

Evaluation of rapid screening methods in the diagnosis of urinary tract infection

Prakash N.¹, Arundathi H. A.², Halesh L. H.³, Siddesh K. C.⁴

^{1,2}Assistant Professor, ³Professor & HOD, ⁴Associate Professor, Dept. of Microbiology, ^{1,3,4}Shimoga Institute of Medical Sciences, Shimoga, Karnataka, ²Kanachur Institute of Medical Sciences, Deralakatte, Karnataka, India

***Corresponding Author:**

Email: drarundathi88@gmail.com

Abstract

Introduction: Urinary Tract Infection (UTI) is one of the most common bacterial infection encountered in clinical practice. Even though urine culture is considered as the gold standard in detection of bacteriuria, many alternative screening methods like urine microscopy, chemical analysis using dipstick method and Gram's staining have been evaluated which are more economical and rapid. Hence, this study was undertaken to evaluate various screening methods in the rapid diagnosis of Urinary Tract Infections.

Objectives: To evaluate Gram staining of urine, leucocyte esterase and nitrite dipstick test methods in the rapid diagnosis of Urinary Tract Infections.

Materials and Methods: All urine samples received at Microbiology laboratory, McGann hospital, attached to Shimoga Institute of Medical Sciences, during study period was subjected to Gram staining, leucocyte esterase and nitrite dip stick test along with culture. Results of these tests were compared to culture results. Sensitivity and specificity of the tests was determined taking culture as standard.

Results: A total of 560 urine samples were processed. Culture showed significant bacteriuria in 210 cases. The sensitivity of urine microscopy was 70.9%, leucocyte esterase test was 98% and nitrite test was 74.8%. Whereas the specificity of urine microscopy was 70%, leucocyte esterase test was 79.1% and nitrite test was 98.8%. Similarly, the sensitivity of combined leucocyte esterase and nitrite tests was 90.9% and specificity was 97.4%. Sensitivity and specificity of all the three screening tests combined were 95.7% and 94.8% respectively.

Conclusion: Screening tests such as, Urine microscopy by Gram's staining, Leucocyte esterase and nitrite dipstick test in combination can be used routinely to exclude bacteriuria. Urine culture can be employed only in the screening tests positive cases and in symptomatic cases irrespective of test results, thereby making diagnosis of UTI cost effective and rapid.

Keywords: Bacteriuria, Leucocyte esterase, Nitrite test, UTI, Urine microscopy.

Introduction

Urinary tract infections are one among the most common bacterial infections and account for a significant part of the workload in clinical microbiological laboratories. UTI can be either symptomatic or asymptomatic. Patients with significant bacteriuria who have symptoms referable to the urinary tract are said to have symptomatic bacteriuria whereas asymptomatic bacteriuria (ABU) is a condition characterized by bacteriuria without classical symptoms attributable to the urinary tract.¹

Even though there are various methods for screening, urine culture is considered as the gold standard test for detecting bacteriuria. However, culture is expensive and takes 24-48 hr to obtain results. To overcome these problems, many alternative screening methods have been evaluated, including urine microscopy, chemical analysis using a dipstick method, Gram staining, dipslide urine culture and bioluminescence. Each method has advantages and disadvantages in terms of sensitivity, specificity, adaptability, capital investment, running costs, automation and convenience for its use.²

Dipstick test, where detection of leucocyte esterase and nitrite is employed, is one of the qualitative diagnostic method used to detect UTI and have the advantage of being easy to perform, interpret, can be carried out in primary care giving facilities and result

can be obtained immediately. Thus, this study was conducted to evaluate the diagnostic accuracy of the rapid dipstick test alone and in combination with Gram stain of uncentrifuged urine to predict urinary tract infection in comparison with the gold standard urine culture method.²

Materials and Methods

1. Source of data: The present study was conducted in Microbiology laboratory, McGann hospital, attached to Shimoga Institute of Medical Sciences, Shimoga for a period of 6 months from July 2015 to December 2015. All urine samples were included in the study.

2. Method of collection of data: Culture

A semi-quantitative calibrated loop technique was adopted for the primary isolation of the organism. A loopful of well-mixed uncentrifuged urine was streaked on to the surface of Blood agar and MacConkey agar. After incubating aerobically for 24 h at 37°C, colony forming unit (CFU) per millilitre of urine was calculated. Significant bacteriuria was taken as $\geq 10^5$ cfu/ml. Samples showing growth of 2 or more bacteria were noted and repeat culture was asked for. The bacterial isolates in samples showing significant bacteriuria were identified by standard procedures and

subjected to antibiotic susceptibility test according to CLSI guidelines.^{3,5}

Gram's staining of urine

A drop of uncentrifuged well mixed urine was taken on a clean grease-free slide and stained by Gram's Method of staining and examined under the oil immersion objective of the microscope. Presence of ≥ 1 bacteria per oil immersion field correlates with significant bacteriuria of $\geq 10^5$ cfu/ml of urine.^{4,5}

Leukocyte esterase test and Nitrite test

Uncentrifuged urine specimens were tested with colorimetric test for the presence of nitrite and leukocyte esterase activity, following the manufacturer's instructions. Briefly, the test strip was dipped in the urine sample and taken out immediately. The strips were blotted with a blotting paper to remove excess urine. Comparative reading was taken at 1 minute and 2 minute intervals for the LET and NT respectively.

