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Comparative diagnostic performance of conventional and modified gram staining versus culture for urinary tract infection: A protocol

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Abstract:

Urinary tract infections (UTIs) require rapid diagnostics to guide early empiric therapy and avoid culture delays. This prospective cross-sectional study will comparatively evaluate 330 suspected UTI urine samples in a tertiary care setting. We will determine the sensitivity and specificity of conventional versus modified Gram staining against quantitative culture. Modified Gram staining of centrifuged deposits is hypothesized to show superior diagnostic accuracy over conventional methods. This fast, cost-effective screening technique aims to improve timely clinical decision-making and antibiotic stewardship.

Keywords: Urinary tract infection (UTI), gram staining, diagnostic effectiveness, urine culture, modified gram stain, sensitivity and specificity

Background:

UTIs are one of the commonest bacterial infections in the world making it highly morbid and a primary cause of antibiotic prescriptions [1]. The world burden is very high as an estimate of 150 million cases is diagnosed each year. Diagnosis should also be accurate and timely to avoid complications and contain antimicrobial resistance. UTI diagnosis is best done by urine culture, which gives conclusive results of pathogens and their susceptibility to antibiotics [2]. Given the 24-48 hour delay of cultures, rapid diagnostic techniques are essential; recent retrospective studies confirm the high predictive value of Gram staining for early UTI diagnosis [3]. Quick diagnostic techniques such as gram staining are providing fast results. Traditional gram staining is easy and cheap with fluctuating sensitivity (40-70%), which is usually not present in low bacterial counts infection [4]. The goals of modified gram staining methods include enhanced sensitivity due to concentration of microorganisms, *e.g.* centrifuged deposit method, where sensitivities of up to 85-95% have been reported [5]. Although this has the potential, modified gram staining is still not used fully and there are no standard and comparative studies that have compared both conventional and modified against culture in large cohorts, especially in tertiary care centres in resource-limited countries such as India [6]. Therefore, it is of interest to determine the diagnostic usefulness between conventional and modified Gram staining and culture in the diagnosis of urinary tract infections by patients attending a tertiary care facility.

Materials and Methods:**Study design:**

A comparative, cross-sectional, laboratory study.

Setting:

The Microbiology Laboratory of Jawaharlal Nehru Medical College & AVBRH, Sawangi (M), Wardha will be used as the study site.

Study population:

The population of the study will include patients of any age and gender with clinical suspicion of UTI (*e.g.*, dysuria, frequency, urgency, suprapubic discomfort, flank pain, fever or catheter-associated bacteriuria) and has been referred to urine culture.

Sample size:

The approximated sample size is 330 urine samples. The calculation is done on the basis of anticipated sensitivities of conventional (60-65%) and modified Gram staining (85-90%) with confidence interval 95, power 80 and margin of error 10 and the 10% buffer of exclusions [7].

Inclusion criteria:

- [1] Patients with clinical presentation that is indicative of UTI.
- [2] Clean-catch midstream urine samples of non-catheterized patients or aseptically collected catheter urine samples.
- [3] All age patients, both male and female.

Exclusion criteria:

- [1] Antibiotic treatment in the last 48 hours.
- [2] Poorly sampled or overtly contaminated samples.
- [3] Known patients with genitourinary abnormalities.
- [4] Samples those were not large enough to do all three tests.

Sample collection and transport sample collection samples are gathered at specified locations and subsequently moved to other locations to allow analysis. Mid-stream clean urine will be collected in sterile containers following proper patient education. The catheter tubing will have the catheter urine aspirated aseptically. The samples will be marked and carried into the lab in a maximum period of one hour, after collection. All samples will be processed within 4 hours at refrigeration conditions of 4°C if it will take more than one hour to process it [8].

Laboratory procedures:**Macroscopic analysis:**

Both samples will be analyzed in terms of color, turbidity and odor.

Conventional gram stain:

Direct smear in uncentrifuged urine will be prepared, heat fixed and stained by use of standard Gram staining (crystal violet, iodine, decolorizer, safranin). The slide will be observed using oil immersion. The detection of a bacterium ≥ 1 per oil immersion field will be regarded as positive [9]. Adjusted Gram Staining (Centrifuged Deposit Method): 10ml of urine will be centrifuged at 3000 rpm and left to spin in 10 minutes. The supernatant will be thrown away and a smear was made of the sediment which will be Gram-stained and examined as above

[10]. Culture (Gold Standard): The samples will be inoculated on CLED agar, mouse MacConkey agar and Blood agar plates by the use of a calibrated loop (0.001 mL). Plastes are going to be incubated aerobically at 37°C during 18-24 hours. The growth of $\geq 10^5$ CFU/mL will be taken to be significant in case of mid-stream samples and $\geq 10^3$ CFU/mL in catheterized or symptomatic patients [11]. The identification of isolates will be made using standard biochemical tests and antimicrobial susceptibility testing will be conducted according to CLSI guidelines [12].

Data analysis:

The statistical software will be used to analyze data (e.g., SPSS). Parameters of diagnostic efficacy such as sensitivity, specificity, PPV and NPV of both gram staining procedures will be compared to culture as the reference standard. A Chi-square test will be applied to test the statistical significance with a p-value of less than 0.05 to be significant.

Ethical considerations:

In the case of ethical approval, the Institutional Ethics Committee of Jawaharlal Nehru Medical College, Sawangi (M), Wardha will be approached.

Expected outcome:

It is expected that the modified Gram staining would show a better diagnostic sensitivity and general accuracy than the conventional Gram staining [13]. Although urine culture is likely to continue to be an important method of identifying the pathogen and its resistance to different antibiotics, modified gram staining is likely to become a useful, fast and cheap screening method [5, 10]. This would help to initiate proper antibiotic treatment sooner, better manage patients in high-burden environments and can be used in antimicrobial stewardship interventions [14-16].

Conclusion:

The research fills an important gap in the literature, presenting an equivalent, head-to-head the contrast of standard and modified gram staining techniques to culture within a tertiary

care institution. The results will aid in establishing the feasible value of considering the use of modified gram staining as a routine rapid screening test on UTI as a way of streamlining laboratory operations and improving clinical decision making amidst scarcity of resources.

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