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Prevalence of asymptomatic Bacteriuria among Pregnant Women in Sirte City (Libya)

Abdallah I. Abdallah[*1], Mohamed A. Dow[1'2], Mohammed S. Mansour[3], Mabroka Aldalba[3], Huda Abdahamida[3].

[1]Department of Medical Microbiology, Faculty of Medicine, Sirte University, Sirte Libya.
[2]Department of Community Medicine, Faculty of Medicine, Sirte University, Sirte Libya.
[3]Department of Laboratory Medicine, Faculty of Health science, Sirte University, Sirte Libya.

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Corresponding Author

abdallah_abdallah@hotmail.om

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A B S T R A C T

Asymptomatic bacteriuria (ASB) is known as significant presence of pathogenic bacteria more as 10^5 CFU/ml in urine culture of pregnant women without any symptoms indicate of urinary tract infections. Which is may lead to serious complications on health of a pregnant woman, and the fetus if left untreated. The purpose of this study was designed to find the prevalence of asymptomatic urinary tract infection (AUTI) among pregnant women, and also to determine the antibacterial susceptibility of the isolates to various antibiotics to reduce risk factors in pregnancy. However, little is known about asymptomatic bacteriuria in pregnancy in Libya, namely in Sirte city.A total of 265 samples were collected in 7 months study involving women attending antenatal clinics in Sirte City-Libya, mid-stream urine samples were collected for microscopically examination. A colony culture growth were identified by biochemical tests and conventional antibiotic susceptibility tested in microbiology laboratory. Isolates were identified and tested against commonly used antimicrobial drugs using Kirby Bauer disc diffusion method.The prevalence of 265 pregnant women in different age groups were enrolled in this study, only 76 (28.7%) pregnant women had bacteriuria. The organism isolated from the urine sample according to the frequency of occurrence was *Escherichia coli* 32(42.1%), Coagulase negative *Staphylococcus* 11 (14.4%), *Staphylococcus aureus* 9(11.8%), *Klebsiella Pneumoniae* 9(11.8%), *Streptococcus agalactiae* 8 (10.5%), *Enterococcus* spp 5(6.6%), *Proteus mirabilis* 2(2.6%), respectively.The overall prevalence of asymptomatic bacteriuria among pregnant women in the study area was not high, but need to be taken on consider reducing risk of infections. The presence of asymptomatic bacteriuria and their antibiotic susceptibility test results should be taken into consideration during the management of pregnant women who are visiting antenatal care clinic; because there was high resistance to the most commonly used antibiotics. Routine urine culture and

1.0 Inroduction

Asymptomatic bacteriuria (ASB) is defined as presence of actively multiplying bacteria within the urinary tract with absence of any symptoms, resulting in adverse pregnancy Outcomes (Sonkar et al., 2021). Generally, females are more susceptible to UTIs because of the short length of the urethra along with close anal canal. Pregnant women are more commonly affected than normal women due to some

anatomical changes occurring during pregnancy (Sandhya et al., 2019). The association of ASB with adverse outcomes of pregnancy is a matter of concern of infections risks. It can lead to symptomatic UTI in pregnant women by the time, which could have devastating effects on both maternal and

