Original Research Article

THE ROLE OF GRAM STAIN IN IDENTIFICATION OF URINARY TRACT INFECTIONS: A CLINICAL AND LABORATORY PERSPECTIVE

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ABSTRACT

Background: Urinary Tract Infections (UTIs) are one of the most commonly occurring infections in medical practice. Urine culture and sensitivity is the gold standard diagnostic test for UTI, but it takes 24 to 48 hours to conclude the report. Hence, an easy, inexpensive, lesser time consuming Direct Gram's stain (DGS) of urine may be an effective method in the diagnosis of UTI in patients on the same day. The aim of this study is to assess the diagnostic value of direct Gram stain of un centrifuged urine in patients with UTI.

Materials and Methods: A total of 4021 urine samples, sent for culture & sensitivity from January 2024 to December 2024 were included in the study. All the required data were obtained from the data base of our Microbiology department.

Results: Among the 4021 samples received, direct Gram's staining of 2100 samples showed the presence of organism, among 2100 Gram stain positivity, 2018 samples (96%) were culture positive. Escherichia coli 545 (27%) and Klebsiella species 425 (21%) were the commonest isolates. The Sensitivity, specificity, Positive predictive value, Negative predictive value were 95.64%, 91.51%, 91.90% and 95.42% respectively.

Conclusion: The above findings suggest that direct Gram's staining of uncentrifuged urine was an easy, inexpensive means to provide an immediate information about the causative organism & presence of pus cells and also a guiding tool for empirical antibiotic prescription in patients suspected of UTI, thus helping to decrease the burden of morbidity and also antimicrobial resistance in the above situation.

Keywords: Urinary tract infections, Direct Gram's stain, Sensitivity, Specificity, Positive & Negative predictive value.

INTRODUCTION

Urinary tract infections, one of the most common bacterial infections accounting for 30- 35% in the community as well as in hospital settings. Men and women are affected, but the incidence is higher in females with at least one episode of UTI in their lifetime.[1] Urine culture and sensitivity is the gold standard test for detecting Urinary tract infection. Direct Gram staining of uncentrifuged urine provide an immediate information about the causative organism & presence of pus cells.

Although several rapid tests are done for diagnosing UTI, performing an easy, cost effective Gram's staining procedure of urine sample will provide information about various clinical scenarios like probable UTI, previous antibiotic usage, Asymptomatic bacteriuria, anaerobic urethritis, Acute urethral syndrome, contaminants with peri urethral flora, candidal cystitis well in advance compared to urine culture reports.[2]

This study was conducted to emphasize the importance of Direct Gram stain of urine samples and also its diagnostic validity in various clinical

scenarios to guide the clinician in the empirical management of UTI.

Objectives

- To highlight the importance of urine Gram's stain in the initiation of proper antibiotics in suspected UTI
- To determine the various infectious conditions of the urinary tract by Gram's stain other than the UTI caused by cultivable aerobic bacteria.
- To assess the accuracy of Gram stain in UTI by sensitivity, specificity and predictive value.
- To determine the prevalence & bacteriological profile of Urinary tract infections.

MATERIALS AND METHODS

This cross sectional study was conducted in the department of Microbiology for a period of three months from Jan 2025 to March 2025. All the urine samples collected by any method in a sterile condition sent for culture & sensitivity from all the wards and outpatient departments with probable diagnosis of UTI from January 2024 to December 2024 were included in the study. Immediately after receiving the sample, the following procedures were done in our Microbiology lab to diagnose UTI.

Gram's staining: one drop of well mixed uncentrifuged urine is kept on a clean glass slide without spreading, allowed to dry, heat fixed and Gram's staining was done.[3] The stained smear was examined for the presence of organisms and pus cells. The following grading was used for quantification.

