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## Impact of Pre-analytic Practices on Diagnostic Accuracy of Urine Cultures- A Retrospective Study

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ARTICLE INFO	ABSTRACT
<p><b>Article type:</b> Research Article</p> <p><b>Article history:</b> Received 02 May 2025 Revised 24 Jun 2025 Accepted 29 Jul 2025 Published 23 Aug 2025</p> <p><b>Keywords:</b> Asymptomatic bacteriuria, Complete urine analysis, Contamination.</p> <p>*Corresponding Authors: Sukanya Sudhaharan: Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad, India. Tel: +91-8801753241, E-mail: sukanyavimala@gmail.com.</p>	<p><b>Background:</b> Several pre-analytic factors adversely affect the diagnostic accuracy of urine cultures. The aim of our study is to identify and evaluate preanalytic practices associated with urine specimens and assess their impact on the accuracy of urine culture microbiology.</p> <p><b>Methods:</b> A retrospective study was conducted from January 2019 to June 2019 on urine cultures showing discrepant results. This included 225 patients whose culture showed growth of a single pathogen with no pus cells in the Gram stain. The details regarding the sample type, repeat cultures sent, and complete urine analysis (CUE), were analyzed.</p> <p><b>Results:</b> Of the 225 samples, 208(93.4%) were clean catch and 17 (3.1%) were catheter catch. Of 17 patients with catheter catch, urine culture and CUE were done in 12 (70.5%) patients. CUE was normal in 10 (83.3%) of the patients. The culture was sent within 1-7 days in 12 (70.5%) patients. Of 208 patients with clean catch urine culture, CUE was done in 174 (83.6%) patients. CUE was normal in 162 (93.1%) of patients. The culture was sent in 1-7 days in 146 (70.1%) patients. <i>Escherichia coli</i> was the predominant organism isolated in clean and catheter catch. Of 450 polymicrobial cultures, Gram stain showed pus cells with/without organisms in 66(14.6%) of cases.</p> <p><b>Conclusion:</b> In the majority of the urine cultures, there was no correlation between microscopy and culture. The samples would have been sent without proper indication and collected improperly. A positive urine culture alone is insufficient for the diagnosis of UTI, it has to be correlated with microscopy and clinical history.</p>

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## Introduction

Urinary tract infection (UTI) is the most common bacterial infection. Urinary tract infections are reported to be the second most common infections affecting up to 15% of women in any given year and 50% of women during their lifetime. Urine cultures often make up the largest portion of the workload for a hospital-based microbiology laboratory and are the most frequent sample presented to microbiology laboratories and are probably the most common microbiology procedure where the specimen is self-collected by the patient (1, 2).

Pan culturing of urine cultures without proper indication, and improper collection of specimens leads to false positive results leading to antibiotic overuse in hospitals.

Asymptomatic bacteriuria (ASB) is a common condition in which bacteria are present in the urine without related symptoms or pathologic consequences (3). ASB increases with age and is more common in older than young adults (4) and in 7–10% of men and 17–20% of women over 75 years of age (5).

The use of antibiotics to treat ASB is a significant contributor to antibiotic overuse in hospitalized and nursing home patients, especially among patients with urinary catheters.

Translating evidence-based guidelines on ASB and catheter-associated urinary tract infection (CAUTI) into bedside decision-making when facing a patient with a positive urinalysis or urine culture can be difficult for healthcare professionals (6). Appropriately managing the factors affecting the pre-analytic phase of urine culture contributes significantly to meaningful culture results that ultimately affect patient diagnosis and management. The aim of our study is to identify and evaluate preanalytic practices associated with urine specimens and assess their impact on the accuracy of urine culture microbiology.

## Materials and Methods

A retrospective study was conducted in January 2019-June 2019 with Inclusion criteria of positive cultures with growth of isolated pathogens with Gram stain showing no pus cells/organisms Polymicrobial growth with Gram stain showing pus cells with/without organism. The details regarding the demographic details, type of sample, repeat cultures sent, complete urine analysis (CUE), the time interval between admissions to culture requisition, and organisms isolated were analyzed.

