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Comparative Evaluation Of Rapid Methods For Detection Of Significant Bacteriuria

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Abstract

Introduction - Number of urine samples received for cultures to diagnose UTI constitute a significant burden on clinical microbiology lab. For this screening tests are employed to rule out UTI. Current study was undertaken to evaluate the various screening tests for their efficacy compared to the gold standard semi-quantitative culture technique.

Materials and Methods - A total of 96 samples were subjected to 5 screening tests: wet mount examination, Gram stain, nitrite test, catalase test and TTC test. All samples were also subjected to semi-quantitative culture. Positivity rate, Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated for all five screening tests.

Results - Of the 96 samples included, 59.3% (57 samples) showed significant growth (≥10 CFU/ml) of a single type of microorganism on semi-quantitative culture. The corresponding positivity rate for Gram stain was 56.25%, wet mount examination 57.29%, catalase test 61.45%, Nitrite test 38% and TTC test 55.20%. The Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) varied for the five screening tests, with Gram stain demonstrating the best figures (Sensitivity - 89.4%, Specificity - 92.3%, PPV - 94.4% and NPV - 85.7%.)

Discussion/Conclusion – Gram. staining was found to have maximum sensitivity, specificity, PPV and NPV among the five rapid methods which were studied. Using one of the various screening methods is a rational and cost-effective approach towards ruling out UTI and rapid diagnosis of UTI for starting the empirical treatment.

Keywords: Gram stain, screening tests, semi-quantitative culture, UTI

Introduction

Infections of the urinary tract are second only in frequency to respiratory infections [1]. However, the number of specimen requests for UTI far exceed the requests for respiratory tract infections in a Microbiology laboratory [1]. Although semi-quantitative culture is the best method to confirm UTI, it consumes time and material and is labour-intensive [1]. Culture may not be widely available in some regions. Urine cultures represent 40 to 70 % of the specimens sent for examination to clinical microbiology laboratories [2]. Although the prevalence of urinary infections may vary in different patient populations approximately 80% of the urine cultures are negative [2]. For these reasons urine samples are screened for presence or absence of bacteriuria/pyuria, which reduces the work load and cost on resources for quantitative urine culture [2].

Rapid diagnostic tests can rule out negative samples, are economical, save valuable time and thus useful in laboratories with a high work load. Screening is also required in special circumstances where it is difficult to identify UTI on basis of clinical criteria alone but where early diagnosis and prevention of complications afford significant benefit (e.g.: children and post renal transplant patients) [3]. Many tests have been proposed to screen for bacteriuria in voided urine including microscopic examination of urine (centrifuged or uncentrifuged), chemical tests of urine (by nitrite, catalase, tetrazolium, glucose or enzymes) and rapid culture techniques (dipstick culture, agar cup or dip slide). The most advantageous test would be both sensitive and specific and give rapid result [4].

The present study was undertaken with the aim of prospectively studying and comparing the most promising rapid test among the following five tests for significant bacteriuria in urine specimen: wet film examination, gram staining, nitrite test, catalase test, triphenyl tetrazolium chloride (TTC) test. Each method was reviewed with respect to its methodology, validity and performance characteristics like sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). For their evaluation semi quantitative culture method with10^5 CFU/ml as threshold level of significant bacteriuria was used.

Materials And Methods

The study was carried out over a period of two months on urine samples received in Microbiology laboratory from inpatient wards and outpatient departments. Approval from Institutional Ethics Committee was taken. It was a comparative study of 5 rapid methods for detection of significant bacteriuria by using semi-quantitative culture methods as reference. After taking informed consent of the patients, collection of urine samples from patients with symptoms of urinary tract infection such as urgency, frequency, burning sensation, fever with chills or pain/pressure in the back or abdomen was done. A total of 96 samples fulfilling these inclusion criteria were studied. Patients were instructed to collect a clean catch, midstream urine sample in a sterile, wide-mouthed, leak-proof container [5]. Each sample was subjected to the five rapid screening tests and semi-quantitative culture. Specimens with polymicrobial growth were excluded from the study.

