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Original Research Article

A retrospective study of predictive value of Gram staining in the diagnosis of urinary tract infection

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ABSTRACT

One of the most common infection among humans is Urinary Tract Infection that almost every person will get atleast once in their lifetime. Timely diagnosis and treatment are necessary to reduce the complications from UTI particularly among patients with comorbidities. Urine culture is considered as the gold standard diagnostic test for UTI, however it is costly and time consuming. In this study we evaluated Urine Gram stain that can be used as screening test that is also cost effective with less turn around time. The Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive value (NPV) of Urinary Gram stain were assessed with gold standard culture and sensitivity that showed the point-of-care Gram stain and pyuria can be used as rapid diagnostic test for UTI, which can be carried out quickly at low cost.

1. Introduction

The most common bacterial infection encountered in clinical practices is Urinary Tract Infection both in community as well as in hospital settings. The presence of pus cells, bacteria in Gram stain and culture are important in the adequate management of UTIs.[1] A Gram stain of urine is an easy, inexpensive method with fast turn around time to provide an immediate information about the causative organism of the urinary tract infection. To avoid undue delay for starting empirical therapy for suspected patients of urinary tract infection and to decrease the burden of morbidity caused by chronic UTIs, Gram staining of the uncentrifuged urine is a useful.[2] In this study, all 1500 uncentrifuged urine specimens were subjected to Gram's stain which is then compared to semi quantitative gold standard urine culture tests which showed a better sensitivity of 93.25%, specificity 91.67%, PPV 95.8% and NPV 86.9% thus proving Gram stain as a reliable screening test in diagnosis of UTI.

2. Materials and Methods

2.1. Study design and place of study

This Retrospective study was conducted in Bacteriology section of the Department of Microbiology, K.A.P.V. Trichy government medical college, Trichy, from January 2013 to December 2013. After getting clearence from ethical committee from K.A.P.V. Trichy government medical college, Trichy a total of 1500 uncentrifuged mid-stream urine samples within one hour of collection, were processed for Gram staining and subsequently semi quantitative urine culture.

2.2. Exclusion criteria

Patients on antibiotic treatment were excluded from the study.

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2.3. Procedure

2.3.1. Gram's staining

Danish pathologist Christian Gram in 1884 first described Gram stain which was later slightly modified. The Gram stain is a very important preliminary step in the initial identification of bacteria by differentiating it into either the gram-positive or the gram-negative group.[3,4] Gramstain guides physicians to start empirical therapy can be initiated for patients without awaiting for culture results.

2.4. Procedure

0.05ml of well mixed urine was placed on a clean glass slide, left for air drying, heat fixed and then Gramstaining done (as described by WHO, 2013) as follows: Application of the Primary Stain (crystal violet) to a Heat-Fixed Smear of Bacterial Culture for 1min. After washing primary stain, Add Gram's Iodine and place for 1min. Third step involves Decolorization with 95% Ethyl Alcohol for 1min followed by final step of Counterstain with Safranin for 1min.[3,5]

For interpretation of Gram stain, atleast 20 fields of the smear were examined under oil immersion objectives (100X). According to Kass concept Significant Bacteriuria denotes presence of more than or equal to 1 bacteria per oil immersion field, which corresponds to 100,000 organisms / ml of urine. Pyuria was defined as positive when five leukocytes per oil immersion field and noted in all Gram stain.[4,6,7] A point-of-care Gram stain was deemed positive if pus cells were present, and any organisms observed. This correlates with a colony count of = 10^5 CFU/ml.[5,8]

The morphology of bacteria observed and quantified as follows:

Q uantitation system of gram stain		
S.No.	**Numerical/Descriptive**	
1	1+/ Rare	Less than one bacteria per oil immersion field
2	2+/Few	one bacteria seen per oil immersion field
3	3+/ Moderate	2-10 bacteria seen per oil immersion field
4	4+/Many	>10 bacteria seen per oil immersion field

3. Bacterial Culture

Urine culture and Antimicrobial susceptibility testing: The urine cultures were processed on CLED agar[9] based on procedure of the Calibrated Loop/Surface Streak Method.

1. Re-mix the urine sample, Remove the cap and dip the end of a sterile 1-μL inoculating loop (white) into the urine and spread the inoculum over the surface of a cysteine lactose electrolyte-deficient (CLED) agar that is considered as standard media for urine culture.[6]
2. Make a single streak across the centre and spread the inoculum evenly distributed in a cross-zigzag arrangement to the primary streak.
3. Incubate the plates aerobically at 35–37 ∘C for at 18–24 h

Cultures were considered positive if the cultures yielded $\geq 10^5$ bacterial colonies. Urine Gram stain findings were correlated with urine culture results.[10,11] Gram staining was evaluated with urine culture by calculating sensitivity, specificity, positive predictive value and negative predictive values respectively.