## Results

A total of 530 patients showed growth of single pathogens. In 225 patients (42.4%) Gram stain showed no pus cells /organisms. In 208 (93.4%) patients the sample was clean catch midstream urine and was catheter catch in 17 patients (3.1%). Of 17 patients with catheter catch, the male-to-female ratio is 2.5:1, and the median age is 55 years. 6 /17 (35.2%) were in the age group of 60-70 years. Urine culture and CUE were done in 12 /17 (70.5%) patients, CUE was not sent in 5/17 (29.4%) patients. CUE was normal in 10/12 (83.3%) and high in 2/12 (16.6%).The Interval of time between admissions to request for culture for catheter catch urine samples was given in Table 1. *Escherichia coli* was the predominant organism isolated in 6/17 (35.2%) followed by *Klebsiella pneumoniae* in 3/17 (17.6%) patients. Extended spectrum beta lactamase producers (ESBL) producers were found in 5 /17 (29.4%) followed by multidrug resistant in 5/17 (29.4%) patients. (Table 2).

Of 208 patients with clean catch, the male-to-female ratio is 1.3:1 and the median age is 50 yrs. 48/208 (23%) were in the age group of 50-60 years. Urine culture and CUE were done in 174/208 (83.6%) patients and CUE was not sent in 34/208 (16.3%) patients.

**Table 1.** Interval of time between admissions to request for culture for catheter catch urine samples.

Variable	With CUE	Isolated cultures	Total
<b>Days from admission to culture requisition</b>			
Same day	1	0	1 (5.8%)
1-7 days	9	3	12 (70.5%) *
> 7 days	2	2	4 (23.5%)
Total	12	5	17

\* (6 cultures sent after 1 or 2 days of admission)

**Table 2.** Pathogens isolated from catheter catch urine samples.

Pathogens	Number (%)	Sensitive	ESBL *	Multi-drug resistant (MDR) †
<i>Escherichia coli</i>	6 (35.2%)	0	4	2
<i>Klebsiella pneumoniae</i>	3 (17.6%)	1	0	2
<i>Pseudomonas aeruginosa</i>	1 (5.8%)	0	0	1
<i>Acinetobacter baumannii</i>	1 (5.8%)	1	0	0
<i>Proteus vulgaris</i>	1 (5.8%)	0	1	0
<i>Serratia marcescens</i>	1 (5.8%)	1	0	0
<i>Enterococcus faecalis</i>	3 (17.6%)	0	0	0
<i>Trichosporon asahii</i>	1 (5.8%)	-	-	-
<b>Total</b>	<b>17</b>	<b>3 (17.6%)</b>	<b>5 (29.4%)</b>	<b>5 (29.4%)</b>

**Table 3.** Interval of time between admissions to request for culture for clean catch midstream urine samples.

Variable	With CUE	Isolated cultures	Total
<b>Days from admission to culture requisition</b>			
<b>Same day</b>	24	5	29 (13.9%)
<b>1-7 days</b>	122 (86 cultures sent on one/two days after)	24 (20 cultures sent on one/two days after)	146 (70.1%)
<b>&gt; 7 days</b>	28	5	33(15.8%)
<b>Total</b>	<b>174</b>	<b>34</b>	<b>208</b>

**Table 4.** Organisms isolated from clean catch midstream urine samples.

Pathogens	Number	Sensitive	ESBL*	MDR <sup>†</sup>
<i>Escherichia coli</i>	131 (62.9%)	20	84	27
<i>Klebsiella pneumoniae</i>	26 (12.5%)	10	4	12
<i>Pseudomonas aeruginosa</i>	13 (6.25%)	9	0	4
<i>Proteus mirabilis</i>	1 (0.4%)	0	0	0
<i>Morganella morganii</i>	3 (1.4%)	0	0	0
<i>Enterobacter cloacae</i>	1 (0.4%)	0	0	1
<i>Citrobacter freundii</i>	2 (0.9%)	1	0	0
<i>Serratia marcescens</i>	3 (1.4%)	1	0	2
<i>Enterococcus faecalis</i>	20 (9.6%)	0	0	0
<i>Enterococcus faecium</i>	9 (4.3%)	0	0	9
<b>Total</b>	<b>208</b>	<b>41 (19.7%)</b>	<b>88 (42.3%)</b>	<b>55 (26.4%)</b>