fetal health (Smaill, 2007; Quadri et al., 2019). The danger of bacteriuria is that it does not always present with symptoms (Sonkar et al., 2021). However asymptomatic bacteriuria during pregnancy if left untreated may lead to complications like pyelonephritis, which might lead to delivery of premature or low-birth-weight infants. (Farshad et al., 2010; Tadesse et al., 2014; Sultan et al., 2015; Sandhya et al., 2019). However Adequate and early diagnosis and treatment reduces the prevalence of these obstetric complications (Schnarr and Smaill, 2008; Jain et al., 2013; kamel et al., 2018). Urine culture is the golden standard diagnostic technique for ASB which occurs during pregnancy (Al-senani N, 2011). Bacteria identified from urine of patients with asymptomatic bacteriuria usually comes from the normal flora of the gut, vagina and per urethral region (Nicolle E, 2003; Cheesbrough M, 2006). UTIs may arise more often in women than men because of the shortness of female urethra, and in a patient exposed to urinary catheters and/or bacteria on contaminated urological instruments, during sexual intercourse, and fluid which may enter into the genitourinary area without previous host colonization. (Nicolle E., 2003; Cheesbrough M, 2006; Ali et al., 2018). Recent studies showed that both Gram-negative and Gram-positive bacteria are predominantly responsible for ASB during pregnancy worldwide. The most common organism responsible for about 75-90% of bacteriuria in pregnancy is Escherichia coli (Perera et al., 2012; Abderrazzack et al., 2015). In additional to other microbial agents include Staphylococcus aureus, Coagulases-negative Staphylococcus, Klebsiella pneumoniae, Proteus mirabilis, group B Streptococcus, Pseudomonas aeruginosa, Staphylococcus saprophyticus, and Enterococcus faecalis (Rizvi et al., 2011; Aernan et al., 2016; Ayoyi et al.,2017). Early treatment of ASB prevents pyelonephritis and reduces the risk of preterm deliveries (Kazemier et al., 2015). Many countries have early routine screening and treatment for ASB as part of antenatal care guidelines. There is a debate on whether treatment of ASB improves neonatal outcomes and whether antibiotic treatment is associated with adverse pregnancy outcomes. However, there is insufficient evidence to support these associations (Force et al., 2019; Wingert et al., 2019). It is important therefore to screen pregnant women and offer treatment to mothers diagnosed with ASB. This will prevent later development of obstetric complications (Smail and Vazquez, 2015; Angelescu et al., 2016; Jain et al., 2013; Nteziyaremye et al., 2020). In general, Most UTIs are caused by bacteria and are treated by antibiotics. However there is increased development of microbial resistance to antibiotics (Jain et al., 2013, Sujatha and Nawani, 2014). Also, antimicrobial treatment of ABS will reduce the risk of having a low birth weight baby from 15% to 5% and pyelonephritis from 35% to 4% (Perera et al., 2012; Awoke et al., 2021). Recently, worldwide bacteriurias are virulent and capable of acquiring multidrug resistance to general antimicrobial used. For example, Escherichia coli are Gram-negative bacteria which can generate large-spectrum of beta-lactam enzymes making them resistant to most beta-lactam antibiotics (Abderrazzack et al., 2015). A rate of antimicrobial resistance varies according to geographic locations and they are directly proportional to the use and misuse of antimicrobials (Ali et al., 2018; Awoke et al, 2021). This study aimed to determine the prevalence of asymptomatic bacteriuria and antibiotics sensitivity pattern among pregnant women attending clinics in Sirte City-Libya

2.0 Materials and Methods

2.1 Study design, period and area:

A pregnant women were attending to Laboratory Clinics for routine urine analysis checking on base of specialist request, Urine samples were collected for bacterial isolates and antibiotic susceptibility patterns in Sirte city, Libya. This Study was carried out from 1st 1st of September 2019 to 30th of April 2020 in the Department of Microbiology, Medicine College, Sirte University.

2.2 Collection of urine samples:

Urine specimens were collected by standard mid-stream "clean catch" method from each pregnant woman who was instructed by nurse to reduce the chance of contamination. About 5 to 10ml of mid-stream urine samples were collected from every pregnant woman in a sterile screw-capped, wide-mouth container that was covered with tight-fitting lids. It was then delivered to Microbiology laboratory and then inculcated on culture and were identified using standard microbiological Methods.

2.3 Bacterial culture and identification:

A total of 265 specimens were cultured on dried plates of Sheep Blood agar, MacConkey's agar and Cystine Lactose Electrolyte Deficient agar (CLED), by standard loop method and the plates were incubated at 37°C overnight. The organisms were identified by classical Microbiology methods from the samples which showed significant bacteriuria growth.

2.4 Antimicrobial susceptibility testing:

Antibiotic sensitivity testing was done in the Microbiology Department using Kirby-Bauer disc diffusion test on Muller-Hinton agar medium (Oxoid, UK). The following antibiotics were investigated in this study are ampicillin (10mcg), amoxyclav (20/10mcg), amikacin (30mcg), clindamycin (2mcg), cefipime (30mcg), ceftriaxone (30mcg), cefuroxime (30mcg), ciprofloxacin (5mcg), cotrimaxazole (25mcg), erythromycin (15mcg), fosfomycin (200mcg), penicillin G (10units), imipenem (10mcg) and meropenem (10mcg).