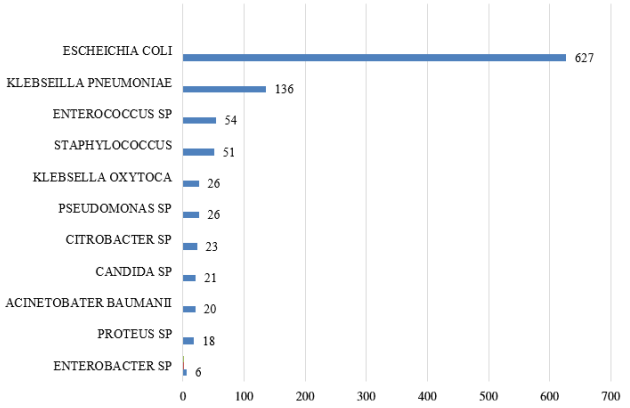

Fig. 1: Urinary pathogens isolated from significant uti specimens

Table 1: Distribution of urinary pathogens with percentage

Urinary pathogens	**Number of isolates (1008)**	**Percentage**
Escheichia coli	627	62.2%
Klebseilla pneumoniae	136	13.4%
Enterococcus SP	54	5.3%
Staphylococcus SP	51	5.05%
Klebsella oxytoca	26	2.5%
Pseudomonas SP	26	2.5%
Citrobacter SP	23	2.2%
Candida SP	21	1.9%
Acinetobater baumanii	20	1.8%
Proteus SP	18	1.5%
Enterobacter SP	6	0.5%

Table 2: Sensitivity, specificity, and predictive values for gram stain

	A.Gramstain			
	Positive culture	**Negative culture**	**Total**	Sensitivity93.2% Specificity 91.67% Positive predictive value 95.8% Negative predictive value 86.9%.
Positive	940	41	981	
Negative	68	451	519	
Total	1008	492	1500	

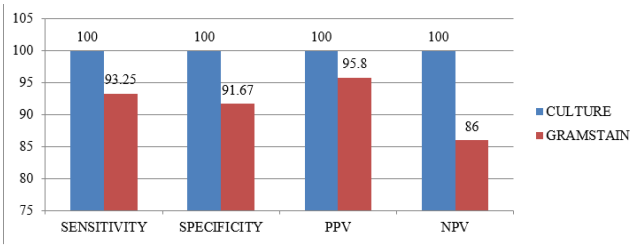

Fig. 2: Showing sensitivity, specificity, positive predictive Value (PPV) and negative predictive value (NPV) of gram staining and culture test of urine.

Table 3: Sensitivity, specificity, and predictive values for pyuria

	B.pyuria			
	Positive culture	**Negative culture**	**Total**	Sensitivity 89.2% Specificity87.6% Positive predictive value 91% Negative predictive value87%.
Positive	900	71	971	
Negative	108	431	539	
Total	1008	492	1500	

Table 4: Sensitivity, specificity, and predictive values – overall urine analysis

C.overall analysis (gramstain & puscells)				Sensitivity 93.2% Specificity, 91.67% Positive predictive value 95.8% Negative predictive value 86.9%
	Positive culture	**Negative culture**	**Total**	
Positive	940	41	981	
Negative	68	451	519	
Total	1008	492	1500	

4. Discussion

The main advantage of urine Gram stain test is providing information about the infecting bacterium and thus guiding the physician for selecting empirical Antimicrobial therapy.[2,9] Physicians have to distinguish UTI from other diseases that have a similar clinical presentation, that the laboratory examination of urine specimens accounts for a large part of the workload in many hospital-based laboratories. Urine cultures are the most common type of culture, accounting for 24%– 40% of submitted cultures in many clinical laboratories.[4,9]

In our study of 1500 urine samples, 37.6% were from males and 60.9% from females which is in concordance with a study by Akeela et al in which among 618 urine samples, 43.04% were from males and 56.95% from females. Among the urine cultures with significant microbial growth, 65.2% were from females and 31.7% from males. This is in concordance to the increased prevalence of UTI in women; the main reason being anatomical and physiological differences between the two sexes.[6,9]

In our study, among culture isolates Escherichia coli was the commonest organism isolated (62.2%), Followed By *Klebseilla Pneumoniae*(13.4%), *Staphylococcus Sp*(5.05)%, *Enterococcus Sp* (5.3%) *Pseudomonas Sp*(2.5%), Klebsiella Oxytoca(2.5)% *Enterobacter Sp*(0.5%), *Proteus Sp*(1.5%) *Acinetobater Baumanii* (1.8%), *Candida Sp*(1.9%), *Citrobacter Sp*(2.2%). Table 1,Figure 1Our results are inconcordance with following studies: Ali M et al., showed that organisms isolated from culture were in the order of; *E.coli* (65%) followed by *Proteus spp.*(16.3%), *Pseudomonas spp.* (7.6%), *Enterococcus spp.*(6.8%) and *Klebsiella spp.*(4.3%).[12] Arslan S et al., in his study showed the growth of organisms on urine culture as; 47% *Escherichia coli*, 18.5% *Klebsiella pneumonia*, 10% *Proteus mirabilis,* and 8.5% staphylococci.[13]