CUE was normal in 162/174 (93.1%), high in 12/174 (6.8%). In 42/208 (20.1%) patients repeat cultures were sent, 39/42 (92.8%) patients which was sterile and in 3/42 (7.1%) patients the same growth of observed. The Interval of time between admissions to request for culture for clean catch urine samples was given in Table 3. *Escherichia coli* was the predominant organism isolated in 131/208 (62.9%) followed by *Klebsiella pneumoniae* in 26/208 (12.55) patients. ESBL producers were found in 88/208 (42.3%) followed by multidrug-resistant in 55/208 (26.4%) patients (Table 4). Of 450 polymicrobial cultures, Gram stain with pus cells with/without organisms was 66 (14.6%).

## Discussion

Differentiating asymptomatic bacteriuria from urinary tract infection (UTI) is a common diagnostic challenge among hospitalized patients (7). Data on the epidemiology of bacteriuria and pyuria in inpatient settings are limited (8).

In our study, most of the patients were in the age group of 50 years and there was a male predominance.

Bacteriuria can be detected microscopically using Gram staining of uncentrifuged /centrifuged urine specimens. Gram stain provides the preliminary report to the clinician and helps them in selecting empiric antimicrobial therapy (9). The sensitivity and specificity of Gram stain vary depending on colony counts. It is more sensitive when the colony count is  $>10^5$  CFU/ml, hence can be used in patients with acute pyelonephritis, invasive UTI, etc (9). The sensitivity and specificity are around 97.8% and 80% respectively with culture as the gold standard (10). In the present study, 42.4% (93% were clean catch and 3.1% were catheter catch) of cases Gram stain was negative but the culture showed growth of organisms with colony count  $>1$  lakh CFU/ml. In these cases, it will be difficult to determine whether the patient has an infection or not, without a proper history.

The diagnosis of UTI relies on clinical and laboratory findings, a positive urine culture alone is insufficient. Concurrent illnesses complicate urine culture interpretation in hospitalized patients, thus findings from urinalysis can be a valuable diagnostic aid. Guidelines support using urinalysis and/or urine microscopy to help differentiate UTI from asymptomatic bacteriuria

(3). Urinalysis provides vital information in the clinical management of patients in the emergency department (2). In the present study, study CUE was done in 70.5% of catheterized patients and 83.6% of clean catch samples. In other cases, only urine cultures were sent.

For patients with urinary catheters, the incidence of bacteriuria is 3–8% per day, with nearly all catheterized patients becoming bacteriuric after one month (12). In one study of hospitalized patients, the rate of bacteriuria in catheterized patients was 51% versus 18.6% among non-catheterized patients (8). In a point prevalence study from the US, 67.7% of patients with UTI were catheterized (13). In a study from Canada of cultures without indication, 21% (16 of 76) were from catheterized inpatients, and of them, only 5% had UTI (8). Urine culture is positive 24 to 45% of the time when ureteral stents or urinary catheters are known to be colonized. The post-operative risk of infection in endo-urolurgical surgery in a patient with ureteral stents or urinary catheters is estimated at the diagnosis is missed around 8 to 11% depending on the type of surgery (14). In our study, all the catheterized patients had a colony count of > 1 lakh CFU/ml probably due to bacteriuria as a result of the longer duration of catheterization.

Nearly half of urine cultures ordered without clinical indication are from patients without an indwelling urinary catheter despite having the lowest risk for UTI (2%) and reporting results of urine cultures might do more harm than good (8). A study from Toronto, found that 67.8% of cultures were ordered without a clinical indication 42% were non-catheterized and on analysis, only 2% had symptoms of UTI (8). Around 93.4% of our cases were clean catch samples of which 93.1% of the patients had normal urine microscopy (CUE, Gram stain). Only 6.8% of patients had pus cells in CUE, and the culture showed significant growth. In all these patients whether there was an infection is questionable. As

it was a retrospective study, we could not get the patient's clinical history.