For the purpose of the study, the urine culture was taken as the gold standard test. A positive result for an individual test that was also culture positive was taken as 'True positive'. Sensitivity and specificity for each test was calculated by using the formula:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{All culture positive}} \times 100$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{All culture negative}} \times 100$$

Sensitivity and specificity of the two tests were combined in parallel to improve the diagnostic performance of the combined test. Combination in parallel means that the combined test result was interpreted as positive if any one test was positive, and interpreted as negative only when both the tests were negative.

Results

A total of 560 urine samples were received during the study period. Out of these, 210 samples showed significant bacteriuria by culture method. *E.coli* was the most prevalent organism isolated. Microscopy by Gram stain of uncentrifuged urine detected 149 cases of bacteriuria. Leucocyte esterase test was positive in 157 culture positive cases and Nitrite test in 166 culture positive tests.

Test results are as shown in table 1 and table 3 respectively. The sensitivity and specificity of individual tests and combined tests is shown in table 2 and table 4 respectively.

Table 1: Screening test results

	Culture positive	Culture negative	Total
Urine microscopy(gram stain)			
Positive	149	07	156
Negative	61	343	404
Total	210	350	560
Leucocyte esterase test			
Positive	157	105	262
Negative	53	245	298
Total	210	350	560
Nitrite test			
Positive	166	04	170
Negative	44	346	390
Total	210	350	560

Table 2: Sensitivity and Specificity of screening tests

	Sensitivity (%)	Specificity (%)
Urine microscopy (Gram's staining)	70.9	98
Leucocyte esterase test	74.8	70
Nitrite test	79.1	98.8

Table 3: Combined test results

	Culture Positive	Culture Negative	Total
Leucocyte esterase+nitrite test			
Positive	191	09	200

Negative	19	341	360
Total	210	350	560
Leucocyte esterase+nitrite+urine microscopy			
Positive	201	18	219
Negative	09	332	341
Total	210	350	560

Table 4: Sensitivity and Specificity of combined tests

	Sensitivity (%)	Specificity (%)
Leucocyte esterase + Nitrite test	90.9	97.4
Leucocyte esterase + Nitrite + Urine microscopy	95.7	94.8

Discussion

Bacteriuria can be detected microscopically using Gram staining of uncentrifuged urine specimens, Gram staining of centrifuged specimens or by direct observation of bacteria in urine specimens. Gram stain of uncentrifuged urine specimens is a simple method. This test has the important advantage of providing immediate information as to the nature of the infecting organism and thereby guiding the physician in selecting empiric antimicrobial therapy. The important disadvantage of this test is, it is too labor-intensive.^{4,6}

By nitrite test, bacteriuria is detected chemically where bacteria produce nitrite from nitrate. The biochemical reaction that is detected by the nitrite test is associated with members of the family Enterobacteriaceae (pathogens most commonly responsible for UTIs), but the usefulness of the test is limited because nitrite production is not associated with urinary-tract pathogens such as *S. saprophyticus*, *Pseudomonas* species and *Enterococci* species.^{4,6}

Leucocyte esterase tests are based on the hydrolysis of ester substrates by proteins with esterolytic activity. Human neutrophils produce as many as 10 proteins with esterolytic activity. These proteins react with ester substrates to produce alcohols and acids that then react with other chemicals to produce a colour change that is proportional to the amount of esterase in the specimen. A reagent strip impregnated with buffered in doxyl carboxylic acid ester and diazonium salt is used to detect leucocyte esterase. These tests have the advantage of detecting both esterases in intact leukocytes and esterases released after cell lysis. Therefore, even specimens that have not been preserved properly may yield a positive test result. False positive result may also be obtained from high levels of ascorbic acid and albumin in urine.^{4,6}

In our study, the sensitivity of urine microscopy, leucocyte esterase and nitrite test was 70.9%, 74.8% and 79.1% respectively. The specificity of these tests in the same order was 98%, 70% and 98.8% respectively. The sensitivity and specificity of combined leucocyte

esterase and nitrite tests was 90.9% and 97.4% respectively. Sensitivity and specificity of all the three screening tests combined was 95.7% and 94.8% respectively.

Other studies which have evaluated the efficacy of these screening tests are, study by Nayak et al, who have reported the sensitivity and positive predictive value of dipstick test as 68% and 71.4% respectively.⁷ Jayalakshmi et al who have concluded that dipstick test with both Leucocyte esterase and nitrite detection has advantage as screening test with sensitivity of 82.9%.⁸

Our test results confirm and expand the findings of Titoria A et al¹ and Taneja N et al⁹ who have reported almost the same diagnostic performance of these screening tests and have concluded that these tests when combined are of great advantage in screening urinary tract infection.

Therefore, even though urine culture is the gold standard for the diagnosis of urinary tract infection, due to cost and delay of result in culture, combined dipstick with urine microscopy by Gram's staining, which is rapid and cheaper can be used as the initial step in UTI diagnosis. A positive screening test will require culture and sensitivity testing so that the right antibiotic can be prescribed. This will decrease a huge work burden in the laboratory as well as cost burden on the patients, like 332 culture negative cases which were detected by combined screening tests itself in the present study. There will be few cases which could be missed by screening tests, like 9 out of 210 cases in the present study. This can be tackled by doing culture in symptomatic cases even with negative screening results.

Conclusion

Use of combined screening tests like urine microscopy by Gram's staining, leucocyte esterase test and Nitrite test as a routine laboratory practice for faster diagnosis of UTI, where culture is performed only when combined screening tests are positive or in

symptomatic patients even with negative results appears rational and cost effective.

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