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Antibiotic Sensitivity of Microbial Isolates Causing Asymptomatic Bacteriuria During Pregnancy, in General Heet Hospital, Western Iraq

Gebelik Sırasında Asimptomatik Bakteriüriye Neden Olan Mikropların Antibiyotik Duyarlılığı, Genel Heet Hastanesi, Batı Irak

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ABSTRACT

The goal of this work is to determine the prevalence of asymptomatic bacteriuria (ASB) during pregnancy, identify the causative organisms, and analyze the antibiotic susceptibility pattern of the isolates. This study was conducted on 139 pregnant women in Iraq. Clinically, all the women had no symptoms of a urinary tract infection. Clean catch midstream urine samples were collected from all patients. The microscopic and cultural methods were used to check all urine samples. Identification of isolates was performed using the VITEK 2 system and antibiotic sensitivity was assessed using the same technique. The results showed that 70 (50.36%) of the 139 pregnant women tested positive for ASB. The age group of 15-20 years had the highest prevalence (60.86%) of ASB. The most common etiological agent causing infections among pregnant women was *Staphylococcus* species (66.65%), followed by *Escherichia coli (E. coli)* (10.52%), and *Klebsiella pneumoniae (K. pneumoniae)* (8.77%). Also, the susceptibility pattern of *E. coli* and *K. pneumoniae* showed that most of the isolates were highly sensitive (100%) to piperacillin/tazobactam, ertapenem, imipenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, and tigecyclin. *E. coli* isolates were highly sensitive to cefoxitin (100%). Coliforms *(E. coli and K. pneumoniae)* were highly resistant to β-lactams, including : ampicillin (100%), ceftazidime (100%), cefazolin (100%), ceftriaxone (100%), and ciprofloxacin (100%). Based on the resistance profiles, all isolates of *K. pneumoniae* and *E. coli* (100%) were extended-spectrum beta-actamase producers.

Keywords: Antibiotic susceptibility test, asymptomatic bacteriuria, antibacterial agent, urinary tract infection, pregnancy

ÖZ

Bu çalışmanın amacı, gebelik sırasında asemptomatik bakteriüri (ASB) prevalansını belirlemek, neden olan organizmaları tanımlamak ve izolatların antibiyotik duyarlılık desenini analiz etmektir. Bu çalışma, Irak'ta 139 gebe kadın üzerinde yapılmıştır. Klinik olarak, tüm kadınlarda idrar yolu enfeksiyonu belirtileri bulunmamaktadır. Tüm hastalardan temiz orta akış idrar örnekleri alınmıştır. Mikroskobik ve kültürel yöntemler kullanılarak tüm idrar örnekleri incelenmiştir. İzolatların tanımlanması VITEK 2 sistemi ile yapılmış ve antibiyotik duyarlılığı aynı teknikle değerlendirilmiştir. Sonuçlar, 139 gebe kadından 70'inin (%50,36) ASB için pozitif sonuç verdiğini göstermektedir. On beş ila yirmi yaş grubundaki kadınlar, en yüksek prevalansa (%60,86) sahipti. Gebelerde enfeksiyonlara neden olan en yaygın etyolojik ajan Staphylococcus türleri (%66,65) olup, bunu *Escherichia coli (E. coli)* (%10,52) ve *Klebsiella pneumoniae (K. pneumoniae)* (%8,77) izlemektedir. Ayrıca, *E. coli* ve *K. pneumonin*'nin duyarlılık deseni, izolatların çoğunun piperacillin/tazobactam, ertapenem, imipenem, amikasin, gentamisin, siprofloksasin, levofloksasin ve tigeciklin'e yüksek derecede duyarlı olduğunu (%100) göstermektedir. *E. coli* izolatları, sefoxitine karşı da yüksek derecede duyarlıdır (%100). Koliformlar (*E. coli ve K. pneumoniae*) β-laktamlara karşı yüksek derecede dirençlidir; bunlar arasında ampisilin (%100), seftazidim (%100), sefazolin (%100), seftriakson (%100) ve siprofloksasin (%100) yer almaktadır. Direnç profillerine dayanarak, tüm *K. pneumoniae ve E. coli* izolatlarının (%100) geniş spektrumlu beta-laktamaz üreten organizmalar olduğu bulunmuştur.

Anahtar Sözcükler: Antibiyotik duyarlılık testi, asemptomatik bakteriüri, antibakteriyel ajan, idrar yolu enfeksiyonu, gebelik

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INTRODUCTION

Urinary tract infections (UTIs): "the colonization and multiplication of bacteria in the urinary tract". The presence of bacteria in the collected urine samples is called bacteriuria (1). Moreover, the genitourinary tract is sterile in normal circumstances. Bacteriuria appears when bacteria from a faecal reservoir enter the bladder via urethral ascent (2). In pregnant women, as in women who aren't pregnant, UTIs are classified either as symptomatic infections (acute pyelonephritis and acute cystitis) when bacteria invade urinary tract tissues involving an inflammatory response, or asymptomatic bacteriuria (ASB) when the infection is limited to the growth of bacteria in urine (in general, defined as true bacteriuria in the absence of specific symptoms of acute UTI) (3).

