

# Antibiotic Screening of Urine Culture for Internal Quality Audit at Amrita Hospital, Kochi

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## ABSTRACT

**Introduction:** Urine antimicrobial activity is a seldom analysed laboratory test which greatly impacts the quantification of urine specimens. Presence of antimicrobial activity in the urine reduces the bacterial load in these specimens. Hence, the chances of erroneously reporting insignificant bacteriuria can be reduced on analysis of the antimicrobial activity in urine.

**Aim:** The aim of the study was to measure the antimicrobial activity of urine samples obtained from patients in a tertiary care hospital.

**Materials and Methods:** A total of 100 urine specimens were collected from the study group. Tests like wet mount, Gram staining and culture were performed. Antimicrobial susceptibility testing was done on the bacteria isolated from each specimen. The urine specimens were reported as significant bacteriuria (>105 Colony Forming Unit (CFU)/ml) and insignificant bacteriuria (<105 CFU/ml – clean catch midstream urine; <102 CFU/ml – catheterized urine sample) according to the CFU/ml. *Staphylococcus aureus* ATCC® 25923™ and *Escherichia coli* ATCC® 25922™ were used to identify the presence of

antimicrobial activity in the urine sample by Urine Anti-Bacterial substance Assay (UABA). McNemar test was used for statistical analysis using Statistical Package for the Social Sciences (SPSS) version 21.0.

**Results:** On analysis of the antimicrobial activity of urine sample with the prior antibiotic history of the patients, 17 were true positives and 43 were true negatives. Twenty six of samples with UABA positivity were culture negative and 28 samples with UABA positivity were culture positive. Sensitivity and specificity of the test was 85% and 53.8% respectively. Accuracy of the test was 60%. The p-value of UABA was <0.001. Enterobacteriaceae was the most common bacterial family isolated from the urine specimens. A total of 85% patients responded to treatment.

**Conclusion:** Presence of antimicrobial activity in urine has a great impact on the interpretation of urine culture reports. Identification of urine antimicrobial activity helps in evaluating the quantification of bacterial growth reported in urine culture. It facilitates speedy recovery of patients by early administration of antibiotics.

**Keywords:** Antimicrobial activity, Enterobacteriaceae, Urine anti-bacterial substance assay

## INTRODUCTION

UABA is a simple laboratory test which has a great impact on the interpretation of urine culture reports. Presence of antimicrobial activity in the urine specimens influences the quantification of urine culture and may lead to incorrect labelling of urine samples as “insignificant bacteriuria”. Quantification of urine culture must be accurate as it influences the management of patients with symptoms suggestive of UTI. Presence of significant bacteriuria warrants for treatment in this group of patients and same is not the case in those with insignificant bacteriuria.

This study was aimed at analysing the antimicrobial activity of urine specimens using UABA test and comparing the results of this test with the previous antibiotic history in these patients.

## MATERIALS AND METHODS

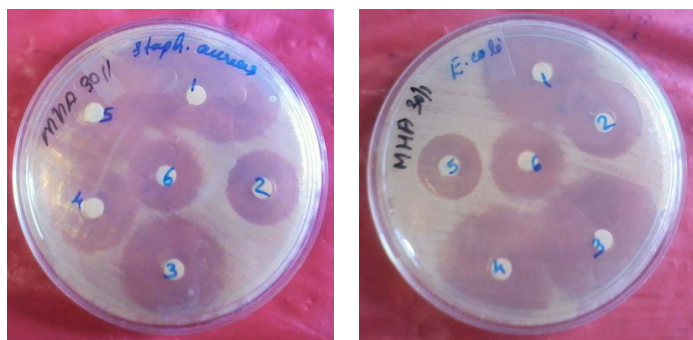
A simple randomised study was conducted with a total of 100 urine specimens over a period of two months from November 2015 to December 2015 from symptomatic inpatients and outpatients of Amrita hospital who were clinically suspected to have Urinary Tract Infection (UTI). Clean catch midstream urine, catheterized urine, suprapubic aspiration, cytology and nephrostomy collection of urine were the wide range of samples collected in this study. Tests like wet mount, Gram staining and bacterial culture onto 5% sheep blood agar and MacConkey agar were performed. Presence of five or more pus cells in the wet mount was taken as an indicator of significant bacteriuria [1]. Biochemical tests such as nitrate reduction, indole production, methyl red and Voges Proskauer reaction, citrate utilization, urease production, mannitol motility test,

sugar fermentation, and amino acid decarboxylation were used for the identification of bacteria. Antibiotic susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) 2015 guidelines by Kirby Bauer disc diffusion method on Mueller Hinton agar plates [2]. Semiquantitative method or standard loop technique was followed for bacterial culture. The following was the gradation used for quantification of urine cultures:

