Frequency of different uro-pathogens causing asymptomatic bacteriuria or bacteriuria without pyuria

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ABSTRACT

Objective: This study determines the frequency of different isolates identified in urine culture, which were identified as asymptomatic bacteriuria on urine RE.

Material and Methods: This descriptive cross-sectional study was conducted at the Department of Microbiology at Izzat Ali Shah Hospital, Wah Cantt from August 2022 to August 2023. A total of 275 urine specimens were included in the study. Mid-stream urine specimens were obtained and routine examination was performed on a fully automated FUS-2000 urinalysis system. Urine culture was performed by inoculating the specimen on CLED and Blood agar plates. The plates were incubated at 37ºC in ambient air for 24-48 hours. Growth was observed and identified based on gram stain and biochemical tests.

Results: Out of 275 specimens, growth was observed in 100 specimens. 175 specimens did not show any growth. Out of the 100 positive urine culture specimens, majority of the isolates belonged to Enterobacterales group. Out of these, 40% were *Escherichia coli* while 25% were *Klebsiella pneumoniae*. Growth of *Staphylococcus saprophyticus* was seen in 12% of specimens. Growth of *Pseudomonas aeruginosa, Enterococcus faecalis, and Streptococcus agalactae was seen in 5% of specimens each,* followed by *Enterobacter cloacae, Enterobacter aerogenes, and Citrobacter koseri* in 2% of specimens each. Growth of *Proteus mirabilis* and *Serratia marcescens* was seen in 1% of specimens each.

Conclusion: In conclusion, among the positive urine cultures, Enterobacterales group dominated followed by Escherichia coli and klebsiella pneumoniae as major isolates. Additionally, Staphylococcus saprophyticus was also identified in a few specimens. These findings highlight the importance of accurate differentiation between urinary tract infection and asymptomatic bacteriuria by correlating urine culture results with routine examination.

Keywords: Asymptomatic bacteriuria, Pyuria, Uropathogens

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INTRODUCTION

A urinary tract infection (UTI) presents as bacteriuria with or without any symptoms [1,2]. Routine screening and treatment for asymptomatic bacteriuria in non-pregnant individuals is not recommended due to its low prevalence, lack of adverse effects, and limited benefit from antibiotic therapy. Some patients, such as those who are pregnant, have just undergone kidney transplant surgery, or are undergoing urological procedures where mucosal bleeding is possible, should be checked for and treated for asymptomatic bacteriuria [1]. The presence of a single morphotype of bacteria in mid-stream voided urine cultures in an individual without symptoms, with 10 [3-5] colony-forming units (CFU) of the bacteria per milliliter of urine, is known as asymptomatic bacteriuria [2]. Females are considered as being more prone to develop asymptomatic bacteriuria due to multiple factors such as shorter urethra, and non-hygienic or non-sterile sample collection techniques [3]. As women age, the prevalence of asymptomatic bacteriuria rises, rising from approximately 1% in schoolgirls to over 20% in older women [4]. Apart

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from symptoms like pain in the suprapubic area, blood in the urine, contractions in the uterus, pain and burning during micturition can be seen in people with symptomatic UTIs [4,5]. Use of urine catheters for longer durations not only leads to the development of bacteriuria without pyuria but may also be the cause of resistance against antibiotics by bacteria [6,7]. To differentiate urethral contamination from bladder bacteriuria, a quantitative bacteriuria threshold has been devised. The following are the most prevalent uropathogens *Enterococcus spp, coagulase-negative Staphylococcus,* and *Staphylococcus saprophyticus* amongst gram-positive isolates. *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter koseri, Proteus mirabilis*, and *Serratia marcescens* are examples of most frequently isolated gram-negative pathogens [2,8]. Studies indicate lower socio-economic states to have higher rates of asymptomatic bacteriuria which are also associated with higher rates of antibiotic resistance as well [8,9,10]. This study aimed to map out the different bacterial isolates causing asymptomatic bacteriuria which may help in establishing a guide to any empirical treatments that may need to be given to any patient experiencing it.