ASB is one of the common infectious conditions in pregnancy that requires antibiotic treatment. Growth above 10^5 colony forming units (CFU) in a midstream urine culture taken from an individual who does not exhibit symptoms or indications of bacteriuria is referred to as ASB (4).

Among active, premenopausal women, the prevalence of ASB is 2-10% (3,5). However, pregnancy-related physiological and anatomical changes to the urinary tract, like displaced bladders, along with immune system modifications, raise the prevalence of ASB and can occasionally result in symptomatic UTIs and other pregnancy complications (such as preterm delivery and pyelonephritis). This matter puts the fetus, newborn, and mother at grave risk (5,6). Pregnant women may be more susceptible to UTIs due to parity, aging, diabetes, urinary tract diseases, sickle cell anemia, and a history of UTIs (6,7).

Preterm labor, pyelonephritis, anemia, low birth weight, amnionitis, toxic septicemia, bacteremia, and pre-eclampsia are some of the issues that can arise from bacteriuria during pregnancy if antibiotics are not administered (6,7). However, therapy for bacteriuria during pregnancy decreases the complications risk. Thus, during pregnancy, the treatment of bacteriuria and screening for early diagnosis in women is essential for preventing the complications associated with bacteriuria (8).

The bacteriuria spread in Iraqi pregnant women was observed to be 48-64.6% (9,10). These percentages are close to the results of our current study. Determining the frequency of UTI, ABS, and the most prevalent pathogenic bacteria is, therefore, an important diagnostic tool in a variety of countries.

This study aims to evaluate the spread of UTI, ASB, and infections associated with bacteriuria in pregnant women in Heet, Iraq. It was also done to determine patterns of bacteria isolated from UTIs and to test antibiotic susceptibility to see which antibiotics might prevent the bacterial isolates that cause ASB in pregnant women from growing.

MATERIALS AND METHODS

Urine Specimen Collection

An appropriate number of 139 pregnant women participated in this investigation who visited the basic care facilities, and admitted to General Heet Hospital's general wards, Anbar, Iraq, who consented to provide us with your data for the current study between October 1, 2021, and March 30, 2021, a period of six months.

Each woman was interviewed for about 15 minutes. The prepared questionnaire was used to interview the pregnant women and obtain data. Nevertheless, the current questionnaire included the following variables: general urine examination, patient complaint, medical and obstetrical history, maternal socio-demographic factors, and acute UTI symptoms. Those taking antibiotics or having taken them for at least 14 days before the presentation were not included in this study. Moreover, the information for each case was recorded in a form prepared for this purpose.

In the current work, the samples of midstream urine were collected from (139) pregnant women a mean age 25.97 ranges (15-40 years),(apparently healthy without any symptoms or signs of UTI), attended the General Heet Hospital were looked at for possible ASB. Moreover, all urine samples were gathered and submitted for regular urinalysis, Gram stain, dipstick, bacterial culture, and urine microscopy.

Asymptomatic Bacteriuria Diagnosis and Culture Test

Un-centrifuged urine samples were incubated at 37°C for 24 hours. For ASB, bacterial growth exceeding 105 CFU/mL was deemed significant. However, the bacterial growth less than 105 CFU/mL was considered contaminated. However, the sample was confirmed negative for ASB if no growth occurred. (ASB is growth of more than 100000 CFU/mL in the bacterial culture of the sample of midstream urine).

Diagnosis Isolation and Antibiotic Susceptibility Test

All isolates were bacteriologically identified, and antimicrobial susceptibility test was investigated using the VITEK 2 system, as mentioned by bioMérieux (11).

Ethical Aspects

The University of Anbar's College of Medicine, the Ethical Committee, examined and approved the study's current protocol. This study was conducted in accordance with the ethical standards of the University of Anbar (approval number: 21, date: 18.03.2025) by the Ethics Committee. Informed consent was obtained from all participants, ensuring they were fully aware of the study procedures, risks, and benefits before voluntarily agreeing to participate.

Statistical Analysis

A p-value of less than 5% was used as the threshold for statistical significance. The goodness of fit test, utilizing the chi-square test (cross tabulation), within non-parametric statistics, was used to determine if there were any significant differences. The chi-square test was utilized to examine the differences between the two test types (the antibiotic types used by the VITEK 2 system).

Results

Severe maternal illness and perinatal morbidity are caused by ASB during pregnancy. This morbidity can be decreased by giving appropriate treatment, and the screening of pregnant mothers. In the current work, the incidence of ASB was 50.36% (Figure 1).

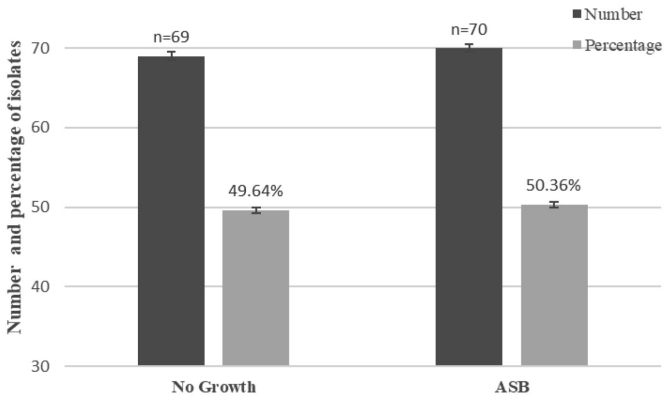

Figure 1. Asymptomatic bacteriuria (ASB) in pregnancy. Microbial growth >105 CFU/mL was considered significant for ASB (total n=139).