**Significant bacteriuria:** Clean catch urine: > or = 105 CFU/ml, Catheterized urine: >102 CFU/ml, Suprapubic aspiration: any CFU/ml

**Insignificant bacteriuria:** Clean catch urine: < 105 CFU/ml, Catheterized urine: <102 CFU/ml [1]

In UABA test, two Mueller Hinton agar plates were used for testing one bacterial isolate. Lawn cultures of *Escherichia coli* ATCC® 25922™ and *Staphylococcus aureus* ATCC® 25923™ were inoculated onto each plate. A sterile cotton swab was dipped into the 0.5 McFarland standard suspensions of the isolated bacterial strains and rotated three times onto both the plates at 60° angles. Whatman grade 1 filter paper was used to prepare discs of size 6 mm. Autoclave was used to sterilize these discs. They were inoculated onto the medium with a distance of 10-12 mm between two discs. One loopful (0.01 ml/1 µl) of urine sample was applied to the corresponding labelled disc. The plates were incubated at 35°-37°C overnight. After incubation, presence of any zone around the disc is taken as UABA positive or antibacterial activity present in urine. If there is no zone around the disc, it is considered as UABA negative [Table/Fig-1,2].



[Table/Fig-1]: UABA test performed using *Staphylococcus aureus* ATCC® 25923™.

[Table/Fig-2]: UABA test performed using *Escherichia coli* ATCC® 25922™.

Comparison of antibiotic screening of urine with previous antibiotic history of patient was done. Urine samples which were positive for UABA test and previous antibiotic history were considered as true positive. Urine samples which were negative for UABA test and previous antibiotic history were considered as true negative. Urine samples with no previous antibiotic history and which were positive for UABA test were considered as false positive. Urine samples with previous antibiotic history and negative UABA test were considered as false negative [3].

## STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 21.0. McNemar test was used for analysis. The p-value of UABA was <0.001.

## RESULTS

Urine specimens were obtained from 100 patients. Most of them were 61-85 years (37%) and 46-60 years (20%) of age. A total of 60% patients were males. Most patients were from areas adjacent to AIMS such as Ernakulam (28%), Thrissur (14%) and Kottayam (11%) [Table/Fig-3]. Among the study patients, 17% patients were from Intensive Care Units (ICU) and 39% patients were from Outpatient Department (OPD). A total of 20% patients had previous history of administration of antibiotics. A total of 22% patients were started on empirical antibiotic therapy after urine sample was sent for analysis. A total of 35% patients had significant bacteriuria and 46% patients had no growth of bacteria on urine culture. UABA was positive in 53% and 54% urine specimens when detected using *Staphylococcus aureus* ATCC® 25923™ and *E. coli* ATCC® 25922™ respectively. Most common organism isolated from the urine specimens belonged to Enterobacteriaceae family (63%). Antimicrobial susceptibility testing showed colistin (17%), meropenem (17%), imipenem (13%) and amikacin (15%) sensitivity among Enterobacteriaceae. Comparison of UABA test and urine culture positivity is shown in [Table/Fig-4]. Comparison of UABA and previous antibiotic history is shown in [Table/Fig-5]. Sensitivity and specificity of the test was 85% and 53.8% respectively. Accuracy of the test was 60%. Positive predictive value of the test was 31.5% and negative predictive value of the test was 93.5%.

## DISCUSSION

UTI is commonly seen in women compared to men and previous studies report that approximately 70% UTIs are seen in women. Almost one in three women who are less than 24 years of age have been on antimicrobial therapy for UTI [4]. The majority of UTIs are caused by *E. coli*. UTI usually resolves without complications if treated with appropriate dosage of antibiotics according to the culture reports. This becomes tricky in cases of patients with previous antibiotic history as it affects the interpretation of urine culture reports [5]. This problem can be alleviated by the usage of UABA test.

In our study, 100 urine samples were analysed for antimicrobial

Determinant	Number	Percentage
<b>Age distribution</b>		
1. 0-15 years	18	18%
2. 16-30 years	13	13%
3. 31-45 years	12	12%
4. 46-60 years	20	20%
5. 61-85 years	37	37%
6. >85 years	0	0%
<b>Sex distribution</b>		
Males	60	60%
Females	40	40%
<b>Residential distribution</b>		
Thrissur	14	14%
Palakkad	3	3%
Kollam	3	3%
Ernakulam	28	28%
Idukki	6	6%
Kannur	3	3%
Thiruvananthapuram	1	1%
Pathanamthitta	8	8%
Alappuzha	8	8%
Kottayam	11	11%
Malappuram	5	5%
Kasaragod	1	1%
Other states	9	9%
<b>Location in the hospital</b>		
ICU	17	17%
Wards	44	44%
Outpatients	39	39%
<b>Previous antibiotic history</b>		
Yes	20	20%
No	80	80%
<b>Quantification of urine</b>		
Significant bacteriuria	35	35%
Insignificant bacteriuria	5	5%
Mixed growth of organisms	14	14%
No growth	46	46%
<b>Empirical therapy</b>		
Ceftriaxone	4	4%
Piperacillin tazobactam	4	4%
Cefoperazone sulbactam	1	1%
Meropenem	3	3%
Ciprofloxacin	2	2%
Levofloxacin	3	3%
Clindamycin	2	2%
Linezolid	1	1%
Vancomycin	1	1%
Colistin	1	1%

[Table/Fig-3]: Demographic profile of study patients.

