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Isolation and Susceptibility of Microorganisms Responsible for Asymptomatic Antenatal Bacteriuria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim & Objective: Determination of prevalence, bacterial profile and antibiotic sensitivity of microorganisms responsible for asymptomatic UTI during pregnancy.
Methodology: The type of study is **c**ross-sectional, Prospective type, was conducted at the PUMHS for Women Nawabshah (SBA) after approval from the Ethical Review Committee and the duration was 2 years from 1st Jan 2021-31st Dec 2022. Samples were collected by non-probability-

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convenient method from all pregnant women without any clinical complaints attending the Outpatient Department of Gynecology and Obstetrics PUMHS Hospital Nawabshah. The midstream urine collected by clean catch method in sterile containers was processed for DR of urine including physical, chemical, and microscopic examination. After the urine detailed report, the samples were sent for, culture by using the following media, blood, Macconkey's, and CLED agar. Growth-positive samples were proceeding for gram staining, biochemical tests, and antibiotic sensitivity reports according to world standard methods for confirmation.

Results: Out of 477 patients 96 (20%) were positive for ASB, with the mean age of the patient being 29.32±5.74 The most common microorganisms found in the study were E.coli 67 (69.7.%), followed by Klebisella in 4(4%), proteus 9(9%), pseudomonas 8(8.3%), staphylococcus aureus 2(2%), staphylococcus saprophyticus in 4(4%), streptococcus pyogen 2(2%).

There was 100% sensitivity found for all isolated microorganisms following culture examination to following antibiotics: Ciprofloxacin, Cefuroxime, Imepenem, Ceftriaxone Amikacin, Meropenem, and Piperacillin-tazobactam; however, 52% and 45% sensitivity of E.coli and 100% sensitivity of streptococci were present for amoxicillin-clavulanic acid and ampicillin respectively.

Conclusion: A rising number of silent antenatal bacteriuria was observed in younger age women with severe maternal and fetal complications in advanced stages of pregnancy and thereafter. It is therefore suggested to routinely perform urine cultures for every suspected pregnant lady during advanced antenatal visits to diagnose and properly treat the asymptomatic bacteriuria in terms of avoiding antibiotic resistance and materno-fetal complications in the near future.

Keywords: ASB; antibiotic susceptibility; asymptomatic bacteriuria; urinary tract infection; antenatal; Isolation.

1. INTRODUCTION

Prokaryotic urinary tract infections are a strong inflictor for approximately more than 150 million population per year, among them a huge number of sufferers are females during pregnancy which could be symptomatic or asymptomatic [1]. Anatomical and Physiological alteration during pregnancy enhances the potentiality for easy bacterial contamination, colonization, and proliferation of microorganisms in the urinary tract [2,3]. Overall global literature-based evidence suggests wide variability in the prevalence of UTI from 2 -47% during pregnancy [4-7]. If asymptomatic bacteriuria during pregnancy is left untreated the rate of development of urinary tract infection is 25% [8]. An Ethiopian study reveals that the rate of UTI following asymptomatic bacteriuria is 70% during pregnancy [9]. The deleterious maternal–fetal outcomes following ASB are pyelonephritis in the mother, and premature birth, low birth weight in newborns [3]. Unfortunately in underdeveloped countries, there are no well-established recommended guidelines for routine antenatal screening for ASB [9]. According to the American College of Obs: and Gynea, the screening is recommended for Asymptomatic Bacteriuria in all pregnant ladies [10]. Earlier screenings and quick and particular treatment of Asymptomatic infection during pregnancy can prevent further associated disorders of urinary tract infection in pregnant women [11,12]. The urine culture at antenatal visits is of gold standard for the diagnosis of asymptomatic bacteriuria [6,13]. In underdeveloped states, the culture of the urine is not practiced in routine antenatal visits in clinics, due to the time factor and high cost involved (48 hrs are required usually) [14]. The antimicrobial drug resistance development by over and non-rational application of antibiotics during pregnancy may lead to several morbid risks to mother and child [5]. There is a strong need to improve the diagnosis with accurate antibiotic prescription for materno-fetal well-being and it will have a positive impact on family and state health care costs [5]. There is a wide variation in the incidence of microbial agents and antimicrobial resistance patterns globally [6]. The E.coli or Escherichia coli during pregnancy is the most common isolate responsible for Asymptomatic Bacteriuria [3,7,9,15], followed by Cons [15] group B Streptococci [7], staph Aureus [6] staph aureus [3] CoNS [9]. The purpose of our study was to record the prevalence of ASB and the isolation of microorganisms responsible for asymptomatic UTI in pregnancy and the drug sensitivity patterns of those isolated microorganisms in urine samples of women attending the antenatal outpatient department at People's Medical Hospital and Department of Pathology, People University of Medical and Health Sciences Nawabshah, Sind, Pakistan.

Rationale: Asymptomatic UTI during pregnancy is a silent disease and not routinely diagnosed by antenatal care providers. There is a need to be properly diagnosed based on urine culture reports from microbiological laboratories. These studies are performed in different parts of many countries, with prevalence determination to isolation of bacteria, and their antibiotic sensitivity, but the results are quite variable at every place.