As nearly one third of the urine samples routinely received in the laboratory during the study period showed no significant growth on culture, this results in unnecessary expenditure and delay in patient care. To overcome this by screening tests that can rule out negative samples, are valuable, Time and cost effective thus useful in high-end laboratories. Gram stain test has pitfalls as follows: It is positive only if the concentration of bacteria in the urine is 10^5cfu/ml and It may not detect bacteria at concentrations of 10^2–10^3 cfu/ml, it should not be used in the outpatient setting for patients with complicated UTIs.[10,14]

In the present study un centrifuged urine is more reliable screening test for UTI that is showed by statistical analysis of Sensitivity 93.25%; Specificity 91.6%; Positive predictive value 95.8%; Negative predictive value 86.9%. Our results are in accordance with Taneja N et al, in which he reported Sensitivity of 68.4 %, Specificity of 60.8 %, PPV of 92.7% and NPV of 87.3 % for this test.[4]

Mohamed Ali et al study showed that this test has the sensitivity of 95.7%, specificity of 99.2%, PPV of 99.1% and NPV of 96.2%.[2] A study by SatishSP et al., reported Sensitivity of 89.1%, Specificity of 86%, PPV of 85.4% and NPV of 89.6% with this test.[15]

Matias L et al., study showed that Gram stain had Sensitivity of 92.7%, Specificity of 88.7, PPV of 68.5% and NPV of 97.9%.[16] In a study by Amalia UP et al., this test had Sensitivity of 88%, Specificity of 100%, PPV of 100% and NPV of 90%.[17] By all the above results, our study concludes Urine Gram stain is very reliable screening test for UTI that can guide the physicians in starting empirical treatment for UTI patients.[11,18]

4.1. Statistical analysis

Reserver operating characteristic (ROC) Analysis of Gramstain.

1. ROC analyses were performed in order to define the diagnostic profile of Gram stain in identifying UTI patients among 1500 subjects with gold standard of Urine culture.

Table 5: Gram stain & Urine Culture Cross tabulation

			Urine Culture		Total
			Negative	Positive	
Gramstai	Negative	Count	451	68	519
		% within Urine Culture	91.7%	6.7%	34.6%
	Positive	Count	41	940	981
		% within Urine Culture	8.3%	93.3%	65.4%
Total		Count	492	1008	1500
		% within Urine Culture	100.0%	100.0%	100.0%

2. To this end, Gramstain showed a very good diagnostic profile, describing an AUC of 0.925 (CI: 0.908 to 0.941) with sensitivity 93.25% (CI: 91.53 to 94.72); specificity 91.67%(CI: 88.86 to 93.95); PPV 0.958(CI:0.944 to 0.968); NPV 0.869(CI: 0.840 to 0.893).

The Gram stain method showed significant p value (P<0.0001) to detect UTI patients. The above routine screening test will eliminate the need for cultures in majority of the Negative culture specimens.

Time taken by each test for processing of one sample was calculated and compared and all the microscopic tests were found to be significantly rapid than the culture test. As urine culture takes a minimum of 24 h to show any significant growth, microscopic tests serve as good screening tests for UTIs because of their rapidity particularly in laboratories where a large percentage of urine cultures prove to be negative.

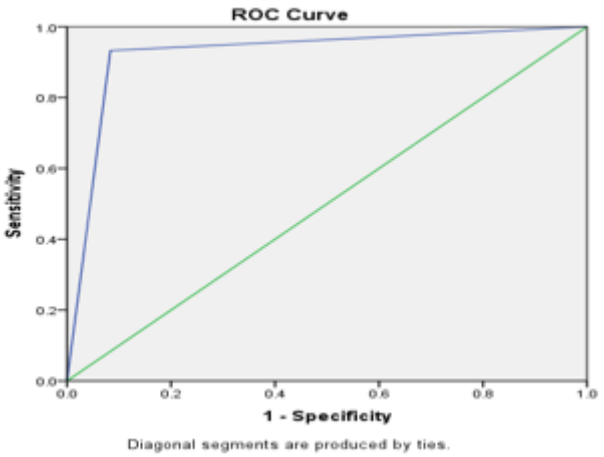

Fig. 3:

5. Conclusion

The Gram stain of Uncentrifuged urine is a very sensitive and specific screening test for diagnosis of UTI. Direct Gram stain smears guides the physician on the initial choice of antibiotics in pending results of culture and sensitivity, Judges Specimen quality, Contributes to the selection of culture media, especially with mixed flora and provides internal quality control when direct smear results are compared to culture results.[9,14] Presence of pus cells in urine gives the clue and supportive evidence of UTI. For clean-catch, un spun urine, the presence of at least one bacteria is likely to indicate a bacterial count of $\geq 10^5$CFU/ml, and the absence of bacteria in several fields on a Gram stain indicates the probability of fewer than 10^4bacteria/ml.[15,17]

Gram stain is a quick and reliable substitute to culture report and in selection of antibiotics for empirical therapy. However, the treating clinicians must look into the local sensitivity pattern of the probable causative agents and in institutions one must follow the recommendation laid by the board of antimicrobial policy.

6. Conflicts of Interest

The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this article.

7. Source of Funding

None.

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