Numerical/ Descriptive	Quantification of Pus cells by American Society of Microbiologist	Quantification of Bacteria(Bailey & Scott)
1+/ Rare	0-5	Less than 1 bacteria per OIF
2+/ Few	5-10	One Bacteria per OIF
3+/ Moderate	11-25	2 – 10 per OIF
4+/ Many	>25	>10 per OIF

Aerobic bacterial culture: Well-mixed, un-centrifuged, undiluted urine is inoculated on to blood agar and MacConkey agar using a calibrated loop that delivers 0.01 ml. The inoculum is done by semi quantitative method with a central streak along the diameter of the plate, followed by streaking across the central line.[2]

The plates are incubated for 18-24 hours aerobically at 35–37∘C. The next day if growth is present, number of colonies are counted & multiplied by factor 100 to get colony forming units (CFU). Further processing with biochemical reactions, Antibiotic sensitivity testing are done to diagnose the infecting organism along with its drug sensitivity pattern and the results interpreted by correlating

with the method of sample collection, presence of organism and pus cells in DGS, clinical history, diagnosis, types of colonies grown and the CFU. [3]

In our study the data regarding age, gender, location from which samples received, probable diagnosis, Direct Gram staining report including quantified organisms and pus cells, organism isolated by culture, antibiotic sensitivity report, colony forming units were obtained from the registers. All the data collected were entered in the Microsoft Excel and statistical analysis was done using SPSS software. Diagnostic test evaluation was also done.

Statistical Analysis

True positive (TP): Direct Gram Stain Positive and Culture Positive.

False positive (FP): Direct Gram Stain Positive and Culture Negative.

True negative (TN): Direct Gram Stain Negative and Culture Negative.

False negative (FN): Direct Gram Stain Negative and Culture Positive.

Sensitivity = TP/ (TP+FN): Probability that Direct Gram Stain will be positive in patients with UTIs (positive culture).

Specificity = TN/ (TN+FP): Probability that Direct Gram Stain will be negative in patients without UTIs (negative culture).

Positive predictive value (PPV): TP/ (TP+FP): Probability that a UTI is present when Direct Gram Stain is positive.

Negative predictive value (NPV): TN/ (TN+FN): Probability that a UTI is not present when Direct Gram Stain is negative.

Ethical clearance was obtained from our Institutional Ethics committee before the commencement of the study.

RESULTS

A total of 4021 urine samples were received over a period of one year, January 2024 to December 2024 from various wards, intensive care units and all the outpatient departments. The gender distribution of the 4021 urine samples were depicted in the Figure1.
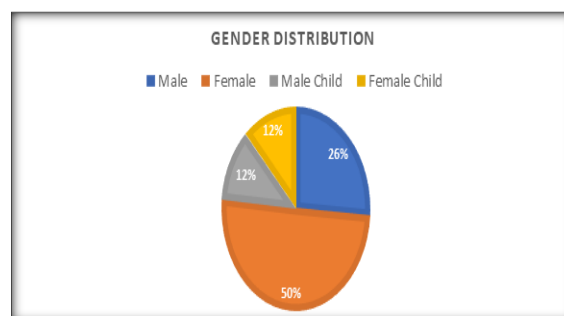

Figure 1: Gender Distribution of the Total Samples (N – 4021)

Figure1: The figure represents the gender distribution of 4021 urine samples.50% of the

samples were from female patients, 26% from the male patients and the remaining 24% was from paediatric patients.
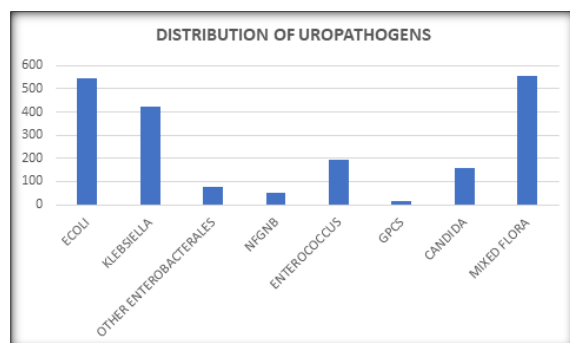