3. Results

3.1 Prevalence of ASB:

Among 265 pregnant women were enrolled in this study, only 76/265 (28.7%) were characterised as bacteriuria, the prevalent of organism isolated showed *E. coli* was the most common 32(42.1%) organism isolated, followed by Coagulase negative *Staphylococcus* 11(14.4%), *Staphylococcus aureus* 9(11.8%), *Klebsiella Pneumoniae* 9(11.8%), *Streptococcus agalactiae* 8(10.5%), *Enterococcus* spp 5(6.6%), *Proteus mirabilis* 2(2.6%), respectively as illustrated in Table 1 and Figure 1.

Table1. Bacteriologic isolates from pregnant women with asymptomatic bacteriuria.

Bacteria isolated from urine samples	Total (%)
E. coli	32 (42.2%)
Coagulase negative *Staphylococcus*	11 (14.4%)
Staphylococcus aureus	9 (11.8%)
Klebsiella pneumoniae	9 (11.8%)
Streptococcus agalactiae	8 (10.5%)
Enterococcus spp	5 (6.6%)
Proteus mirabilis	2 (2.6%)
Total	**76(100%)**

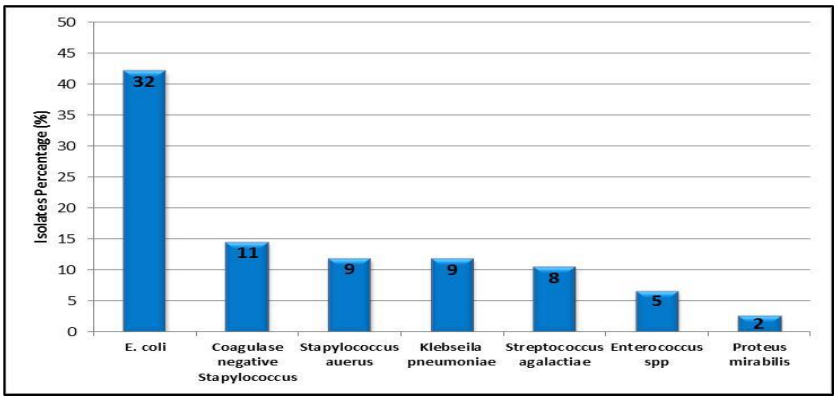

Figure 1. Percentage of pathogenic bacteria causing asymptomatic bacteriuria

Table 1. Shows the distribution of ASB among the pregnant women according to age group, the percentage of bacteria isolates of urine cultural was high in the age group 36-40 years comparing with other age group of pregnant women in this study, followed by age group of 41-45 years and by 31-35 age groups. The lowest rate of infection was recorded among age 20-25 and 26-30 years respectively.

Table 2. Distribution of isolated ASB among pregnant women according to age groups.

	No of bacteria isolates (%)						
Age	*E. coli*	*CN-Staph*	*S. aureus*	*Klebsiella pneumina*	*Streptococcus agalactiae*	*Enterococcus spp*	*Proteus mirabilis*
20-25	2(6.2%)	0(0%)	0(0%)	1(11.1%)	1 (12.5%)	0(0%)	0(0%)
26-30	1(3.1%)	3(27.2%)	2(22.2%)	1(11.1%)	0(0%)	0(0%)	0(0%)
31-35	8(25%)	4(36.3%)	2(22.2%)	0(0%)	1(12.5%)	2(40%)	0(0%)
36-40	13(40.6 %)	2(18.1%)	3(33.3%)	4(44.4%)	3(37.5%)	2(40%)	1(50%)
41-45	8(25%)	2(18.1%)	2(22.2%)	3(33.3%)	3(37.5%)	1(20%)	1(50%)
Total	**32**	**11**	**9**	**9**	**8**	**5**	**2**