Urine was inoculated on Blood agar and MacConkey agar plates using a 1 mm standard loop (delivering 0.01 ml of urine), incubated at 37°C for 18-24 hours and examined daily for 2 days. The identification of organism was done by the standard microbiological procedures [5]. Growth was interpreted as significant if colony count was 10^5 CFU/ml or more (significant bacteriuria) [6].

The following rapid methods were performed:

Wet film examination - 50 microlitre (1 drop) of well mixed, uncentrifuged urine sample was placed on a clean, grease free glass slide and covered by 20 mm × 20 mm cover slip. The wet mount preparation was examined under a high power (40 X) microscope for presence of bacteria and leucocytes or pus cells. The cut off for a significant finding was > 1 pus cell per 7 high power fields (hpf) for pyuria. Presence of squamous or tubular epithelial cells, RBCs, tubular casts and crystals was also noted [7].

Gram staining - Smears were prepared using 2 drops of uncentrifuged urine on a slide, air dried, heat fixed and then stained using Gram's stain [8]. Gram-stained smear was observed under oil immersion objective for number of organisms per oil field, morphology and Gram stain characteristics. A positive microscopic examination was defined as presence of ≥ 2 microorganisms per oil immersion field after observation of at least 20 fields [9].

Nitrite test - 3 ml urine was taken in a sterile test tube and centrifuged this for 15 minutes. The supernatant was decanted. To the precipitate, 0.5 ml of a 10% solution of potassium nitrate was added. This was incubated for one half hour at room temperature. Then, 1 ml of the Griess reagent added. The development of a pink or a red colour in a matter of seconds was considered to be a positive test [10].

Catalase test - 2ml of urine was poured into a sterile test tube. 4 drops of 10% hydrogen peroxide were added and the test was interpreted after 2 min. Production of effervescence represented a positive test [11].

TTC test - 2ml of urine was poured into a sterile test tube and 0.5 ml of the working solution of TTC reagent was added with a sterile pipette, shaken

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thoroughly and incubated at 37°C for 4 hours. At the end of 4 hours the deposit was examined with the naked eye. A positive result was shown by a red precipitate and a negative test by the absence of red precipitate [11].

The results of all the five tests were analysed and correlated with the result of culture.

Statistical analysis - Data including patient profile, results of rapid tests and semi-quantitative cultures were recorded and analysed using Microsoft Excel Software. The sensitivity, specificity and positive predictive value (PPV) and negative predictive value (NPV) were determined using the semi quantitative urine culture as the gold standard (reference test) by the following equations [12]:

1	Sensitivity	=	No. true positives / No. true positives + No. false negatives	x 100
2	Specificity	=	No. true negatives / No. true negatives + No. false positives	x 100

3	Positive Predictive Value	=	No. true positives / No. true positives + No. false positives	x 100
4	Negative Predictive Value	=	No. true negatives / No. true negatives + No. false negatives	x 100

Tables

Table 1: Results of Rapid tests and culture method in 96 clinical samples

	Culture	Wet mount	Gram stain	Nitrite test	Catalase test	TTC test
Positive	57	55	54	38	59	53
Negative	39	41	42	58	37	43

Table 2: Results of wet film examination

Wet mount result	Culture results		
	Positive	Negative	Total
Positive	46	9	55
Negative	11	30	41
Total	57	39	96

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Table 3: Results of Gram staining

Gram staining result	Culture results		
	Positive	**Negative**	**Total**
Positive	51	3	54
Negative	6	36	42
Total	57	39	96

Table 4: Results of nitrite test

Nitrite test result	Culture results		
	Positive	**Negative**	**Total**
Positive	31	7	38
Negative	26	32	58
Total	57	39	96

Table 5: Results of catalase test

Catalase test result	Culture results		
	Positive	**Negative**	**Total**
Positive	49	10	59
Negative	8	29	37
Total	57	39	96

Table 6: Results of TTC test

TTC test result	Culture results		
	Positive	**Negative**	**Total**
Positive	39	14	53
Negative	18	25	43
Total	57	39	96

Table 7: Performance characteristics of rapid tests

Parameter	Wet mount	Gram staining	Nitrite test	Catalase test	TTC test
Sensitivity	80.7	89.4	54.3	85.9	68.4
Specificity	76.9	92.3	82.0	74.3	64.1
PPV	89.1	94.4	81.5	83.0	73.5
NPV	73.1	85.7	55.1	78.3	58.1

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Results

96 urine samples were tested which were obtained from the wards as well as Outpatient department. Of the 96 samples, 57 samples (59.3%) showed significant growth (≥10 CFU/ml) of a single type of microorganism on semi-quantitative culture. No growth was reported in 39 samples (40.6%). The number of samples positive by wet mount, Gram staining, nitrite test, catalase test, TTC test were 55, 54, 38, 59 and 53 respectively (Table 1).

