 4: Prevalence of bacteriuria associated with gestational period.

Trimester period	Total Number	Significant bacteriuria	
		Positives No. (%)	Negatives No. (%)
First trimester	23	7(30.43)	16(69.57)
Second trimester	53	23(43.40)	30(56.60)
Third trimester	64	36(56.25)	28(43.75)
Total	**140**	**66(47.14)**	**74(52.86)**

Bacterial profile and Antibiotic susceptibility test

A total of 66 (34 from asymptomatic and 32 from symptomatic) isolates were identified (Table 5). The most predominant isolate was *E. coli* 15(22.73%), followed by *Staph. aureus* 13(19.7%), *staph. epidermidis* 7(10.61%), *Staph. hemolyticus* 5(7.576%), *K. pneumonia* 5(7.576%), *Staph. saprophyticus*4(6.061%), *P. aeruginosa* 4(6.061%), *M. varies* 3(4.545%), *P. vulgaris* 2(3.03%), *C. koseri* 1(1.515%), *Serratia marcescens* 1(1.515%), *Enterobacter spp*. 1(1.515%), *M. morganii* 1(1.515%), *P. mirabilis* 1(1.515%), *Strep. agalactiae* 1(1.515%), *M. luteus* 1(1.515%), *S. typhimurium* 1(1.515%) The high frequently of *E. coli* also reported in many previous studies in different part of the world (11, 21, 32). The higher prevalent of *E. coli* may be attributed to the reduced genital sanitation by pregnant women who may find it difficult to clean their anus correctly after defecating or clean their genital after urination during their pregnancy (33) , mechanical effect of sexual intercourse facilitate the introducing of *E. coli* into the urethra and bladder, also sexual intercourse changes the normal lactobacillus dominant vaginal flora and encourage the growth *E. coli* in the vagina (34).

Table 5: Bacteria species isolated from the urine of pregnant women with ASB and SB.

No.	Bacteria	ASB(%)	SB(%)	Total (%)
1	*Escherichia coli*	5(33.33)	10(66.67)	15(22.73%)
2	*Staphylococcus aureus*	6(46.15)	7(53.85)	13(19.7%)
3	*Staphylococcus epidermidis*	2(28.57)	5(71.43)	7(10.61%)
4	*Staphylococcus hemolyticus*	5(100)	0(0.0)	5(7.576%)
5	*Klebssiella pneumoniae*	3(60)	2(40)	5(7.576%)
6	*Staphylococcus saprophyticus*	0(0.0)	4(100)	4(6.061%)
7	*Pseudomonas aeruginosa*	3(75)	1(25)	4(6.061%)
8	*Micrococcus varies*	2(66.67)	1(33.33)	3(4.545%)
9	*Proteus vulgaris*	2(100)	0(0.0)	2(3.03%)
10	*Citrobacter koseri*	1(100)	0(0.0)	1(1.515%)
11	*Serratia marcescens*	1(100)	0(0.0)	1(1.515%)
12	*Enterobacter spp.*	1(100)	0(0.0)	1(1.515%)
13	*Morganella morganii*	1(100)	0(0.0)	1(1.515%)
14	*Proteus mirabilis*	1(100)	0(0.0)	1(1.515%)
15	*Streptococcus agalactiae*	1(100)	0(0.0)	1(1.515%)
16	*Micrococcus luteus*	0(0.0)	1(100)	1(1.515%)
17	*Salmonella typhimurium*	1(100)	0(0.0)	1(1.515%)
	Total	**34(51.52%)**	**32(48.48%)**	**66(100%)**

The resistant of uropathogen to most commonly used antibiotics make physicians with narrow spectrum antibiotic for treatment of UTI (35). The *in-vitro* antimicrobial resistance pattern of the isolates against 13 different antibiotics is shown in Table 5. Our result revealed that more than 90% of the isolated strains were resistant to amoxicillin/clavulanic acid and cephalexin. However amikacin was the most potent of all studied antibiotic as 77.3% of uropathogens showed highly degree of sensitive to it. The resistance rates to ≥3 antimicrobial was 98.5% while, only 1.5% were resist to all antimicrobial tested. This designates that multi drug resistance was found to be more to the frequently used antibiotics. Consequently, the object for this alarming phenomenon might be administration of incorrect or abuse of antibiotics for therapies and absence of suitable infection control strategies, which lead to rise the prevalence rate of resistant microorganism in the community.