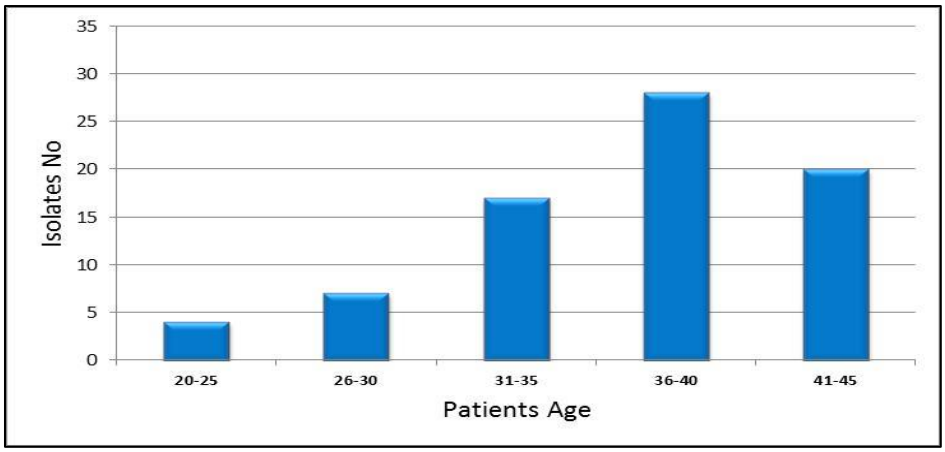

Figure 2. Percentage of bacteria isolates according to patients age group.

Antimicrobial susceptibility of the isolates:

The results in table 3, obtained indicated the antibiotic sensitivity pattern of various isolated strains presented in this study, the susceptibility profile of isolates to commonly used antibiotics ranger between (0.0% to 100%). The most common isolates in this study was *Escherichia coli* indicated (96.9%) susceptibility to Imipenem, (93.8%) to Meropenem, and (84.7%) to amikacin. and least susceptible to Clindamycin and Erythromycin (9.4%). Coagulase-negative *Staphylococcus* also was highly susceptible to Imipenem (100%) and least to Ampicillin, Cefuroxime, Erythromycin and Penicillin G (18.2%). *Staphylococcus aureus* was most susceptible to Imipenem and cefuroxime (88.9%) and least susceptible to Erythromycin and Penicillin G (22.2%). *Klebsiella pneumonia* was most susceptible to Imipenem (100%) and least susceptible to Erythromycin (0%) and Ampicillin, Clindamycin, Cefuroxime, and Penicillin G (11.1 %). *Streptococcus agalactiae* was most susceptible to Imipenem (100%) and Penicillin G (75%), and least susceptible to Erythromycin (12.5%). *Enterococcus* spp was susceptible to Imipenem (100%) and least susceptible to Ampicillin, amikacin, cefuroxime, cotrimaxazole and Erythromycin (20%). *Proteus mirabilis* also was susceptible to Imipenem, amoxyclav, ceftriaxone, and ciprofloxacin (100%), and least susceptible to amikacin, clindamycin, cefuroxime, cotrimaxazole, erythromycin and penicillin G (0%). Most of the bacterial isolates were susceptible to Imipenem (88.9-100%), meropenem and followed by amoxyclav (33.3–93.8%) which was the second most effective antibiotic, and the least option to be used is Ampicillin (20%) for isolates were detected in this study.

Table 3. Patterns of antimicrobial sensitivity results for the bacterial isolated from pregnant women.