As per this work, there was a greater incidence of ASB in the first trimester of pregnancy, 66.66% (8/12 isolates), then 62.96% (17/27 isolates) in the second trimester, and while 42.66% (32/75 isolates) were in the third trimester (Table 1). The 15 to 20 years age group (60.86%) had the greatest rate of ASB (Table 2). This may be because women in this age range engage in sexual activity.

The results of the current research exhibited that species of *Staphylococcus (S.)* (66.65%) were the most frequently occurring causative agents of infection among pregnant women, followed by *Escherichia coli (E. coli*) (10.52%) (Table 3).

In this work, *E. coli* was the second most common (10.52%) bacterium after *Staphylococcus* ssp. (Table 3). Numerous research studies were published. *E. coli* was the most common bacterium found in pregnant women's urine cultures (27,28,29). Moreover, the majority of bacteria in the vaginal and rectal areas are *E. coli*.

The other isolated strains were *Klebsiella pneumoniae* (*K. pneumoniae*) (8.77%), *Kocuria* spp. (3.5%), *Aeromonas hydrophila* (1.75%), *Enterococcus faecium* (1.75%), *Sphingomonas pancimobilis* (1.75%), *Dermacoccus nishinomiyaensis* (1.75%), Mixed Growth [*Staphylococcus hominis* (*S. hominis.)* + *Aerococcus viridans*] (1.75%). Only *Candida albicans* (1.75%) was the fungus that was isolated in this study (Table 3).

Antimicrobial Susceptibility Pattern of Staphylococcal Species Isolates

Our study revealed that linezolid (100%), vancomycin (100%), nitrofurantoin (100%), tigecycline (96.96%), clindamycin (93.93%), teicoplanin (93.93%), rifampicin (93.93%), tobramycin (87.87%), gentamicin (84.84%), levofloxacin (75.75%), moxifloxacin (75.75%) and trimethoprim/sulfamethoxazole (72.72%) were relatively effective antibiotics against *Staphylococcus* uropathogen species (Table 4).

However, isolates of *Staphylococcus* spp. were highly resistant to the β-lactam class [benzylpenicillin (100%), oxacillin (93.93%)], and fusidic acid (78.78%). low resistance to both erythromycin (66.66%) and tetracycline (42.42%) was identified (Table 4). Out of all *Staphylococcus* spp. isolates (n=33), multi-drug resistance (MDR) was recorded for 25 (75.75%) isolates (Table 5).

Pattern of Antimicrobial Susceptibility of Klebsiella pneumoniae and E. coli isolates

In this study, the susceptibility pattern of *K. pneumoniae* and *E. coli* exhibited that most of the isolates were highly sensitive to piperacillin/tazobactam (100%), ertapenem (100%), imipenem (100%), amikacin (100%), gentamicin (100%), ciprofloxacin (100%), levofloxacin (100%), and tigecyclin (100%) (Table 5). Also, *E. coli* isolates were highly sensitive to cefoxitin (100%), (Table 5). In addition, the sensitivity level of *E. coli* and *K. pneumoniae* bacteria to gentamicin, nitrofurantoin, and trimethoprim/sulfamethoxazole was (83.33%, 60%), (66.66%, 60%), and (66.66%, 60%), respectively (Table 5). Likewise, *K. pneumonia* isolates were moderately resistant to cefoxitin (60%) (Table 5).

As a result, this research is required due to the bacteria isolates identified in this study, which have shown high resistance to the majority of routinely used antimicrobial drugs. This follow-up study could explain the molecular foundation for bacteria developing resistance to highly effective antibacterial agents.

Discussion

Severe maternal illness and perinatal morbidity are caused by ASB during pregnancy. In the current work, the incidence of ASB was 50.36% (Figure 1). This result agreed with similar research done in Iraq, Saudi Arabia, and Nigeria where the incidence of ASB was reported from 48-64.6% (7-10,12).

The slight variation in the ratio between the studies above could be the result of variations in the patients' socioeconomic position, social habits, education, and access to community health services (13).

Table 1. Relationship between ASB and the duration of pregnancy

Gestation period	Total no. screened	ASB	
		No.	%
First trimester	12	8	66.66
Second trimester	27	17	62.96
Third trimester	75	32	42.66
Total	114	57	50

ASB: Asymptomatic bacteriuria

Table 2. Relationship between age distribution and ASB during pregnancy

Age in years	Total no. screened	ASB	
		No.	%
15-20	23	14	60.86
21-25	37	16	43.24
26-30	35	17	48.57
31-35	15	8	53.33
35-40	4	2	50
Total	**114**	**57**	**50**