Characteristics	UABA +ve	UABA -ve	TOTAL
Culture +ve	28	26	54
Culture -ve	26	20	46
Total	54	46	100

[Table/Fig-4]: UABA vs urine culture among study patients.

Characteristics	Previous antibiotic history present	Previous antibiotic history absent	TOTAL
UABA +ve	17	37	54
UABA -ve	3	43	46
Total	20	80	100

[Table/Fig-5]: UABA vs previous antibiotic history among study patients.

activity, the presence of bacteria and its antimicrobial susceptibility testing. This study involved patients from various age groups. Most patients belonged to 61-85 years of age. Decreased immune response and use of catheter may be the reasons for increase in number of patients belonging to this age group [6]. Other reasons for UTI to occur more common in elderly people may be incomplete bladder draining due to prostate problems, postmenopausal women with incomplete bladder draining due to prolapsed bladder or bulging of the bladder into the vagina and use of anticholinergic medications [7].

A total of 60% of our study patients were males. This may be because males approach healthcare more than females. Majority of the study patients were from places near AIMS such as Ernakulam and Thrissur. Most of the patients in our study were inpatients (83%). Majority of the urine test request forms analysed during our study did not contain antibiotic history in spite of antimicrobial activity being detected in the urine. The following may be the reasons for this finding: prior intake of antibiotics for infections other than UTI, improper history taking and uneducated patients who are not aware of the names of the consumed drugs and poor recall [3]. The empirical antibiotics commonly used in our study were piperacillin, ceftriaxone, levofloxacin and meropenem compared to other studies where cefepime and meropenem were used for therapy [8].

In this study, a total of 37 (46%) samples were false positive and three (15%) samples were false negative as detected by UABA test. A total of 17 (85%) samples were true positive and 43 (53.8%) samples were true negative results as detected by UABA test. Wilson G et al., report that in their study on UABA, out of 14,680 urine samples, 97.32% were true negative, 0.04% false negative, 1.51% true positive and 1.13% were false positive [3]. A previous study done on UABA reported that only 55% of the patients with UABA positivity had previous antibiotic history [9]. Abu Shagra QM performed urine antimicrobial activity using *Staphylococcus aureus* ATCC® 29737™. He reports 14.4% significant bacteriuria cases out of 500 study patients and urine antimicrobial activity was seen in 19.2% specimens. He also states the presence of urine antimicrobial activity as a reason for identification of sterile pyuria in a number of urine specimens [10]. Cardozo D et al., state that in their study on antimicrobial residue activity in urine, 14/188 urine specimens yielded no bacteria on culture but were positive for antimicrobial residue activity [11]. Organisms most commonly detected in our study were *E. coli*, *Klebsiella*, *Pseudomonas* and *Enterococcus*. This was similar to a previous study done in India [12]. *Streptococcus pneumoniae* was also isolated from one urine specimen. This isolation was in accordance with a study by Krishna S et al., [13]. Enterobacteriaceae group of bacteria were most sensitive to imipenem, meropenem, amikacin and colistin. Non-fermenting Gram negative bacilli were most sensitive to colistin. The Gram positive cocci were most sensitive to tetracycline, doxycycline, vancomycin, and teicoplanin. A similar scenario was also seen in previous studies [14-16].

In this study, 85% patients showed response to treatment and 8% did not show response to treatment. Failure of antibiotic therapy was due to the presence of complicated UTI in these patients. Mortality was 7% in this study. The cause of death in these patients was not due to UTI but as a result of co-morbid conditions such as malignancy, pneumonia, and multiorgan failure. A similar study in 2011 observes that the antibiotic screening of urine culture is a useful quality audit [3].

## LIMITATION

The results of this study cannot be extrapolated to the larger population as it was conducted over a period of two months with a sample size of 100 urine specimens. We find the need for a larger study to understand the impact of urine antimicrobial activity on the interpretation of urine culture.

## CONCLUSION

UABA can be used to screen urine specimens for the presence of antimicrobial activity in urine. It is a useful tool which has a great impact on the interpretation of urine culture reports and management of patients with UTI.

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