MATERIAL AND METHODS

This descriptive cross-sectional study was carried out at the Department of Microbiology, Izzat Ali Shah Hospital, Wah Cantt, from August 2022 to August 2023 for a period of one year. A sample size of 275 was calculated by keeping a margin of error at 5%, confidence interval at 95%, and prevalence of UTI at 23.3% [11]. Approval from ethical review committee was taken before initiating the study. Written informed consent was obtained from all patients before enrolling them in the study.

A total of 275 mid-stream urine samples from patients of both sexes, ranging in age from 18 to 75, that were being evaluated for asymptomatic bacteriuria were included in the study.

Duplicate samples and samples collected by non-sterile methods or received from outdoor departments were excluded from the study. Urine samples were first analyzed by automated urine analyzer FUS 2000 (DIURI) to find out the physical and biochemical parameters of the sample. Presence of cells was also determined. A significant finding of ≥10 pus cells per high power field (HPF) in the urine sample is considered pathognomonic of a urinary tract infection (UTI).

For culture, all samples were inoculated onto CLED (Oxoid, UK) and Blood (Oxoid, UK) agar plates in quantitative as well as semi-quantitative techniques. In the quantitative technique, a calibrated loop delivering 0.001 mL of urine was used to streak the agar plates to estimate the number of colony-forming units (CFUs) per milliliter of urine. In the semi-quantitative technique, first 10µl of urine is spread vertically across half of plate with a calibrated loop followed by dilution smear with the same loop from top to bottom cross streaking technique to provide an approximation of bacterial growth. The plates were then incubated at 37ºC in ambient air for 24-48 hours. Any pure growth observed was then identified by gram staining and biochemical testing. Catalase and coagulase tests were used to identify gram-positive isolates. Enterococcus spp were then further identified by Lancefield grouping (12) and arabinose fermentation. *Staphylococcus saprophyticus* was identified by using novobiocin diagnostic disc in Muller Hinton agar plates. Gram-negative isolates were identified further by using API 20E and 20NE on basis of positive or negative oxidase test.

The data was analyzed by using Statistical Package for Social Sciences, SPSS version 23. Frequency and percentages were calculated for categorical variables while Mean ± SD were calculated for continuous variables. A p-value of <0.05 was taken as significant

RESULTS

A total of 275 mid-stream urine samples were included in the study. Out of these, 165 samples were from female patients while 110 samples were from male patients. Gender distribution of patients in urinary isolates had shown in Table-I. Of the 275 samples, 100 samples yielded positive bacterial growth of a single morphotype of bacteria. 175 samples did not yield any growth even after 48 hours of incubation. Frequency of different bacterial isolates identified from cultures is shown in Table-II. All of the 275 isolates were analyzed on the

automated FUS 2000 urine analyzer for the presence of pus cells. Among the 275 isolates, 125 exhibited numerous pus cells, while 150 showed no pus cells. 38 samples showed presence of numerous pus cells but no growth on cultures and hence were labeled as false negative, whereas 13 samples were culture positive without any pus cells and were labeled as false positive. 87 samples showed positive cultures positive as well as presence of pus cells and were labeled as true positive. 137 samples did not show any growth or any pus cells and hence were labeled as true negatives. The sensitivity of uro-pathogen was 69.60%, specificity was 91.33, PPV 87.0%, NPV was 78.29% and diagnostic accuracy was 81.45% as shown by following Table-III

Table-I: Gender distribution (n=275).

Gender	N (%)
Females	165(60%)
Males	110(40%)
Total	**275**

Table-II: Bacterial isolates from positive cultures (n=100).

Urinary isolates	N (%)
Escherichia coli	40 (40)
klebsiella pneumoniae	25 (25)
Staphylococcus saprophyticus	12 (12)
Pseudomonas aeruginosa	5 (5)
Enterococcus fecalis	5 (5)
Streptococcus agalactiae	5 (5)
Enterobacter cloacae	2 (2)
Enterobacter aerogenes	2 (2)
Citrobacter koseri	2 (2)
Proteus mirabilus	1 (1)
Serratia marcescens	1 (1)
Total	100

Table-III: Odds ratio and diagnostic accuracy of urinary isolates.