ASB: Asymptomatic bacteriuria

Table 3. The isolated pathogenic bacteria from pregnant women with ASB

No.	Isolates		No.	%
1	***Staphylococcus* species**		**38**	**66.65**
	Staphylococcus haemolyticus		12	
	Staphylococcus epidermidis		10	
	Staphylococcus hominis	**Coagulase-negative *staphylococci* (CoNS)**	9	
	Staphylococcus warneri		1	
	Staphylococcus capitis		1	
	Staphylococcus lentus		1	
	Staphylococcus aureus		4	
2	***Escherichia coli***		**6**	**10.52**
3	***Klebsiella pneumonia***		**5**	**8.77**
4	***Kocuriae* spp.**		**2**	**3.5**
	Kocuriae varians		1	
	Kocuriae kristinae		1	
5	Aeromonas hydrophila		**1**	**1.75**
6	***Enterococcus faecium***		**1**	**1.75**
7	***Sphingomonas pancimobilis***		1	**1.75**
8	***Dermacoccus nishinomiyaensis***		1	1.75
9	**Mix growth (*Staphylococcus hominis* + *Aeroccocus viridans*)**		**1**	**1.75**
10	Candida albicans		1	1.75
Total			**57**	**100**

ASB: Asymptomatic bacteriuria

Moreover, one of the major risk factors for symptomatic bacteriuria is ASB. Also, ASB constitutes around 40% of pyelonephritis and 30% of cystitis in unscreened pregnant women. Moreover, ASB has been indirectly linked to anemia and preeclampsia (9,14).

As per this work, there was a greater incidence of ASB in the first trimester of pregnancy, 66.66% (8/12 isolates), then 62.96% (17/27 isolates) in the second trimester, and while 42.66% (32/75 isolates) were in the third trimester (Table 1). This corresponds with prior studies by Chandel et al. (15) and is attributed hormonal changes occurring prior to anatomical changes. In this respect, Azami et al. (6) confirmed in their study that the highest and lowest prevalence of ASB were noticed in the 1st trimester (11.7%) and 3rd trimester (6.1%), respectively (6). In another study (12,16,17), the 3rd trimester was observed to have a greater prevalence of the condition being studied. As pregnancy progresses, urinary stasis increases, leading to a higher incidence of bacteriuria in the final trimester (17). This, combined with poor cleaning techniques and the heavily distended belly of pregnant women in their third trimester, may illustrate the high spread of bacteriuria noticed among pregnant women in their 3rd trimester (12). According to research results and other studies, monitoring for ASB is recommended to be conducted in each of the three trimesters of pregnancy in order to avoid the major consequences that might result from ASB during pregnancy (13). The 15 to 20 years age group (60.86%) had the greatest rate of ASB (Table 2), and this almost matches previous research findings (9,13). This may be because women in this age range engage in sexual activity.

The results of the current research exhibited that species of *Staphylococcus (S.)* (66.65%) were the most frequently occurring causative agents of infection among pregnant women, followed by *E. coli* (10.52%) (Table 3). In a similar study by Almukhtar (18) in Iraq, and Kalgo et al. (19) in Nigeria, the most common isolates were *Staphylococcus* species, with incidences of 20.6% and 51.6% respectively.

The most common *Staphylococcus* species uropathogens were *Staphylococcus* This result is consistent with separate scientific works achieved in northern Iraq by Assafi et al. (20) and Al-Naqshbandi et al. (21). Gram-positive cocci known as coagulase-negative staphylococci (CoNS), are grouped due to their shared absence of the virulence factor coagulase (22). When it comes to CoNS, the species most commonly found together are *S. epidermidis* and *S. haemolyticus*, which are clinically defined species. Other species that have historically been included in this group include *S. hominis, S. capitis, S. warneri,* and *S. lentus*. (23), can be caused cystitis in young sexually active women, they have been found to make up a noteworthy percentage in the current study as well (24). The findings indicate that this organism may be becoming more well-known as a potential cause of UTIs during pregnancy, probably related to its occurrence as part of normal vaginal flora (25).

Prior researchers regarded the coagulase-negative *Staphylococcus* that were identified from the urine sample, as contaminants and did not assign them any importance. However, a considerable number of coagulase negative *Staphylococcus* have been identified as the agents of UTIs in recent years (26). Additionally, because the urine of pregnant women contains high levels of albumin and amino acids, it serves as a suitable substrate supporting the growth of most infections. Additionally, pregnant women's defenses are weakened during pregnancy, leaving them more vulnerable to infections, particularly *Staphylococcus* species (18).

In this work, *E. coli* was the second most common (10.52%) bacterium after Staphylococcus ssp. (Table 3). Numerous research studies were published. *E. coli* was the most common bacterium found in pregnant women's urine cultures (27-29). Moreover, the majority of bacteria in the vaginal and rectal areas are *E. coli*. Pregnancy may increase the risk of contracting a UTI from *E. coli* due to changes in function and anatomy as well as challenges with maintaining personal hygiene (30). Because it promotes the colonization of certain bacterial strains, Urine stasis during pregnancy is the main cause of increased isolates of *E. coli* (31).

The other isolated strains were *Klebsiella pneumoniae* (*K. pneumoniae*) (8.77%), *Kocuria* spp. (3.5%), *Aeromonas hydrophila* (1.75%), *Enterococcus faecium* (1.75%), *Sphingomonas pancimobilis* (1.75%), *Dermacoccus nishinomiyaensis* (1.75%), Mixed Growth [*Staphylococcus hominis* (*S. hominis.)* + *Aerococcus viridans*] (1.75%).