Antibiotics Agents tested														
Isolates (no.)	AST patt	AMP	AMC	AMK	CLD	CTR	CRX	CIP	COT	ERY	FOS	PENG	IMP	MEM
E.coli (32)	S(%) R(%)	5(15.6) 27(84.4)	14(43.8) 18(56.2)	27(84.4) 5(15.6)	3(9.4) 29(90.6)	25(78.1) 7(21.9)	22(68.7) 10(31.3)	26(81.2) 6(18.8)	5(15.6) 27(84.4)	3(9.4) 29(90.6)	19(59.4) 13(40.6)	4(12.5) 28(87.5)	31(96.9) 1(3.1)	30(93.8) 2(6.2)
CN-staph (11)	S(%) R(%)	2(18.2) 9(81.8)	9 (81.8) 2 (18.2)	9(81.8) 2(18.2)	6(54.5) 5(45.5)	7(63.6) 4(36.4)	2(18.2) 9(81.8)	9(81.8) 2(18.2)	5(45.5) 6(54.5)	2(18.2) 9(81.8)	5(45.5) 6(54.5)	2(18.2) 9(81.8)	11(100) 0(0)	6(54.5) 5(45.5)
S. aureus (9)	S(%) R(%)	2(22.2) 7(77.8)	5(55.6) 4(44.4)	6(66.7) 3(33.3)	7(77.8) 2(22.2)	8(88.9) 1(11.1)	3(33.3) 6(66.7)	6(66.7) 3(33.3)	5(55.6) 4(44.4)	1(11.1) 8(88.9	6 3	1(11.1) 8(88.9	8(88.9) 1(11.1)	3(33.3) 6(66.7)
K. pneumon (9)	S(%) R(%)	1(11.1) 8(88.9)	3(33.3) 6(66.7)	6(66.7) 3(33.3)	1(11.1) 8(88.9)	2(22.2) 7(77.8)	1(11.1) 8(88.9)	3(33.3) 6(66.7)	2(22.2) 7(77.8)	0(0) 9(100)	5(55.6) 4(44.4)	1(11.1) 8(88.9)	9(100) 0(0)	6(66.7) 3(33.3)
S. agalactiae (8)	S(%) R(%)	2(25) 6(75)	4(50) 4(50)	2(25) 6(75)	4(50) 4(50)	4(50) 4(50)	2(25) 6(75)	1(12.5) 7(87.5)	5(62.5) 3(37.5)	1(12.5) 7(87.5)	4(50) 4(50)	6(75) 2(25)	8(100) 0(0)	5(62.5) 3(37.5)
Enterococcus (5)	S(%) R(%)	1(20) 4(80)	3(60) 2(40)	1(20) 4(80)	2(40) 3(60)	3(60) 2(40)	1(20) 4(80)	3(60) 2(40)	1(20) 4(80)	1(20) 4(80)	4(80) 1(20)	3(60) 2(40)	5(100) 0(0)	4(80) 1(20)
P. mirabilis (2)	S(%) R(%)	1(50) 1(50)	2 (100) 0 (0%)	0(0) 2(100)	0(0) 2(100)	2(100) 0(0)	0(0) 2(100)	2(100) 0(0)	0(0) 2(100)	0(0) 2(100)	1(50) 1(50)	0(0) 2(100)	2(100) 0(0)	1(50) 1(50)

AMP:
ampicillin, AMC: amoxyclav, AMK: amikacin, CLD: clindamycin, CTR: ceftriaxone, CRX: cefuroxime, CIP: ciprofloxacin, COT: cotrimaxazole, ERY: erythromycin, FOS: fosfomycin, PENG: penicillin G, IMP: imipenem and MEM: meropene

