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Original Article

Microbiology Section

Catalase Test and Gram Staining of Uncentrifuged Urine for the Diagnosis of Urinary Tract Infection: A Cross-sectional Study

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ABSTRACT

Introduction: Urinary Tract Infection (UTI) is caused by the abnormal growth of the pathogen in the urinary tract. Urine Microscopy and culture is still the gold standard for the isolation of bacteria. However, screening tests are cost-effective and more practical in managing UTIs.

Aim: To determine rapid screening tests (Gram Staining and Catalase test) for detection of UTI keeping culture as a gold standard.

Materials and Methods: The cross-sectional study was done in the Department of Microbiology, Jawaharlal Nehru Medical College; Acharya Vinoba Bhave Rural Hospital, Sawangi Meghe, Wardha, Maharashtra, India, for a period of one year from August 2019 to September 2020. In this study, 100 urine samples were processed by screening tests such as the Catalase test and Gram stain, followed by culture.

Results: Of total 100 urine samples 51 were from males and 49 from females. Positive predictive value of the catalase test was 55.31% and Gram's stain was 78.26%. In contrast, the negative predictive value of catalase was 69.81%, and Gram stain was 88.88%. The sensitivity and specificity of the catalase test was 61.90% and 63.79%, respectively and the sensitivity and specificity of Gram stain was 85.71% and 82.75%, respectively.

Conclusion: Gram stain had the highest sensitivity, 85.71%, and specificity of 82.75% compared to the catalase test. *Candida* spp. was the most frequently isolated from a urine culture, followed by *Enterococcus* spp. *E. coli, and Klebsiella* spp. were also commonly isolated from people.

Keywords: Culture, Rapid screening tests, Urine microscopy

INTRODUCTION

The UTI is defined as the invasion of pathogens to the urinary tract tissues extending from the renal cortex to the urethra which includes prostate, urinary bladder, kidney [1]. UTIs are among the most common bacterial infections and account for a significant part of the workload in clinical microbiology laboratories [2]. The term "UTI" refers to a broad group of conditions, including urinary tract inflammation and symptomatic or silent microbial invasive infections [3]. The UTI is the most common occurring infection in the population after Upper Respiratory Tract (URT) infection [4]. The fluids and waste products in typical urine are sterile and devoid of germs. A person's hydration and food intake will determine how much urine they produce [5]. The host urethra contains bacteria that colonise its transitional epithelium, rendering the upper urinary tract sections sterile [6].

The UTIs are considered to be one of the most common microbial infections. Due to shorting of the urethra, females are more susceptible to UTI infection, and half of the women will have UTI during their lifetime [7].

The resistance pattern increases worldwide, and conditions vary according to geographical and regional locations [8]. High-risk UTI patients such as catheter patients, pregnant women, and older patients [9]. Clinically, it is difficult to decide as one test is unreliable for confirming a UTI. The advantages of urine microscopy are bacteria, pus cells, Red Blood Cells (RBC), casts, leukocytes, and other cellular elements were directly observed. Gram's staining added benefits to deciding whether there is a urinary infection or bacteria and cells are contaminants (e.g. of vaginal origin) and for guiding antibiotic therapy by observing an organism's morphology and staining property [10-12]. UTI is an inflammatory response of the urethra to the invasion of pathogenic microorganisms [13]. Urine specimens submitted for cultures are approximately 80% culture-negative [14]. It is estimated that there are about 150 million UTI per annum worldwide [15]. There are many screening procedures available, including Gram staining, Catalase test, Nitrate test, Wet mount microscopy, and Triphenyl Tetrazolium Chloride (TTC). Screening tests are rapid and low-cost tests, but their performance characteristics are still questionable [16]. The objective of this study was to evaluate efficiency and to compare catalase test and Gram staining for detection of UTI keeping culture as a gold standard.

MATERIALS AND METHODS

The cross-sectional study was done in the Department of Microbiology, Jawaharlal Nehru Medical College; Acharya Vinoba Bhave Rural Hospital, Sawangi Meghe, Wardha, Maharashtra, India, for a period of one year from August 2019 to September 2020. In this study, 100 urine samples were processed by screening tests such as the catalase test and Gram stain, followed by culture. The study was duly approved by Institutional human Ethical Committee (IEC) (IEC Approval No: DMIMS (DU)/IEC/2019/7935).