2. MATERIALS AND METHODS

The type of study is Cross Sectional-Prospective type, by non-probability (convenient) sample technique, and was conducted at the Pathology and Outpatient Department of Obs:&Gynea: PUMHS for Women Nawabshah (SBA)). The duration of the study was January 2021-December 2022.

Sample Collection Criteria: The women attending OPD for routine antenatal visits with no any urinary tract complaints were included, the pregnant lady with symptoms of Urinary Tract Infection and currently taking antibiotic treatment were excluded.

Following an informed written consent form taken from pregnant lady a sterile container was used to take the sample of urine quantity of 10-15 ml by clean–catch method. The sample was sent to the Pathology department for Urine Detailed Report, Culture for growth, and antibiotic sensitivity of microorganisms under strict laboratory standards by using multiple media like blood agar, an Enriched & differential media, Cystine Lactose Electrolyte Deficient agar cystine lactose electrolyte deficient which is specific for micro-organisms in urine, and Mac Conkey's agar a differential media based on lactose fermentation. The samples with positive growth cultures were forwarded for Grams staining, Bio-chemical tests of samples like Triple sugar iron/TSI test, Christensen urea agar, Simon Citrate agar test, string test, catalase test, coagulase test, and for evaluation of antibiotic sensitivity by using specific Mueller Hinton test under world standard methods. Blood agar plate: There is mammalian blood (normally horse or sheep) in blood agar plates (BAPs) generally at a concentration of 5% to 10%. Blood agar plate (BAPs). Cystine lactose electrolyte deficient (CLED)agar, Triple Sugar Iron (TSI) medium has a pH-sensitive dye the phenol red, Simmons' citrate agar is used to differentiate the gram-negative bacteria on the basis of citrate utilization, Christensen's urea medium is used for the growth and detection of bacteria produces urease, the Mueller Hinton agar media, The Catalase test: which is utilizes to ascertain the catalase-positive bacteria, Coagulase test is positive when there is clot formation following incubation of bacteria with plasma. The string test is positive when Klebsiella colonies form a pink-colored string after being touched by a wire-loop and then taken back.

Data Analysis: The data collected on Performa was designed especially and analyzed by using the 22 version of Statistical Package for the Social Sciences. The percentages of bacteria isolated were recorded and the antibiotic sensitivity was calculated. The results were presented in tables.

3. RESULTS

The total of 477 patients, the Mean age was 29.32± 5.74 years, and the recoded prevalence of ASB was 96(20%) Table 1. The samples of urine revealed mostly no growth 381(80%). The organisms isolated from cultures were E.coli 67 (69.7%), followed by Klebsella in 4(4%), proteus 9(9%), pseudomonas 8(8.3%), staph: Aureus 2(2%), staph: Saprophyticus in 4(4%), and the strept: pyogen 2(2%) were the least one Table 2.

There is 100% sensitivity to ciprofloxacin, Cefuroxime, Imepenem, Amikacin, Ceftriaxone, Meropenem, and Piperacillin-tazobactam; while E.coli is 52% sensitive to Amoxicillin, Clavulanic acid and 45% sensitivity to Ampicillin while streptococci were 100% sensitive to both antibiotics mentioned above Table 3.

Table 1. Total number of positive cases

Total number of cases		
No of patient	**Positive case**	**Negative case**
477	96(20%)	381(80%)

Table 2. The number of microorganism positive following the culture of urine

	Distribution of Culture Positive Organisms						
Organisms isolated	***E. coli***	***Klebseilla***	***Proteus***	***Pseudomonas***	***Staphylococcus aureus***	***Staphylococcus saprophyticus***	***Streptococci***
Number percentage	67(69.7%),	4(4%),	9(9%),	8(8.3%),	2(2%),	4(4%),	2(2%).

Table 3. Antibiotic susceptibility of positive microorganism

Antibiotics	***E. coli***	***Klebsella***	***S. saprophyticus***	***S. aureus***	***Proteus***	***Pseudomonas***	***Sterptococci***
AMC	35	1	0	0	2	0	2
	52%	25%			22%		100%
AMP	30	0%	1	0	0	0	2
	45%		25%				100%
CXM	67	4	4	2	9	4	2
	100%	100%	100%	100%	100%	50%	100%
AMK	67	4	4	2	9	8	2
	100%	100%	100%	100%	100%	100%	100%
CTR	67	4	4	2	9	8	2
	100%	100%	100%	100%	100%	100%	100%
CIP	67	4	4	2	9	8	2
	100%	100%	100%	100%	100%	100%	100%
TZP	67	4	4	2	9	8	2
	100%	1O0%	100%	100%	100%	100%	100%
MERO	67	4	4	2	9	8	2
	100%	100%	100%	100%	100%	100%	100%
IMP	67	4	4	2	9	8	2
	100%	100%	100%	100%	100%	100%	100%