4.0 Discussion

defined as common cause of adverse maternal and pregnancy outcomes precisely pyelonephritis, low birth weight, preterm premature rupture of membranes and preterm labour, only around 10% of pregnant women will develop symptoms of a UTI, while others have asymptomatic bacteriuria, which may lead to serious infections complication, if not diagnosed and treated. (Ahmad *et al*., 2011), but Asymptomatic bacteriuria in pregnant women complications risk will be reduced by quick early routine screen and treat policy in pregnant women (Wingert *et al*., 2019). Worldwide about 30-40 untreated pregnant women with ASB will develop acute pyelonephritis in late pregnancy which is associated with significant morbidity for the mother and fetus and thus exact screening and treatment of bacteriuria in order to avoid further complications (Mwei *et al*., 2018). The prevalence of ASB among pregnant women in our study in Sirte city showed out of 265 only 76 (28.7%) were carried bacteriuria, which is low comparing with other study in Tripoli Libya by Ben Ashur *et al*., 2021 about (60%), while found to be high comparing with other study in Benghazi Libya by Almehdawi *et al*., 2017 about (13.3%), and almost similar in study in Alkhoms by Tamalli *et al*., 2013 about (30%) Also we found to be high comparing with some studies worldwide by Sujatha and Nawani, 2014 (7.3%), Mukherjee *et al*, 2014 (8.4%), Jayalakshmi and Jayaram, 2008 (7.4%), while our study were in agreement with studies done by Khan *et al*., 2015 (23.4%) and Patnaik *et al*., 2017 (25.3%), Nabbugodi *et al*., 2015 (26.7). Also in this study regarding the ages we found, the highest prevalence rate of ASB in women was reported in the age group (36-40) years and the lowest was in the age group (20-25) and (26-30) years, which nearly similar to other studies on bacteriuria in pregnant women (Mwambete and Msigwa., 2017; Ben Ashur *et al*., 2021). overall the isolates rate was showed that the highest isolated organisms were *E. coli* 32/76 (42.1%) followed by other common bacteria causing UTI in pregnant women, Coagulase negative *Staphylococcus* (14.4%), *Staphylococcus aureus* (11.8%), *Klebsiella pneumoniae* (11.8%), *Streptococcus agalactiae* (10.5%), *Enterococcus* spp (6.6%), *Proteus mirabilis* (2.6%), this finding is most consistent with many of the previous studies in Libya, which determined the *E. coli* are the most common urinary tract associated bacteria (Tamalli *et al*., 2013; Almehdawi *et al*., 2017; Ben Ashur *et al*., 2021). In addition to other gram negative and positive bacteria (Enayat *et al*., 2008; Sujatha and Nawani, 2014; Kamel *et al*., 2018; Sonkar *et al*., 2012). This is in agreement with other studies on ABS worldwide (Nabbugodi *et al*., 2015; Ayoyi *et al*., 2017; Gohar *et al*., 2019; Sonkar *et al*., 2021; Awoke *et al*., 2021). Coagulase negative *Staphylococcus*, *Staphylococcus aureus* were the second and third most common bacteria isolated of pregnant women in this study followed by *Klebsiella pneumoniae*, *Streptococcus agalactiae*, *Enterococcus* spp, *Proteus mirabilis* isolated from urine culture. Similar finding have been reported on prevalence of ASB in Libya by Ben Ashur *et al*,. 2021, while other studies in Egypt by Al-Kamel *et al*., 2018, Kenya by Nabbugodi *et al*., 2015; Ayoyi *et al*., 2017 and in India by Ankur *et al*., 2015, showed that the *E. coli* was the most common isolated organism followed by *Staphylococcus aureus* and *Klebsiella pneumonia*. Regarding the antimicrobial susceptibility pattern of the bacteriuria, we found that the isolated organisms highly susceptible to Imipenem were considered the most effective antibiotics against the most common organisms causing asymptomatic bacteriuria in this study, which were in agreement with other studies in India by Prasanna *et al*., 2015; and Sandhya *et al*., 2019, followed by meropenem, amoxyclav, ceftriaxone and ciprofloxacin, however *E. coli* isolates and other isolates were shown high level of resistant to Penicillin G, erythromycin, clindamycin and cotrimaxazole, respectively. This was found in agreement with other studies by Arredondo-García and Amábile-Cuevas, 2008; Ayoyi *et al*., 2017; Gohar *et al*., 2019; Emami *et al*., 2020.

However the antibiotic susceptibility patterns against UTI are shown to be different in different geographical locations. Moreover previous clinical trials have shown that antibiotic treatment significantly reduces the risk of serious complications associated with UTI in pregnancy worldwide (Smaill, 2007; Moyo *et al*., 2010). Furthermore, Routine early investigation, diagnosis and treatment of UTI infection in pregnant women are essential to avoid its complication and reduce risks.

5.Conclusion

The prevalence of ASB among pregnant women in Sirte City shown that E coli found to be the common isolates organism form urine culture among the pregnant women, beside other gram negative and positive bacteria, which may lead infection complications for pregnancy. This study recommend, all pregnant women should be screened by urine culture to detect asymptomatic bacteriuria at their first visit to prevent overt urinary tract infections (UTI) and other complications in both mother and fetus to prevent risks, and used the safe and effective antibiotics to reduce the dangers.

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