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 at least 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.

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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 clearance 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. Gram stain 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.

For interpretation of Gram stain, at least 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. 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 $\geq 10^5$ CFU/ml.

The morphology of bacteria observed and quantified as follows:

| Q. No. | Numerical/Descriptive | 1 | 2 | 3 | 4 |
|-------|-----------------------|---|---|---|---|
| 1     | 1+/Rare               | 1 |   |   |   |
| 2     | 2+/Few                |   | 2 |   |   |
| 3     | 3+/Moderate           |   |   | 3 |   |
| 4     | 4+/Many               |   |   |   | 4 |

### 3. Bacterial Culture

Urine culture and Antimicrobial susceptibility testing: The urine cultures were processed on CLED agar 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.

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. Gram staining was evaluated with urine culture by calculating sensitivity, specificity, positive predictive value and negative predictive values respectively.

### Table 1: Distribution of urinary pathogens with percentage

| Urinary pathogens      | Number of isolates (1008) | Percentage |
|------------------------|---------------------------|------------|
| *Escherichia coli*     | 627                        | 62.2%      |
| *Klebsiella pneumoniae*| 136                        | 13.4%      |
| *Enterococcus SP*      | 54                         | 5.3%       |
| *Staphylococcus*       | 51                         | 5.05%      |
| *Klebsiella oxytoca*   | 26                         | 2.5%       |
| *Pseudomonas SP*       | 26                         | 2.5%       |
| *Citrobacter SP*       | 23                         | 2.2%       |
| *Candida SP*           | 21                         | 1.9%       |
| *Acinetobacter 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                     | Sensitivity 93.2% | Specificity 91.67% | Positive predictive value 95.8% | Negative predictive value 86.9%. |
|---------------------------------|-------------------|--------------------|---------------------------------|---------------------------------|
| Positive culture                |                   |                    |                                 |                                 |
| Negative culture                |                   |                    |                                 |                                 |
| Total                           | 940               | 41                 | 981                             |                                 |
| Positive                        | 68                | 451                | 519                             |                                 |
| Negative                        | 1008              | 492                | 1500                            |                                 |
was the commonest organism isolated (62.2%), Followed
sexes.
anatomical and physiological differences between the two
prevalence of UTI in women; the main reason being
from males. This is in concordance to the increased
microbial growth, 65.2% were from females and 31.7%
from females. Among the urine cultures with significant
urine samples, 43.04% were from males and 56.95%
with a study by Akeela et al in which among 618
males and 60.9% from females which is in concordance
many clinical laboratories.
laboratories. Urine cultures are the most common type of
for a large part of the workload in many hospital-based
diseases that have a similar clinical presentation, that
therapy.
guiding the physician for selecting empirical Antimicrobial
information about the infecting bacterium and thus
The main advantage of urine Gram stain test is providing
Sensitivity, specificity, and predictive values – overall
Table 4: Sensitivity, specificity, and predictive values – overall
Table 3: Sensitivity, specificity, and predictive values for pyuria
Table 4:

| Positive culture | Negative culture | Total  |
|------------------|------------------|-------|
| B.pyuria          |                  |       |
| Positive         | 900              | 71    | 971  |
| Negative         | 108              | 431   | 539  |
| Total            | 1008             | 492   | 1500 |

Sensitivity 89.2%
Specificity 87.6%
Positive predictive value 91%
Negative predictive value 87%.

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

| Positive culture | Negative culture | Total  |
|------------------|------------------|-------|
| C.overall analysis (gramstain &puscells) |                  |       |
| Positive         | 940              | 41    | 981  |
| Negative         | 68               | 451   | 519  |
| Total            | 1008             | 492   | 1500 |

Sensitivity 93.2%
Specificity 91.67%
Positive predictive value 95.8%
Negative predictive value 86.9%.

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. 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.

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.

In our study, among culture isolates Escherichia coli
was the commonest organism isolated (62.2%), Followed
by Klebsiella Pneumoniae(13.4%), Staphylococcus
Sp(5.05%), Enterococcus Sp (5.3%) Pseudomonas
Sp(2.5%), Klebsiella Oxytocca(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 in concordance 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%).
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.

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 $10^5$cfu/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.

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.

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%. 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.

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%. In a study by Amalia UP et al., this test had Sensitivity of 88%, Specificity of 100%, PPV of 100% and NPV of 90%.

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.

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

| Gram stain | Count | % within Urine Culture |
|------------|-------|------------------------|
| Negative   | 451   | 91.7%                  |
| Positive   | 41    | 8.3%                   |
| Total      | 492   | 100.0%                 |

| Urine Culture | Negative | Positive | Total |
|---------------|-----------|----------|-------|
| Count         | 489       | 940      | 1500  |
| % within Urine Culture | 97.3%  | 92.3%  | 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.

5. Conclusion

The Gram stain of Uncentrifuged urine is a very sensitive and specific screening test for diagnosis of UTI. Direct Gram stain smear 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. 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 ≥10^5 CFU/ml, and the absence of bacteria in several fields on a Gram stain indicates the probability of fewer than 10^4 bacteria/ml.

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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