

ISOLATION AND SUSCEPTIBILITY OF MICROORGANISMS RESPONSIBLE FOR ASYMPTOMATIC ANTENATAL BACTERIURIA

ABSTRACT:

Aim & objective: Determination of prevalence and bacterial profile and antibiotic sensitivity of microorganisms responsible for asymptomatic UTI during pregnancy.

Methodology: Cross-Sectional-Prospective Setting: This study was conducted in the Department of Pathology Peoples University of Medical & Health Sciences (PUMHS) for Women Nawabshah (SBA) **Duration of Study:** 2 years

Study Design: Cross-Sectional-Prospective Setting: This study was conducted in the Department of Pathology Peoples University of Medical & Health Sciences (PUMHS) for Women Nawabshah. Samples were collected from the pregnant women attending the Outpatient Department of Gynecology and Obstetrics PUMHS Hospital Nawabshah.

Material and methodology All midstream clean catch collected urine samples in sterile containers processed for urine detailed report (physical, chemical, and microscopic examination). All the urine samples are further processed for cultures on blood agar, macconkey's agar, and CLED agar. All the positive growth cultures were further processed for gram staining, biochemical tests like Triple sugar iron (TSI) agar, Simon citrate agar, Christensen urea medium, string test, coagulase test, catalase test, and antibiotic sensitivity report on Mueller Hinton agar according to world standard methods.

Approval date Jan 2021.

Sample Size: The sample size is 477.

Sample Technique: Non-probability- Convenient. Samples were taken from the Gynecology and Obstetrics outpatient department of PUMHS Hospital Nawabshah.

Results: Out of 477 patients the mean age of the patients was 29.32 ± 5.74 years. The total prevalence of asymptomatic bacteriuria (ASB) was $n=96$ (20%). The organisms isolated from cultures were E.coli $n=67$ (69.7%), klebsiella $n=4$ (4%), proteus $n=9$ (9%), pseudomonas $n=8$ (8.3%), staphylococcus aureus $n=2$ (2%), staphylococcus saprophyticus $n=4$ (4%), streptococcus pyogen $n=2$ (2%).

The organisms isolated from urine cultures were all 100% sensitive to ciprofloxacin, imipenem, amikacin, ceftriaxone, cefuroxime, 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 asymptomatic bacteriuria during antenatal period was observed in younger age women with severe maternal and fetal complications in advance stages. It is

therefore suggested to routinely perform the urine cultures for every single suspected pregnant lady during advanced antenatal visits to diagnose and properly treat the asymptomatic bacteriuria in terms of avoiding avoid antibiotic resistance and materno-fetal complications in near future.

Keywords (1) Isolation (2)Antibiotic susceptibility (3) Asymptomatic bacteriuria (4)Urinary tract infection (5) Antenatal

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 suggest wide variability in the prevalence of UTI from 2 -47% during pregnancy.^{4,5,6,7} 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 Obstetricians and Gynecologists, screening for ASB is recommended in all pregnant women.¹⁰ Early screening, prompt and quality treatment of ASB during pregnancy can prevent further complications of bacteriuria in pregnant women.^{11,12} The urine culture at antenatal visits is of gold standard for the diagnosis of asymptomatic bacteriuria.^{6,13} In developing countries, the urine culture test is not routinely practiced in antenatal clinics, due to the time factor and high cost involved (48 hrs are required usually).¹⁴ The antimicrobial drug resistance development by over and incorrect use of antibiotics during pregnancy may lead to several morbid risks to mother and child.⁵ If we can improve diagnostics and related antibiotic prescribing, we can also influence fetal development and possibly long-term health with the results of this study. All of them impact future healthcare costs. Next to that, if we could decrease the number of tests for an accurate diagnosis, costs could be saved.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 Cons¹⁵ group B Streptococci⁷, staph Aureus⁶ staph aureus³ CoNS⁹. The purpose of this study was to determine the prevalence 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.

MATERIAL AND METHODS: STUDY DESIGN: The study is Cross Sectional-Prospective type, by non-probability (convenient) sample technique, and was conducted at the Department of Pathology, Peoples University of Medical & Health Sciences for Women Nawabshah (SBA)). The duration of the study was January 2021-December 2022. Samples were collected from the pregnant women attending the outpatient department of Gynecology and Obstetrics, PUMHS Hospital Nawabshah.

SAMPLE COLLECTION CRITERIA: All the Pregnant women without urinary tract symptoms, pregnant women with symptomatic UTI and currently taking antibiotic treatment were excluded.

Following informed written consent from pregnant women a midstream urine sample of 10-15 ml in a sterile container by clean-catch method. The specimen was processed for physical, chemical, and microscopic examination and culture on the blood agar (Enriched & differential media), CLED agar (specific for urine micro-organisms), and Mac Conkey's agar (differential media based on lactose fermentation). All the positive growth cultures were further processed for gram staining, and bio-chemical tests including Triple sugar iron (TSI) agar, Simon citrate agar, Christensen urea medium, string test, coagulase test, catalase test and antibiotic sensitivity report on Mueller Hinton agar 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%. BAPs (blood agar plate).CLED (cystine lactose electrolyte deficient)agar, Triple Sugar Iron (TSI) test: The TSI that holds a pH-sensitive dye (phenol red), agar, Simmons' citrate agar: is used to differentiate the gram-negative bacteria on the foundation of citrate consumption, Christensen's urea medium: This medium is recommended for the detection of urease-producing bacteria, Mueller Hinton agar medium, Catalase test: It is used to detect catalase-positive bacteria, Coagulase test: This test is positive when bacteria are incubated with plasma and a clot is formed. String test: This test is positive when colonies of klebsiella are touched with a wire loop and taken back it forms a pink-colored string.