Culture	Urine Pus Cells		Total	p-value
	Positive	Negative		
Positive	87 (TP)	13 (FP)	100	0.001
Negative	38 (FN)	137 (TN)	175	
Total	**125**	**150**	**275**	

Sensitivity= TP/(TP+FN) = 87/(87+38)*100=69.60 %
Specificity= TN/(TN+FP) = 137/(137+13)*100=91.33%
Positive Predictive Value= TP/(TP+FP)* 100= 87/(87+13)= 87.0%
Negative Predictive Value= TN/(TN+FN)*100=137/(137+38)= 78.29%
Diagnostic Accuracy=(TP+TN)/All patients*100 = (87+137)/275=81.45%

DISCUSSION

If pyuria is not seen on urine analysis, asymptomatic bacteriuria (ASB) is established on urine culture when a bacterial count of ≥10^5 colony-forming units [CFU]/mL) is found in the urine. ASB is a common observation in female populations and in many women or men with genitourinary tract disorders that either introduce a foreign body in the urinary tract or hinder voiding [6,7]. According to the above-mentioned criterion, a specimen from a mid-stream urine (MSU) of asymptomatic women with consistently high levels of bacteriuria as much as 10^5 CFU/mL pointed towards a UTI, whereas lower colony counts indicated bacterial contamination [8,9].

Pathogenesis of asymptomatic bacteriuria is attributed to multiple reasons which includes host factors such as urinary catheter usage, surgeries of the urinary tract or non-sterile sample collection. Pathogen factors such as microbiota attachment via fimbriae adhesions is also an important cause for the persistence of symptomatic or asymptomatic infection [13-16].
The findings of the current study align with those of a comparable study conducted in the United States in 2021 where about 45% patients showed positive cultures in the absence of any symptoms. Another similarity to this study was the isolation of *E. coli* as the most frequent pathogen [17] and a similar research study conducted in Denmark in 2020 revealed a positive culture percentage of 42% with *E. coli* as the most common isolated organism [18].

The urine samples were initially assessed for leukocyte counts using a fully automated FUS-2000 urinalysis hybrid based on flow cytometry principles before undergoing culture and sensitivity testing. The sensitivity of uro-pathogen detection was determined to be 69.60%, with a specificity of 91.33%, a positive predictive value (PPV) of 87.0%, a negative predictive value (NPV) of 78.29%, and a diagnostic accuracy (DA) of 81.45%. These results were compared with those of a research study conducted in India. Out of total 216 pregnant female subjects, 36 tested positives for ASB. And similar to the current study, *Escherichia coli* (61.1%, n=22) was the dominating isolate followed by Cons (16.7%, n=6) and *S. aureus* (8.3%, n=3) [19].

This research aimed to comprehensively identify and characterize the bacterial isolates associated with asymptomatic bacteriuria, a condition characterized by the presence of bacteria in the urine without accompanying symptoms of urinary tract infection. By mapping out the diverse range of bacterial species involved in asymptomatic bacteriuria, the study sought to shed light on the microbial landscape of this condition.

CONCLUSION

In conclusion, among the positive urine cultures, Enterobacterales group dominated followed by Escherichia coli and klebsiella pneumoniae as major isolates. Additionally, Staphylococcus saprophyticus was also identified in a few specimens. These findings highlight the importance of accurate differentiation between urinary tract infection and asymptomatic bacteriuria by correlating urine culture results with routine examination.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none

AUTHORS CONTRIBUTION

Naila Iqbal: Main idea and concept
Muhammad Zeeshan Khalid: Data analysis
Abdul Rehman: Result writing
Amber Jamil Siddiqi: Data collection
Humera Javed: Critical review
Saira Salim: Proofreading and revisions

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