Conclusion

It can be concluded that the prevalence of ASB and SB bacteriuria among pregnant women were (43.28%) and (50.68%) respectively. The Prevalent organisms were still *Escherichia coli* but lower percentage than observed by previous researchers. *Staph. aureus* was a second predominant isolates. Most of isolated strains were sensitive to amikacin and azithromycin, in contrast more than 80% of isolates were resistant to amoxicillin-clavulanic acid, cephalexin, amoxicillin, penicillin G, nitrofurantoin. Hence, urine culture should be done periodically so as to reduce bacteriuria and multi-drug resistance bacteria, as well as prevent misuse or overuse of antibiotics.

Table 6 : Antimicrobial resistance pattern among isolated uropathgen from pregnant.

Isolated Bacteria	No.	AK(%)	AX	TMP	F	CN	AMC	NA	CL	RA	AZM	TE	VA	P
Escherichia coli	15	14(93.3)	14(93.3)	8(53.3)	8(53.3)	6(40)	14(93.3)	10(66.7)	15(100)	11(73.3)	3(20)	5(33.3)	13(86.7)	13(86.7)
Staphylococcus aureus	13	3(23.1)	10(76.9)	7(53.9)	13(100)	10(76.2)	12(92.3)	11(84.6)	12(92.3)	9(69.2)	4(30.8)	8(61.5)	12(92.3)	11(84.6)
Staphylococcus epidermidis	8	8(100)	6(75)	4(50)	5(62.5)	4(50)	8(100)	6(75)	6(75)	1(12.5)	3(37.5)	5(62.5)	5(62.5)	6(75)
Staphylococcus hemolyticus	5	0(0)	4(80)	2(40)	4(80)	1(20)	5(100)	4(80)	4(80)	2(40)	2(40)	2(40)	0(0)	4(80)
Klebssiella pneumoniae	4	0(0)	4(100)	0(0)	3(75)	3(75)	4(100)	1(250	4(100)	3(75)	3(75)	2(50)	4(100)	4(100)
Staphylococcus saprophyticus	4	1(25)	3(75)	2(50)	3(75)	2(50)	2(50)	2(50)	3(75)	3(75)	2(50)	2(50)	3(75)	3(75)
Pseudomonas aeruginosa	4	0(0)	4(100)	4(100)	4(100)	1(25)	4(100)	3(75)	4(100)	4(100)	1(25)	3(75)	4(100)	4(100)

Micrococcus varies	3	0(0)	2(66.7)	3(100)	3(100)	0(0)	3(100)	2(66.7)	3(100)	2(66.7)	1(33.3)	1(33.3)	1(33.3)	1(33.3)
Proteus vulgaris	2	0(0)	2(100)	2(100)	2(100)	0(0)	2(100)	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)	2(100)
Citrobacter koseri	1	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)
Serratia marcescens	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	0(0)	(0)	1(100)	1(100)
Enterobacter spp.	1	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)	1(1000	1(100)
Morganella morganii	1	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)
Proteus mirabilis	1	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)
Streptococcus agalactiae	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
Micrococcus luteus	1	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)
Salmonella typhimurium	1	0(0)	1(100)	0(0)	1(100)	0(0)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	0(0)	1(100)
Total	**66**	**28**	**55**	**36**	**53**	**30**	**61**	**46**	**60**	**41**	**24**	**32**	**51**	**55**

AK: Amikacin, Ax: Amoxicillin, TMP: Trimethoprim, F: Nitrofurantoin, CN: Gentamicin, AMC: Amoxicillin-clavulanic acid, NA: Nalidixic acid, CL: Cephalexin, RA: Rifampin, AZM: Azithromycin ,TE: Tetracycline, VA: Vancomycin , P: Penicillin

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