Inclusion criteria: A total of 100 urine samples were collected from suspected cases of UTI; voided midstream urine/catheter specimen/ suprapubic aspiration were included in this study.

Exclusion criteria: Samples such as catheter tips were excluded, samples collected more than two hours before processing, and improperly stored samples were excluded from the study.

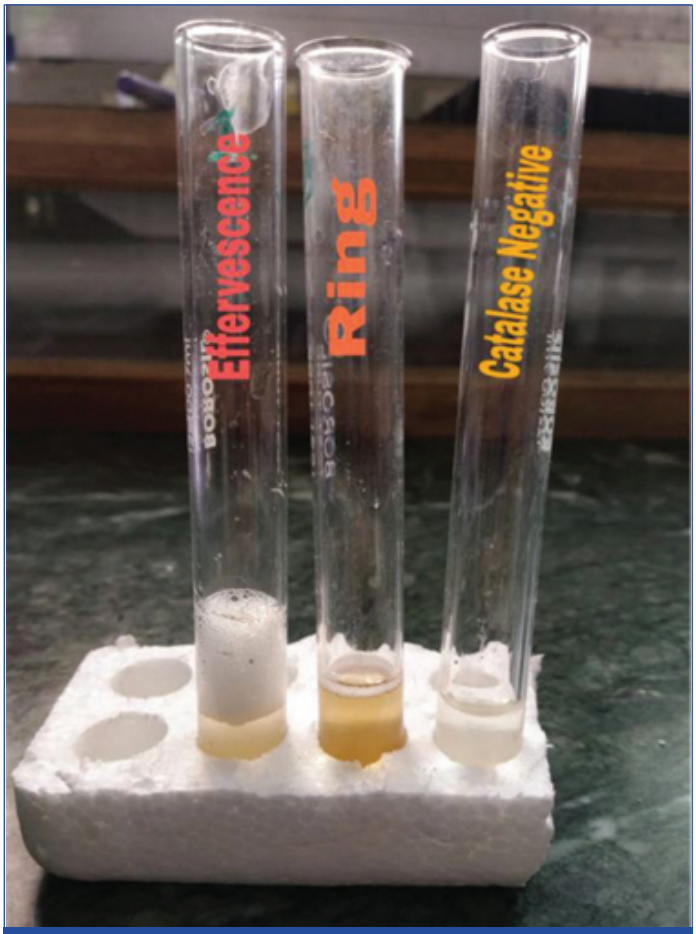
Methodology

Sample collection: A leak-proof sterile container was given to the patient, and they were requested a 10-20 mL midstream urine specimen after explaining the importance of collecting a sample. First, midstream urine was taken. The male patient was instructed to wash their hands before collecting, and a female patient cleaned

around the area of the urethral opening with clean water and swiped with a sterile gauze pad before collecting urine. Urine was collected from the held apart of labia [17].

Macroscopy: The macroscopic examination was done with the naked eye to observe the appearance, colour, and deposit. A cloudy appearance may be due to the presence of bacteria, casts, crystals, leukocytes, and proteins [18].

Screening test: Catalase test: In the test tube, 2 mL of urine was mixed with four drops of 10% hydrogen peroxide. Five seconds were spent gently shaking the mixture. A successful test was considered to have occurred when a whole ring or layer of effervescence formed on the liquid's surface within one to two minutes [19] [Table/Fig-1].



[Table/Fig-1]: Catalase test "Effervescence" or "Ring" consider a Catalase test Positive. No Effervescence or No Ring consider a Catalase test Negative.

Control positive:-*Staphylococcus aureus* ATCC 25923

Control negative:-*Enterococcus* spp. ATCC 51299

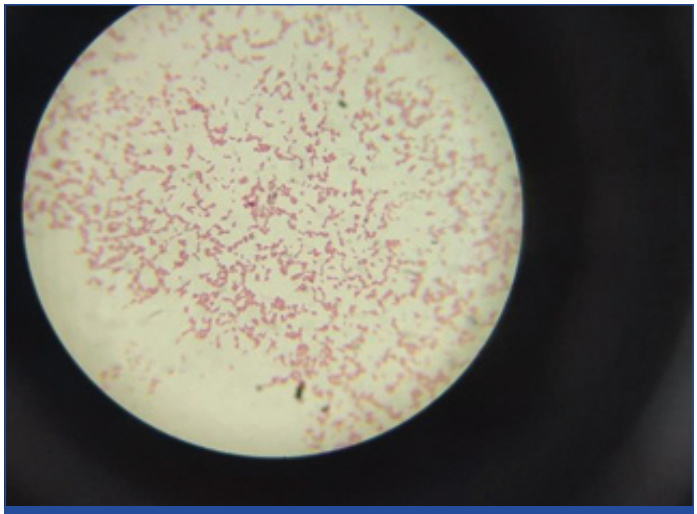
Microscopy: Gram's staining: The presence of ≥1 bacteria in 20 fields on an oil immersion field correlated with significant bacteriuria of $\geq 10^5$ colony forming unit (cfu)/mL of urine [20] [Table/Fig-2].