Figure 2: Distribution of Uro-Pathogens among the Urinary Isolates

Figure 2: The figure represents the pathogens isolated from the urine samples. The most common uropathogen is Escherichia coli 545 (27%), Klebsiella species 425 (21%), other

Enterobacterales 76 (4%), Non fermenting Gram negative bacilli 49 (3%), Enterococcus 194 (10%), other Gram Positive cocci like Methicillin sensitive and resistant Staphylococcus 16 (1%), Candida species 157 (7%) and Peri-urethral flora 556 (27%).
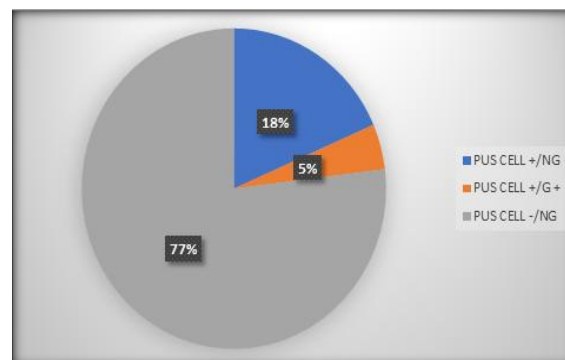

Figure 3: Distribution Of Urinary Pathogens among Negative Direct Gram's Stain Samples: (n = 1921)

Table 1: Distribution Of Urinary Pathogens among Direct Gram's Stain Positive Samples: (n = 2100)

Name of the organism	Culture Positive		Culture Negative
	Presence of Pus cells	No Pus cells	
Escherichia coli	495	19	
Klebsiella species	404	9	
Proteae family	26	1	
Citrobacter species	36	0	
Enterobacter species	8	0	
Non fermenting GNB	41	2	170
Enterococcus	151	26	
MSSA & MRSA	13	0	
Candida Species	143	0	
Peri- urethral Flora	68	488	
Total	1385	545	

Table 1: The table describes the association between culture positivity and negativity among the direct Gram stain positive samples (with organisms).The direct Gram stain showed the presence of organism in 2100 samples/4021 (52%). Culture was positive in 1930/2100 (92%) and culture negative in 170 (8%).

Table 2: Distribution Of Urinary Pathogens among Negative Direct Gram's Stain Samples: (n = 1921)

	Culture Positive	Culture Negative
Escherichia coli	31	
Klebsiella species	12	
Proteae family	2	
Citrobacter species	2	
Enterobacter species	1	
Non fermenting GNB	6	1833
Enterococcus	17	
MSSA & MRSA	3	
Candida Species	14	
Total	88	

Table 2: The table describes the association between culture positivity and negativity among the direct Gram stain negative samples (without organisms). Culture was positive in 88/1921 (5%).

Table 3: Diagnostic Test Evaluation (n= 4021)

Direct Gram Stain	Culture Outcome		Total	
	Culture Positive	Culture Negative		
Organism Present	1930 (True Positive)	170 (False Positive)	2100	Positive Predictive Value = 91.90%
Organism Absent	88	1833	1921	Negative Predictive Value =

	(False Negative)	(True Negative)		95.42%
Total	2018	2003	4021	

DISCUSSION

In this study a total of 4021 urine samples were received for culture & sensitivity from various inpatient and outpatient departments of our hospital for a period of one year from January to December 2024. Among the samples received, 50% of the samples were from female patients, 26% from the male patients and the remaining 24% was from paediatric patients with equal gender distribution. This gender distribution is similar to a study done by Pritam Pardeshi and S Ramalatharani et al.[4,5]

Among the total 4021 samples processed, 2018 samples (50%) were culture positive. The most common uropathogen is Escherichia coli 545 (27%), Klebsiella species 425 (21%), Enterococccus 194 (10%), Candida species 157 (7%) other Enterobacterales 76 (4%), Non fermenting Gram negative bacilli 49 (3%), Methicillin sensitive and resistant Staphylococcccus 26 (1%), and Peri-urethral flora 556 (27%). Ramalatharani et al study showed similar pattern of uropathogen isolation.[5]

In our study, a significant bacteriuria of 105CFU/ml of urine is grown in all samples whose direct Gram's stain grading of organisms is few (one/oil immersion field) and above.[2,5] Presence of organisms in the direct Gram's stain examination of the total urine samples were 2100 (Gram positive & Gram negative). Among these Gram's stain positive samples with organism, pus cells were seen in 1385 samples in which 1317 samples shows growth of uro-pathogens that confirms the patients who have definite UTI and 68 samples shows mixed growth of peri urethral flora.