DATA ANALYSIS: The collection of data on specially designed Performa for this study was analyzed by using the SPSS 22 version. During data analysis percentage of bacteria isolated and their antibiotic sensitivity was calculated. The results were presented in tables.

RESULTS

Out of 477 patients the mean age of the patients was 29.32 ± 5.74 years. The total prevalence of asymptomatic bacteriuria was $n=96(20\%)$ Table no: 01. The samples of urine revealed mostly no growth $n=381(80\%)$. However, the remaining organisms isolated from cultures were mostly E.coli $n=67(69.7\%)$, klebsella in $n=4(4\%)$, proteus $n=9(9\%)$, pseudomonas $n=8(8.3\%)$, staphylococcus aureus $n=2(2\%)$, staphylococcus saprophyticus in $n=4(4\%)$, streptococcus pyogen $n=2(2\%)$ Table no: 02.

The organisms isolated from urine cultures were all 100% sensitive to ciprofloxacin, imipenem, amikacin, ceftriaxone, cefuroxime, 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. Table no: 03

Table no: 01 Total number of positive cases

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

Table no 02: 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: 03 Antibiotic susceptibility of positive microorganism

antibiotics	E.coli	Klebsella	S. Saprophyticus	S.aureus	Proteus	Pseudomonas	Sterptococci
AMC	35 52%	1 25%	0	0	2 22%	0	2 100%

AMP	30 45%	0%	1 25%	0	0	0	2 100%
CXM	67 100%	4 100%	4 100%	2 100%	9 100%	4 50%	2 100%
AMK	67 100%	4 100%	4 100%	2 100%	9 100%	8 100%	2 100%
CTR	67 100%	4 100%	4 100%	2 100%	9 100%	8 100%	2 100%
CIP	67 100%	4 100%	4 100%	2 100%	9 100%	8 100%	2 100%
TZP	67 100%	4 100%	4 100%	2 100%	9 100%	8 100%	2 100%
MERO	67 100%	4 100%	4 100%	2 100%	9 100%	8 100%	2 100%
IMP	67 100%	4 100%	4 100%	2 100%	9 100%	8 100%	2 100%

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 bacteriuria⁶. Asymptomatic bacteriuria during pregnancy remains untreated and is associated with increased morbidity developing acute pyelonephritis in the mother, and low gestational age, low birth weight, increased neonatal mortality 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 bacteriuria¹⁷. The prevalence varies from different parts of the world, from the UK 3.75%² and 35.5 from Ghana¹⁸, 4-7% from Canada¹⁹, 4.9% from Australia,² 10% from Egypt,²⁰ 12% from Bangladesh²¹, 15.6% from Ethiopia²², 17.9% from UAE⁷, 16.7% from India¹⁵, 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 different parts of the world have found that asymptomatic bacteriuria is caused by Staph hemolytic or aerobic gram-negative bacilli and suggest earlier treatment with antibiotics. In our study the most common organism isolated was E coli followed by Proteus and pseudomonas; Klebsiella etc. Most of the studies found 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. In a study by Jain et al, E. coli was the most common organism isolated at 37.6% followed by Enterococcus species in 21.1%. Celen et al have found 76.6% E coli and Klebsiella in 14.6% of patients with increased sensitivity and specificity for fosfomycin and nitrofurantoin i-e; 99.2% and 88% respectively. Tadesse et al have found the most commonly isolated organism to be Staphylococcus in 32.6%, after this Escherichia coli in 26.1%, and Staphylococcus aureus in 13% with increased susceptibility for norfloxacin i-e; 64.7% and minimum for ampicillin ie; 17.6% ²⁷.

The emphasis posed on the practice of urine culture during antenatal visits of pregnant ladies at OPD clinics before suggesting antibiotics to set the susceptibility and decrease the resistance of bacteria to simple antibiotic treatment. The results of this study suggest that after sending a culture of urine to each pregnant symptomatic lady with positive urine D/R, 97% safe practices can be achieved and will also decrease blind treatment burden by eluding preventable antibiotic medicine. Shortly, further studies and practices are strongly required to be implemented for pregnant women, showing positive urine cultures with few risk factors like previous infection history of UTI.

Asymptomatic bacteriuria during pregnancy is increasingly found in younger ages with increasing gestational age and parity. Therefore every pregnant woman coming for antenatal care, urine cultures should be routinely practiced to properly diagnose the asymptomatic bacteriuria and should be treated with proper antibiotics to prevent the increasing resistance of those antibiotics that can be used during pregnancy and to avoid materno-fetal complications.

In contrast, over-treating pregnant women with antibiotics may also cause harm. Overuse and incorrect use of antibiotics are the main causes of antimicrobial resistance. Moreover, the unnecessary exposure of the unborn child to antibiotics may also not be without risks. It has recently been found that prenatal exposure to antibiotics can probably lead to alterations in the differential methylation at regulatory regions of imprinted genes.⁵ If we can improve diagnostics and related antibiotic prescribing, we can also influence fetal development and possibly long-term health with the results of this study. All of them impact future healthcare costs. Next to that, if we could decrease the number of tests for an accurate diagnosis, costs could be saved.⁵

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