Table 5. Result of minimal inhibitory concentration (MIC) of antimicrobial susceptibility test for isolates of *Staphylococcus ssp.* by VITEK2 system

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																	MDR
			FOX	BEN (a)	OXA (a)	GEN (b)	TOB (b)	LVX (c)	MOX (c)	ERY (d)	CLI (e)	LZD (f)	TEC (g)	VAN (g)	TET (h)	TGC (i)	NIT (j)	FA (k)	RIF (l)	SXT (m)	
1	P44	*S. haemolyticus*	+	R	R	S	S	R	I	R	S	S	S	S	R	S	S	R	S	S	5* (+)
2	P47	*S. haemolyticus*	*No results																	
3	P49	*S. haemolyticus*	*No results																	
4	P54	*S. haemolyticus*	+	R	R	I	S	R	I	R	S	S	S	S	R	S	S	R	S	S	5* (+)
5	P59	*S. haemolyticus*	+	R	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S	R	3* (+)
6	P63	*S. haemolyticus*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S	3* (+)
7	P64	*S. haemolyticus*	+	R	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	R	6* (+)
8	P79	*S. haemolyticus*	+	R	R	S	S	R	R	R	S	S	S	S	R	S	S	R	R	R	7* (+)
9	P89	*S. haemolyticus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
10	P91	*S. haemolyticus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
11	P95	*S. haemolyticus*	+	R	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	5* (+)
12	P104	*S. haemolyticus*	+	R	R	S	S	R	I	R	S	S	S	S	R	S	S	R	S	S	5* (+)
13	P5	*S. epidermidis*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	2* (-)
14	P29	*S. epidermidis*	+	R	R	S	I	R	I	R	R	S	S	S	R	S	S	R	S	R	7* (+)
15	P31	*S. epidermidis*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	2* (-)
16	P46	*S. epidermidis*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1* (-)
17	P51	*S. epidermidis*	*No results																	
18	P67	*S. epidermidis*	+	R	R	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S	2* (-)
19	P68	*S. epidermidis*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	3* (+)
20	P72	*S. epidermidis*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S	3* (+)
21	P80	*S. epidermidis*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	R	5* (+)
22	P98	*S. epidermidis*	+	R	R	R	I	S	S	S	S	S	S	S	S	S	S	R	S	S	3* (+)
23	P25	*S. hominis*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	R	S	R	4* (+)
24	P36	*S. hominis*	+	R	R	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S	3* (+)
25	P62	*S. hominis*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	2* (-)
26	P66	*S. hominis*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
27	P71	*S. hominis*	+	R	R	S	S	S	S	R	S	S	R	S	S	S	S	R	S	R	5* (+)
28	P77	*S. hominis*	+	R	R	S	S	S	S	R	S	S	I	S	S	S	S	R	S	S	3* (+)
29	P104	*S. hominis*	+	R	R	S	S	R	I	R	S	S	S	S	R	S	S	R	S	S	5* (+)
30	P106	*S. hominis*	*No results																	
31	P112	*S. hominis*	*No results																	
32	P27	*S. aureus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
33	P35	*S. aureus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	R	5* (+)
34	P58	*S. aureus*	+	R	R	S	S	S	S	R	S	S	S	S	R	S	S	R	S	S	4* (+)
35	P110	*S. aureus*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1* (-)
36	P11	*S. capitis*	-	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1* (-)

Table 5. Continued

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																		MDR
			FOX	BEN (a)	OXA (a)	GEN (b)	TOB (b)	LVX (c)	MOX (c)	ERY (d)	CLI (e)	LZD (f)	TEC (g)	VAN (g)	TET (h)	TGC (i)	NIT (j)	FA (k)	RIF (l)	SXT (m)	
37	P34	*S. warneri*	-	R	S	S	S	S	S	S	R	S	S	S	S	R	S	S	R	S	4* (+)
38	P102	*S. lentus*	+	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1* (-)
No.&% R			31 (93.93)	33 (100)	31 (93.93)	3 (9.09)	2 (6.06)	8 (24.24)	3 (9.09)	22 (66.66)	2 (6.06)		1 (3.03)		14 (42.42)	1 (3.03)		26 (78.78)	2 (6.06)	9 (27.27)	25 (75.75)
No.&% S			2 (6.06)		2 (6.06)	28 (84.84)	29 (87.87)	25 (75.75)	25 (75.75)	11 (33.33)	31 (93.93)	33 (100)	31 (93.93)	33 (100)	19 (57.57)	32 (96.96)	33 (100)	7 (21.21)	31 (93.93)	24 (72.72)	8 (24.24)
No.&% I						2 (6.06)	2 (6.06)		5 (15.15)				1 (3.03)								