4. DISCUSSION

The prevalence of asymptomatic bacteriuria varies between 3.75 to 45.3% around the different parts of the globe from developed to underdeveloped countries [2,3,4]. Our study had a 20% prevalence of asymptomatic bacteriuria, while a study conducted in year 2009 in Lahore Pakistan showed 7.3% of women developing asymptomatic bacteriuria [6]. Asymptomatic bacteriuria during pregnancy remains untreated and is related to increasing morbidity to developing acute pyelonephritis in the mother, and in neonates with increasing chances of lower gestational age, lower birth weight, and increased mortality rate in neonates. Screening of pregnant women visiting antenatal clinics including urine cultures routinely recommended to rule out asymptomatic urinary tract infection and treated with proper antibiotic prescription following culture-positive results. Julius and Celen have found an incidence of ASB to be 3.5% to 8.5% [3,16] while another study report from Nigeria showed a higher 86.% prevalence of asymptomatic bacteriuria [17]. The prevalence varies from different parts of the world, from the UK 3.75% [2] and 35.5 from Ghana [18], 4-7% from Canada [19], 4.9% from Australia [2], 10% from Egypt, [20] 12% from Bangladesh [21], 15.6% from Ethiopia [22], 17.9% from UAE [7], 16.7% from India [15], few Indian studies showed such a huge variability in prevalence as 8% [23] to 16-17% [24] to 25.3% [25]. Such a wide variation in results from different parts of the world and even from a single country may be due to different sample sizes, geographical differences, community social habits, availability of health-related facilities, and their proper consumption. A large number of studies from various areas of the globe have documented that ASB is caused by Staphylococcus hemolytic and aerobic bacilli of gram-negative group and earlier and particular antibiotic treatment was suggested. The commonest microorganism isolated in our study was E coli then Proteus, pseudomonas and Klebsiella etc. *E. coli* is the commonest organism causing asymptomatic UTI during pregnancy [7,15], but the congruent results were found at Ghana [17] where gram-positive S.aureus 64% and CoNS 70% are responsible for causing asymptomatic bacteriuria in pregnancy. Proteus is the 2nd common organism in our study but Group B streptococci at UAE [7] and CoNs are found at India [15] respectively. All the common microorganisms cultured were 100% sensitive to most antibiotics. The most commonly isolated organism Ecoli was 52% sensitive to Amoxicillin-clavulonic acid and 45% to Ampicillin but was sensitive to all the other antibiotics. As Penicillin and cephalosporin are safe during pregnancy, all our patients respond well to treatment. Higher resistance was detected against Ampicillin, Ceftriaxone, Cotrimoxazole, Erythromycin, and Nitrofurantoin was detected by Ezekiel [26]. The higher resistance may be due to irrational use and availability of antibiotics. The study by Jain showed that E. coli was the more common causative organism isolated and was 37.6% then *Enterococcus species* in 21.1% on the 2nd commonest organism. Celen reported in his study that the commonest organism was E coli 76.6% followed by 14.6% *Klebsiella*, but the sensitivity and specificity for fosfomycin and nitrofurantoin were greater than 99.2% and 88%. Tadesse in his study found *Staphylococcus* at 32.6%, most common followed by *Escherichia coli* at 26.1%, Staphylococcus Aureus at 13% with increased sensitivity to norfloxacin 64.7%, and 17.6% at for ampicillin [27].

It is therefore proposed that urine culture be practiced on antenatal visits of pregnant women at the outpatient Department before prescribing antibiotics to avoid bacterial resistance development to drugs safe in pregnancy. The results of this study suggest that after sending a urine culture of each symptomatic pregnant lady with positive urine D/R, 97% safe practices be achieved and may also decrease blind treatment burden by avoiding antibacterial drugs. In short, there is a need for moreover research and practices to be executed for pregnant ladies, with positive reports of urine cultures with few risk factors like previous history of Urinary Tract Infections.

5. CONCLUSION

The younger age pregnant ladies were increasingly affected by ASB with advancing pregnancy and parity. Therefore urine cultures should be routinely practiced during antenatal visits to diagnose the ASB and treated with prompt and proper antibiotics in order to avoid the increasing resistance of those antibiotics that can be used during pregnancy and to avoid materno-fetal complications.

On the other hand, improper use of antibiotics may cause harm to mother and child. The main reason for increasing resistance to simple antibiotics that can be used during the antenatal period to treat ASB was excessive and improper

use. There is also a risk to the fetus with dispensable exposure to antibiotics. It was observed recently that exposure to antibiotics to the mother during pregnancy may lead to alterations in the differential methylation at regulatory regions of imprinted genes [5]. By improving the diagnosis and properly prescribed antibiotics, fetal development will be smooth. All these steps will affect the future healthcare expenses. When we avoid the huge list of tests and specify them for accurate diagnosis, costs will be reduced and saved [5].

ETHICAL APPROVAL

The study was conducted at the PUMHS for Women Nawabshah (SBA) after approval from the Ethical Review Committee and the duration was 2 years from 1st Jan 2021-31st Dec 2022.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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