Urine culture: A sterile 4 mm calibrated loop was used to get the proper amount of specimen and it delivered 0.01 mL volume, it was used to inoculate urine sample to the culture media (Blood agar and MacConkey agar) (to avoid wastage of media, Cystine-Lactose-Electrolyte-Deficient (CLED) agar was not used in the current study) [19].

STATISTICAL ANALYSIS

Sensitivity, specificity, and positive and negative values were calculated according to the following formulae: [20].

- **Sensitivity=**true positive/(true positive+false negative)
- **Specificity=**true negative/(true negative+false positive)
- **Positive predictive value (PPV)=**true positive/(true positive+false positive)



[Table/Fig-2]: Gram Negative bacilli were seen in Gram stain (Under 100X).

- **Negative predictive value (NPV)=**true negative/(true negative+false negative)

RESULTS

Out of 51 males the results for positive catalase test, Gram stain and culture was 24, 25 and 22, respectively. On the other hand, out of 49 females, positivity of catalase test, Gram stain and culture were 23, 21 and 20, respectively as shown in [Table/Fig-3]. In catalase test, out of 100 samples, 47 samples showed catalase positive in which 26 were True positive and 21 were false positive whereas 37 showed true negative and 16 showed false negative in comparing with culture. In Gram stain. out of 100 samples, 46 samples shows positive organism seen in which 36 were true positive and 10 were false positive whereas 48 showed true negative and 6 were false negative as shown in [Table/Fig-4]. The sensitivity and specificity of catalase test and Gram stain is shown in [Table/Fig-5]. In this study out of 100 samples, 42 sample showed culture positive in which different organisms were isolated. Among 42 positive samples, *Candida* spp. (30.95%) were most isolated which was followed by *Enterococcus* spp., *Klebsiella* spp. as 23.81% and 16.67%, respectively, *Acinetobacter* spp. were isolated only in two sample whereas Methicillin-Susceptible *Staphylococcus aureus* (MSSA) and Coagulase-negative *Staphylococcus* (CoNS) were isolated in only one sample each shown in above [Table/Fig-6].

Gender	Catalase test		Gram stain		Culture	
	Positive	Negative	Positive	Negative	Positive	Negative
Male (51)	24	27	25	26	22	29
Female (49)	23	26	21	28	20	29
Total (100)	47	53	46	54	42	58

[Table/Fig-3]: Showing the distribution of patients based on catalase test, Gram's stain and culture as gold standard method.

Test	TP (Screening test positive and culture positive)	FP (Screening test positive and culture-negative)	TN (Screening test negative and culture-negative)	FN (Screening test negative and culture positive)
Catalase test	26	21	37	16
Gram stain	36	10	48	6

[Table/Fig-4]: Showing positivity and negativity of gram stain and catalase test with culture.
TP: True positive; FP: False positive; TN: True negative; FN: False negative

Test	Sensitivity	Specificity	PPV	NPV
Catalase test	61.90%	63.79%	55.31%	69.81%
Gram stain	85.71%	82.75%	78.26%	88.88%

[Table/Fig-5]: Showing sensitivity, specificity, P.P.V and N.P.V of catalase test and Gram stain.
PPV: Positive predictive value; NPV: Negative predictive value

S. No.	Type of organisms	Number of organisms	%
1	Methicillin-Susceptible *Staphylococcus aureus* (MSSA)	1	2.38
2	*Klebsiella* spp.	7	16.67
3	*Escherichia coli*	8	19.05
4	*Candida* spp.	13	30.95
5	*Enterococcus* spp.	10	23.81
6	*Acinetobacter* spp.	2	4.76
7	Coagulase-negative *Staphylococcus* (CoNS)	1	2.38
Total		42	100

[Table/Fig-6]: Organisms isolated from urine culture.