*No Results: The results of Antimicrobial Susceptibility Test have not been read by the VITEK 2 system due to a technical error in system. MDR: Multidrug resistant, (a)- β-Lactams class, (b)- Aminoglycosides class, (c)- Quinolones class, (d)- Macrolides class, (e)- Lincosamides class, (f)- Oxazolidinones class, (g)- Glycopeptides class, (h)- Tetracyclines class, (i)- Glycylcyclines class, (j)- Nitrofurans class, (k)- Fusidane class, (l)- Ansamycins class, (m)- Folate pathway inhibitors class, (*)- Classes of antibiotics which resistance by *Staphylococcus ssp.* Isolate.
FOX: Cefoxitin Screen, BEN: Benzylpenicillin, OXA: Oxacillin, GEN: Gentamicin, TOB: Tobramycin, LVX: Levofloxacin, MOX: Moxifloxacin, ERY: Erythromycin, CLI: Clindamycin, LZD: Linezolid, TEC: Teicoplanin, VAN: Vancomycin, TET: Tetracycline, TGC: Tigecycline, NIT: Nitrofurantoin, FA: Fusidic Acid, RIF: Rifampicin, SXT: Trimethoprim/Sulfamethoxazole. R: Resistant, I: Intermediate and S: Sensitive; by Vitek 2 system, (+): Positive, (-): Negative.

Only *Candida albicans* (1.75%) was the fungus that was isolated in this study (Table 3). From setting to setting, differences and similarities in etiological agents of ASB exist. It might be because each location has different environmental conditions, socioeconomic and educational attainment levels, and personal cleanliness practices (32).

Because pregnancy increases the risk of significant consequences, treatment for ASB is advised. When a pregnant woman with ASB goes untreated, up to 40% of these women develop acute pyelonephritis, which can have major consequences for the fetus as well as the mother (29).

Antibiotics should thus be given just when necessary, with proper patient education on the need for compliance, because failing to take antibiotics for the full period indicated can also lead to resistance (4). This fragment requires additional context to form a complete sentence. For example: “in order to treat and eliminate infections in pregnant women, healthcare providers often prescribe antibiotics.” When treating ASB, the practitioner must consider the antimicrobial susceptibility pattern of UTI bacteria to choose and employ the most potent antimicrobial drug (32).

Antimicrobial Susceptibility Pattern of Staphylococcal Species Isolates

The bulk of *Staphylococcus* spp. in their investigation showed resistance to different antibiotics such as oxacillin, benzylpenicillin, erythromycin, and tetracycline, ranging from 60 to 100% (35).

Therefore, these medications would not be appropriate for the empirical treatment of ASB in expectant mothers. This was confirmed by the present research, where the vast majority of isolates (93.93%) were positive for cefoxitin screen. These tests are suitable for assessing the degree of oxacillin resistance in all CoNS species that is mediated by the *mecA* gene (11,36).

On the other hand, cefoxitin screen (oxacillin resistance) is a marker for *MRS*, involving *methicillin-resistant coagulase-negative staphylococci (MR-CoNS)* and *MRS aureus (MRSA*) (37) (Table 4). Antibiotic misuse and self-medication may be the cause of the rise in antibiotic resistance. Additionally, it is said that the rates of antimicrobial resistance among frequently isolated uropathogens are increasing to several routinely used drugs; their susceptibility varies depending on the time and location (38).

Out of all *Staphylococcus* spp. isolates (n=33), MDR was recorded for 25 (75.75%) isolates (Table 5). An MDR isolate is one that is resistant to agents in three or more antimicrobial categories (39). A similar result was reported in Northeast Ethiopia (72.4%) (40), Eastern Uganda (72.4%) (41), Western Ethiopia (74.4%) (33), and Somaliland (75%) (42).

Widespread antibiotic usage (inappropriate medication use, taking antibiotics for the wrong length of time, or using antibiotics when they are not needed, including for viral illnesses) and lax antibiotic monitoring are linked to the rise in MDR. This causes bacteria to choose antibiotic resistance mechanisms (41). MDR strains can also emerge from a biological mechanism that confers resistance to multiple drugs, from the genetic linkage of genes that confer resistance to various antibiotics on a chromosome or plasmid, or from the evolution of multiple mutations that confer resistance to multiple antibiotics in a host (43).

Table 5. Result of minimal inhibitory concentration (MIC) of antimicrobial susceptibility test for isolates of *Escherichia coli* and *Klebsiella pneumonia* by VITEK 2 system

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																
			ESBLs	AM (a)	P/T (a)	CZ (a)	FOX (a)	CEF (a)	CRX (a)	CP (a)	ERP (a)	IMI (a)	AMI (b)	GEN (b)	CIP (c)	LVX (c)	TGC (d)	NIT (e)	SXT (f)
1	**P21**	***Escherichia coli***	**+**	**R**	**S**	**R**	**S**	R	R	R	S	S	S	R	S	S	S	I	R
2	P26	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	**S**	**S**	**I**	**R**
3	P45	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
4	P57	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
5	P60	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
6	P78	***Escherichia coli***	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
No.&% R			100	100		100		100	100	100				16.66					33.33
No.&% S					100		100				100	100	100	83.33	100	100	100	66.66	66.66
No.&% I																		33.33	