Also among these 2100 samples, 545 samples shows growth without any pus cells in the direct Gram's stain examination, of which 488 showed mixed growth with more than 3 types of colonies that are contaminants of urine samples with peri-urethral flora which mandates proper sample collection method of clean catch mid-stream urine. The remaining samples without pus cells showed growth of 57 uro-pathogens. Out of these, 55 samples were taken from antenatal mother with growth showing 10^{5}CFU/ml of urine diagnosing asymptomatic bacteriuria in these patients.

About 170 samples showed no growth in culture out of 2100 DGS positive samples. The organisms seen in direct Gram's stain out of 170 samples are Gram negative bacilli (35) associated with pus cells in 21 samples and Gram positive cocci (25) associated with pus cells in 18 samples. The probable cause for the above scenario could be anaerobic urethritis. Gram positive bacilli with morphotype of Lactobacilli without any pus cells are seen in 108 urine samples of female patient that indicates vaginal flora contamination of urine. Two urine samples from male patients showed presence of Gram negative diplococci associated with pus cells

that clearly reveals Gonococcal urethritis in those patients.

The direct Gram's stain findings of 1921 samples were negative without organism. In this, absence of definite UTI were diagnosed in 1478 patients whose DGS of urine samples shows no pus cells. The remaining 443 samples examined showed pus cells without organisms, in which 88 samples gave growth of uro-pathogens of 10^2 – 10^3 CFU/ml of urine that discloses the diagnosis of Acute urethral syndrome. Sterile pyuria were seen in 355 samples that showed pus cells without organisms, and with no growth in culture. Common causes could be prior antibiotic usage, non- gonococcal urethritis, trichomonas vaginalis urethritis, tuberculous infections of urinary tract and very rarely could be a viral urethritis.

Urine culture is taken as the gold standard tests for diagnosis of UTI in our study. The sensitivity, specificity, PPV & NPV of Urine Gram's stain in our study is 95.64%, 91.51%, 91.90% & 95.42% respectively. These statistical values are similar to a study done by Dr. Manoj Kumar et al, Amalia Utami Putri.[6,7]

Viraj wiwanitkit et al study compared Urine Gram's stain and urine microscopy with culture. The sensitivity, specificity, PPV & NPV of Urine Gram's stain was 96.2%, 93.0%, 94.3% and 95.2% which was higher than urine microscopy in his study and the values were similar to our study.[8]

Amit Padmakar Khekade et al. study compared two screening tests, urine Gram's stain and catalase test with urine culture and found that the urine Gram's stain sensitivity, specificity, PPV & NPV are higher than catalase test.[9]

The urine sample with ≤10^{4}CFU/ml shows absence of organism in direct Gram's stain.[10]

CONCLUSION

The direct Gram's staining of urine sample is an easy, cost effective reliable technique that provide same day information about nature of the infecting organism, presence of pus cells to decide about initiating empiric antibiotic treatment according to our institutional antibiotic policy. It quickly identifies the culture negative samples and circumvents the excessive use of media, technical staffs & the overnight incubation period.

The Gram's staining results of urine sample will provide additional information about various infectious conditions of the urinary tract other than the UTI caused by cultivable aerobic bacteria as discussed, well in advance when compared to urine culture reports. The direct Gram's stain of urine samples with organism ≤ 10^{4}CFU/ml is negative and presence of ≥10^{5}CFU/ml of urine samples shows at least one organism /OIF in direct Gram's stain.

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