Frequent urine culturing as part of a diagnostic evaluation for fever (ie, “pan-culturing”) may increase the detection of asymptomatic bacteriuria and funguria that do not require treatment (7). A study from Canada found that of urine cultures without indication 30% of urine cultures were from non catheterized patients at hospital admission and 7% were from catheterized patients at hospital admission (8). In our study, we found that in both cases of clean and catheter catch, most of the samples (70.1% of clean catch & 70.5% of catheter catch) were sent within 7 days of admission: in them, the majority (72.6% of clean catch & 50% of catheter catch) are sent within 1 or 2 days of admission. As this is a retrospective study we were unable to conclude whether they were sent with a proper indication or sent as a part of the routine investigation after admission.

If urine cultures are ordered without clinical indication it leads to the detection of ASB that results in unnecessary therapy in more than 50% of patients (8). More than 60% of the patients are treated empirically for UTI in emergency departments (15). In retrospective studies involving hospitalized patients and residents of long-term care facilities, 32.8%–41.0% of patients with ASB receive antimicrobial therapy (8). Misdiagnosis of asymptomatic bacteriuria or funguria as CAUTI is a major cause of inappropriate antimicrobial use (7). The rates of inappropriate treatment of ASB were reduced from 48% to 12% when urine cultures were not reported in asymptomatic patients (8). In our study, multidrug-resistant Gram-negative bacteria were predominantly isolated which may lead to inappropriate treatment of the patients.

Treatment of ASB has been associated with the emergence of resistant organisms and subsequent UTI risk among women with recurrent UTIs (5). Hence, reducing the treatment of asymptomatic bacteriuria (ASB), or isolation of bacteria from a urine specimen in a patient without urinary tract

infection (UTI) symptoms, is a key goal of antibiotic stewardship programs.

In our retrospective study, 14.6% of the cases showed pus cells in the urine but there was polymicrobial growth in culture; probably due to improper collection where the sample has to be repeated to know the pathogen causing infection. A review of the results of 2000 ED-sourced midstream urine (MSU) samples indicated contamination by squamous epithelial cells ( $\geq 10$  cells per field) in 41.5% of samples from women and 5.4% of men (2). False positivity is high due to contamination of urine samples during collection, especially in women. This is mostly because patients were not aware of proper collection techniques (2). In catheterized patients, the technique of collection is more important. Most of them are collected from urobags which could give a false positive result. In our prospective study, all the catheterized samples were collected from urobags which showed the growth of organisms with a colony of  $> 10^5$  CFU/ml. Apart from sample collection, delays in the transport of samples also lead to false positive results. In our prospective study, there was a delay in transport in 8% of patients. All the factors should be kept in mind while collecting urine for culture which would reduce contamination and hence false positive/negative results. Sample contamination can lead to diagnostic ambiguity or incorrect diagnosis and inappropriate treatment. This in turn may lead to poorer patient outcomes and increases the misuse of antibiotics and overall resistance. The need to repeat samples incurs additional costs, prolongs time for diagnosis and treatment, and can increase patient anxiety and time spent in the hospital (2).

## Conclusion

From this study, we conclude that the in majority of the urine cultures, there was no correlation between microscopy and culture. The samples would have been sent without proper indication

and collected improperly. A positive urine culture alone is insufficient for the diagnosis of UTI, it has to be correlated with microscopy and clinical history. Proper indication, collection, preservation, storage, and transport, of urine are the major factors affecting the pre-analytic phase of urine culture. Hence, managing the pre-analytic factors for urine cultures helps in the generation of meaningful culture which will help in proper patient diagnosis and management.

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There was no funding for this research.

## Ethics approval and consent to participate

This study involved the samples which were routinely sent to microbiology for culture and the data was collected retrospectively from the results of the culture. Samples were not collected specifically for the study. The data was collected retrospectively and clinical details were not collected. Hence ethical approval was not taken.

## Conflict of interest

All the authors declare no conflict of interest.

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