No.	No. of isolate	Type of Isolate	Result of (MIC) of antimicrobial susceptibility test																
			ESBLs	AM (a)	P/T (a)	CZ (a)	FOX (a)	CEF (a)	CRX (a)	CP (a)	ERP (a)	IMI (a)	AMI (b)	GEN (b)	CIP (c)	LVX (c)	TGC (d)	NIT (e)	SXT (f)
1	**P3**	***K. pneumoniae***	**+**	**R**	**S**	**R**	R	R	R	R	S	S	S	S	S	S	S	S	R
2	**P37**	***K. pneumoniae***	**+**	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
3	**P42**	***K. pneumoniae***	**+**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**S**	S	S	S	S	S	S	S	S
4	**P76**	***K. pneumoniae***	**+**	**R**	**S**	**R**	**R**	**R**	R	R	S	S	S	I	S	S	S	I	R
5	**P83**	***K. pneumoniae***	**+**	**R**	S	R	S	R	R	R	S	S	S	R	S	S	S	R	S
No.&% R			100	100		100	60	100	100	100				20				20	40
No.&% S					100		40				100	100	100	60	100	100	100	60	60
No.&% I														20				20	

R: Resistant, I: Intermediate and S: Sensitive; by VITEK 2 system, (+): Positive, (-): Negative. (a)- β-Lactams class, (b)- Aminoglycosides class, (c)- Quinolones class, (d)- Glycylcyclines class, (e)- Nitrofurans class, (f)- Folate pathway inhibitors class.

ESBLs: Extended-spectrum β-lactamases, AM: Ampicillin, P/T: Piperacillin/Tazobactam, CZ: Cefazolin, FOX: Cefoxitin, CEF: Ceftazidime, CRX: Ceftriaxone, CP:Cefepime, ERP: Ertapenem, IMI: Imipenem, AMI: Amikacin, GEN: Gentamicin , CIP: Ciprofloxacin, LVX: Levofloxacin, , TGC: Tigecyclin, NIT: Nitrofurantoin, SXT: Trimethoprim/Sulfamethoxazole

Pattern of Antimicrobial Susceptibility of *Klebsiella pneumoniae* and *E. coli* isolates

Similar results from earlier research conducted in Iraq (35), Uganda (41) Indonesia (44), and India (45) have been published coliforms (*E. coli and K. pneumoniae*) were highly resistant to β-lactams, involving ampicillin (100%), cefazolin (100%), ceftazidime (100%), ceftriaxone (100%), and ciprofloxacin (100%). Likewise, *K. pneumonia* isolates were moderately resistant to cefoxitin (60%) (Table 5).

Similarly, in a multicenter study by Seni et al. (46) from Tanzania reported *E. coli* and *K. pneumoniae* These isolates showed strong resistance to Ampicillin, with resistance rates of 94.5% and 98%, respectively. Our finding was relatively higher than a meta-analysis study by Chelkeba et al. (29). Ampicillin resistance was found in around 80% and 75% of *E. coli and K. pneumoniae* species, respectively.

Also, in the published work which was done in Iraq by Naqid et al. (35), there were significant findings. *K. pneumoniae* showed strong resistance to ampicillin and ceftriaxone, and exhibited resistance patterns resembling those of *E. coli.*

β-lactam antibiotics, such as cephalosporins and penicillins, are generally used to treat UTIs throughout the gestational period and are thought to be safe during pregnancy (47). In recent years, these drugs have not been suitable for empirical treatment of ASB in these pregnant women. The confirmation of this is provided by the results of the current study; according to the resistance profiles, 100% of the isolates of *K. pneumoniae* and *E. coli* were extended-spectrum beta-lactamase (ESBL) producers (Table 6).

ESBLs have been detected predominantly in *Klebsiella* spp. and *E. coli* among the enterobacteriaceae. ESBLs clinically diminish the effectiveness of β-lactam, especially cephalosporins, and are linked with prolonged hospital stays and increase significant morbidity and mortality (47,48). The gram-negative isolates' synthesis of extended spectrum β-lactamase, their acquisition of resistance genes, the downregulation of receptors, and drug efflux, are the causes of their resistance to β-lactam antibiotics.

This is primarily, because using third-generation cephalosporins puts selective pressure on the body (41). In the present study, multidrug resistance was not seen among *E. coli* and *K. pneumoniae* isolates.

The authors are currently conducting research to identify and describe virulence and resistance genes responsible for MDR *in Staphylococcus spp.* and ESBLs in gram-negative bacteria associated with ASB in pregnant women in Iraq. MRS, including MRSA and MRCoNS strains, has become an emerging threat to humans, considerably contributing to morbidity, mortality, and socioeconomic expenses (49). The *mec*A gene is acquired by MRSA and MRCoNS,

Table 6. Result of minimal inhibitory concentration (MIC) of antimicrobial susceptibility test for isolates of *Escherichia coli* and *Klebsiella pneumonia* by VITEK2 system

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																
			ESBLs	AM (a)	P/T (a)	CZ (a)	FOX (a)	CEF (a)	CRX (a)	CP (a)	ERP (a)	IMI (a)	AMI (b)	GEN (b)	CIP (c)	LVX (c)	TGC (d)	NIT (e)	SXT (f)
1	P21	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	R	S	S	S	I	R
2	P26	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	I	R
3	P45	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
4	P57	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
5	P60	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
6	P78	*Escherichia coli*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
No.&% R			**100**	**100**		**100**		**100**	**100**	**100**				**16.66**					**33.33**
No.&% S					**100**		**100**				**100**	**100**	**100**	**83.33**	**100**	**100**	**100**	**66.66**	**66.66**
No.&% I																		**33.33**	