Sensitivity, Specificity, PPV and NPV for the five screening tests was calculated by comparing the results of the 96 samples for the five screening tests with the reference test (semi-quantitative culture). The values for the four parameters (Sensitivity, Specificity, PPV and NPV), expressed in percentage, were arrived at as follows:

a = No. of samples positive by rapid tests as well as semi-quantitative culture = true positives

b = No. of samples positive by rapid tests but negative by culture method = false positives

c = No. of samples negative by rapid tests but positive by culture method = false negatives

d = No. of samples negative by rapid tests as well as culture method = true negatives

For wet mount examination, the sensitivity, specificity, PPV and NPV were 80.7%, 76.9%, 89.1% and 73.1% respectively (Table 2). Gram stain results showed sensitivity of 89.4%, specificity of 92.3%, PPV of 94.4%, and NPV of 85.7% (Table 3). The Sensitivity, Specificity, NPV and PPV values of nitrite test were observed to be 54.3%, 82%, 81.5% and 55.1% respectively (Table 4). The Sensitivity, Specificity, NPV and PPV values of catalase test was found to be 85.9%, 74.3%, 83.0% and 78.3% respectively (Table 5). The Sensitivity, Specificity, PPV and NPV of TTC test was found to be 68.4%, 64.1%, 73.5%, and 58.1% respectively (Table 6). The performance characteristics (sensitivity, specificity, PPV and NPV) of each of the rapid screening tests were compared with each other (Table 7). Gram staining showed maximum values of sensitivity (89.4%), specificity (92.3%), PPV (94.4%) and NPV (85.7%).

Discussion

The positivity rate of Gram stain (56.25%), wet mount examination (57.29%), catalase test (61.45%) and TTC test (55.20%) compared favourably with semi-quantitative culture (59.3%). Comparative positive rate for Nitrite test was low (39.58%) (Table 1). However, when the five screening tests were compared on the basis of the four parameters of Sensitivity, Specificity, PPV and NPV, variation in the efficacy of each screening test was observed. Gram stain was observed to be the best among the screening tests, especially with respect to Specificity (92.3%) and PPV (94.4%). The performance characteristics for all other screening tests faltered on one or more of the parameters compared to Gram stain. Wet mount examination had a high PPV (89.1%) but a lower Specificity (76.9%) and NPV (73.1%). Nitrite test had a poor Sensitivity (54.3%) and NPV (55.1%). While catalase test was found to be good enough on all four parameters, with the positivity rate being the highest among the five screening tests. However, Gram stain stood out as a better screening test compared to catalase test after taking into account all four parameters. TTC test, just like the Nitrite test, suffered from a low Sensitivity (68.4%) and NPV (58.1%), apart from a low Specificity (64.1%).

Conclusion

Specimen for urine culture constitute a major work load in Microbiology laboratory. Majority of the urine specimen submitted for cultures turn out to be negative. This can be a strain on resource-starved settings. Hence, using a screening method appears to be a rational and cost-effective approach towards ruling out UTI, especially in laboratories with high work load. Since a screening test rules out infection, it saves time since it is rapid. It also saves cost in processing of all urine samples received by the laboratory for semi-quantitative cultures since the screening tests in general are economical. However, it is imperative to select a screening test with a good sensitivity and specificity so that infections are not missed and at the same time the screening test does not give false positive results that will add to the time and cost involved. Screening tests also aid in rapid diagnosis of UTI for starting the empirical treatment. In the present study, validity of Gram stain was found higher compared to other rapid diagnostic tests and can be recommended as a preferable screening test for UTI.

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