DISCUSSION

Various screening tests such as catalase test and Gram stain were evaluated to diagnose UTIs by keeping culture as the gold standard. In this study, a rapid urine screening test was performed, which indicated high suspected cases of UTI and it could help in earlier presumptive diagnosis. However, culture isolation is considered the gold standard for diagnosing UTIs. The culture of urine results takes a minimum of 24-48 hours for confirmation.

The present study included a bacteriological analysis of 100 urine samples with suspected UTI cases. Out of 100 urine samples, 51 were male patients, and another 49 were female, and the semiquantitative culture method showed, 42 (42%) significant bacteriuria. Out of 42 significant bacteriuria, 22 (52.38%) were male, and 20 (47.61%) were female patients. Gram stain had the highest sensitivity in this investigation (85.71%). On the other hand, Gram staining enables a suppositional identification of the organisms that cause UTIs. Negative samples are quickly tested for a diagnosis, which saves time and money and is useful in high-end laboratories.

There is no test that can diagnose UTI with 100% sensitivity and specificity [21]. Fatima A et al., in a study among the urine culture, significant growth was found in 52.38% of males and 47.61% of females [22]. Catalase test showed 61.90% sensitivity and 63.79% specificity in this study which was less than the study of Naik P and Pinto MJ [23]. In 21% of the cases in this investigation, a false-positive result was seen. This is caused by the presence of renal tissue and inflammatory cells, both of which can create catalase. RBCs can also induce false-positive results, as was previously noticed by Naik P and Pinto MJ [23]. False-positive of Gram stain was found like 10 in this study, similar to the study of Naik P and Pinto MJ [23]. However, Gram stain is an easy and quick procedure with more information on different organisms such as Gram-positive and Gram negative, bacilli or cocci.

Urine culture was also performed in this study *Candida* spp. (30.95%) was the most common isolated fungi, followed by *Enterococcus* spp. (23.81%), *E. coli* (19.05), *Klebsiella* spp. (16.67), *Acinetobacter* spp. (4.76%), MSSA and CONS were isolated as 2.38% from one sample each. *E. coli* was the most frequently isolated organism, according to Fatima A et al., [22]. *Candida* spp., MRSA, and *Citrobacter* spp. were the next most frequently isolated organisms. Arslan S et al., [24] studies showed the growth of organisms as *E. coli* (47%) most commonly isolated, followed by *Klebsiella pneumoniae (*18.5) and least was Staphylococci spp.

Another study by Shobha KL et al., [25] showed *E. coli* (38%) as most commonly isolated, followed by *Acinetobacter* spp., *Candida* spp., *Enterococcus* spp., *Klebsiella* spp. and *Enterobacter* spp. Combinations of screening tests will be useful to reduce morbidity related to UTI in resource-limited areas without access to culture facilities or labs with high patient loads. [Table/Fig-7] shows comparison of present study with other studies [22,23,25,26].

S. No.	Author name	Year of publication, place of study	Gram stain	Catalase test
1	Shobha KL et al., [25]	2014, Manipal	59%	53%
2	Ninama AB and Shah PD [26]	2016, Gujarat, India	NA	88.63%
3	Fatima A et al., [22]	2017, Srinagar, India	96.96%	NA
4	Naik P et al., [23]	2019, Goa, India	85.5%	80.6%
5	Present study	2023, Maharashtra, India	85.71%	61.90%

[Table/Fig-7]: Showing various studies and their findings [22,23,25,26].

Limitation(s)

The drawback of this study was that, it was a laboratory based cross-sectional study conducted in a short period of one year, so the results of the patients could not be assessed. Further, it was a unicentre study conducted only in one hospital. It is suggested that to get more reliable results multicentre study with large sample size can be carried out in future.

CONCLUSION(S)

Gram stain had the highest sensitivity, 85.71%, and specificity of 82.75% compared to the catalase test. *Candida* spp. was the most frequently isolated from a urine culture, followed by *Enterococcus* spp., *E. coli,* and *Klebsiella* spp. These screening tests could help eliminate culture for those samples that could yield no growth on culture. The negative sample is diagnostic by rapid test, which economically solves valuable time and thus is helpful in laboratories. UTIs are diagnosed solely based on clinical criteria, however, early detection and complication prevention have been shown to have significant benefits. No 100% sensitivity and specificity test exist for the UTI diagnosis.

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