No.	No. of isolate	Type of isolate	Result of (MIC) of antimicrobial susceptibility test																
			ESBLs	AM (a)	P/T (a)	CZ (a)	FOX (a)	CEF (a)	CRX (a)	CP (a)	ERP (a)	IMI (a)	AMI (b)	GEN (b)	CIP (c)	LVX (c)	TGC (d)	NIT (e)	SXT (f)
1	P3	*K. pneumoniae*	+	R	S	R	R	R	R	R	S	S	S	S	S	S	S	S	R
2	P37	*K. pneumoniae*	+	R	S	R	S	R	R	R	S	S	S	S	S	S	S	S	S
3	P42	*K. pneumoniae*	+	R	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S
4	P76	*K. pneumoniae*	+	R	S	R	R	R	R	R	S	S	S	I	S	S	S	I	R
5	P83	*K. pneumoniae*	+	R	S	S	S	R	R	R	S	S	S	R	S	S	S	R	S
No.&% R			**100**	**100**		**100**	**60**	**100**	**100**	**100**				**20**				**20**	**40**
No.&% S					**100**		**40**				**100**	**100**	**100**	**60**	**100**	**100**	**100**	**60**	**60**
No.&% I														**20**				**20**	

R: Resistant, I: Intermediate and S: Sensitive; by VITEK 2 system, (+): Positive, (-): Negative.
(a) β-Lactams class
(b) Aminoglycosides class
(c) Quinolones class
(d) Glycylcyclines class
(e) Nitrofurans class
(f) Folate pathway inhibitors class.
ESBLs: Extended-spectrum β-lactamases, AM: Ampicillin, P/T: Piperacillin/Tazobactam , CZ: Cefazolin, FOX: Cefoxitin, CEF: Ceftazidime, CRX: Ceftriaxone, CP:Cefepime, ERP: Ertapenem, IMI: Imipenem, AMI: Amikacin, GEN:Gentamicin , CIP: Ciprofloxacin, LVX: Levofloxacin, TGC: Tigecyclin, NIT: Nitrofurantoin, SXT: Trimethoprim/ Sulfamethoxazole ,

leading to resistance. The *Staphylococcus* cassette chromosome mec (SCCmec) carries the mec operon, which contains the *mec*A gene. SCCmec I through XIII have been identified to date (37). Enzyme groups, known as ESBLs and mediated by plasmids, hydrolyze aztreonam, extended-spectrum cephalosporins, and penicillins (48).

As a result, this research is required due to the bacteria isolates identified in this study, which have shown high resistance to the majority of routinely used antimicrobial drugs. This follow-up study could explain the molecular foundation for bacteria developing resistance to highly effective antibacterial agents.

CONCLUSION

The incidence of ASB among pregnant women attending prenatal general wards at General Heet Hospital was relatively high in the study presented here. As a result, it is critical to screen every pregnant woman who visits an antenatal hospital in Iraq and other developing countries for ASB. The majority of bacterial isolates found in pregnant women's urine samples at General Heet Hospital were *Staphylococcus* species, *E. coli*, and *K. pneumoniae*. *Staphylococcus* spp. have been identified as a typical causative agent of ASB in pregnant women. As a result, routine screening for gram-positive bacteria as the causative agent of ASB should be considered. The vast majority of *Staphylococcus* species isolates were sensitive to linezolid, vancomycin, nitrofurantoin, tigecycline, clindamycin, teicoplanin, and rifampicin. The majority of the isolates were resistant to commonly used antimicrobials, particularly to the β-lactams class (benzylpenicillin and oxacillin). The high level of resistance among isolates of *Staphylococcus* spp. that cause ASB limits the use of antimicrobial drugs for therapy. Moreover, the dissemination of MDR isolates poses a risk to medical practice.

The susceptibility patterns of *E. coli* and *K. pneumoniae* showed that most of the isolates showed greater sensitivity to piperacillin/tazobactam, ertapenem, imipenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, and tigecycline. Also, the susceptibility of *E. coli* isolates to cefoxitin was greater compared to other antibiotics tested. Coliforms (*E. coli* and *K. pneumoniae*) were highly resistant to β-lactams, including ampicillin, cefazolin, ceftazidime, ceftriaxone, and ciprofloxacin. As a result, clinicians should base their empirical antibiotic selection on knowledge about the local prevalence of bacterial profiles and antibiotic susceptibility testing in Iraq, rather than adhering to universal guidelines.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the ethical standards of the University of Anbar (approval number: 21, date: 18.03.2025) by the Ethics Committee.

Informed Consent: Informed consent was obtained from all participants, ensuring they were fully aware of the study procedures, risks, and benefits before voluntarily agreeing to participate.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: A.S.J., N.A.B., Concept: A.S.J., N.A.B., Design: A.S.J., N.A.B., Supervision: A.S.J., Resources: M.N.O., Material: A.S.J., N.A.B., Data Collection or Processing: A.S.J., N.A.B., Analysis or Interpretation: A.S.J., M.N.O., Literature Search: A.S.J., M.N.O., Writing: A.S.J., M.N.O., Critical Review: M.N.O.

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