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RESEARCH ARTICLE

EVALUATION OF VARIOUS SCREENING TESTS FOR DETECTION OF URINARY TRACT INFECTIONS

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ABSTRACT

Background: Conventional urine cultures cannot be used routinely on large scale or when there is urgency in diagnosing urinary tract infections (UTI) or where the facilities are not available. In these instances screening tests prove to be more useful. **Aim:** Evaluation of various urinary screening tests for detecting significant UTI for routine use with urine culture as gold standard. **Methods:** 500 samples of mid-stream clean catch urine were collected and processed for various screening tests such as wet mount examination, Gram's stain, catalase test, tri –phenyl tetrazolium chloride test, Griess nitrate test and modified Griess nitrate test. Urine was also cultured by using standard semi-quantitative loop method and results of each test were compared. **Results**: 0f the 500 urine samples, 256 samples were culture positive. Of all the screening tests, evaluated modified Griess nitrate test was found to be most useful while urine wet mount examination found to be the least useful test for screening for urinary tract infection. **Conclusions:** These screening when used together will serve as an aid in diagnosis of urinary tract infections in resource limited settings.

Key words: Urinary tract infections, Urine culture, Screening tests, Wet mount, Modified Griess nitrate test.

INTRODUCTION:

Urinary tract infections (UTI) are amongst the most common infections encountered in clinical practice and also the most common bacterial infection that leads patients to seek medical care. Approximately 10% of humans will have UTI at some time during their illness. This is also the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections[1]. UTI accounts for more than 7 million visits to physician's offices and necessitate or complicate over 1 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations annually[2]. Conventional urine cultures cannot be used routinely on large scale or when there is urgency in diagnosing or where the facilities are not available. In these instances screening tests prove to be more useful. Also screening tests are of more help in busy laboratories so as to reduce their work load and also there will be cost advantage in screening urine in laboratories that receive many culture negative specimens. Samples showing positive screening test only can be further processed for detailed study. The purpose of rapid bacteriuria screening is twofold: (i) to provide accurate information to the physician in a timely manner, which in turn leads to prompt care of the patients; and (ii) to eliminate negative specimens rapidly, allowing the microbiologist to spend more time on positive specimens. The basic requirement of screening tests are that they should show few false positives and false negatives and should be useful to diagnose asymptomatic cases, be easy, reliable, suitable for use on large scale, simple to perform and must be comparable with standard culture methods. With this perspective, the following study was under taken to evaluate the diagnostic accuracy of various screening tests for detection of urinary tract infections taking urine culture by standard semi-quantitative loop method as gold standard.

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MATERIAL AND METHODS:

A total of 500 urine samples were collected from patients of whom 380 were general cases from various out-patient and in-patient departments at Osmania General Hospital, Hyderabad and the remaining 120 samples were collected from pregnant women attending ante-natal clinics at Nayapool Government Maternity Hospital, Hyderabad during the period from June 2007 to June 2008.

Selection of cases:

Cases were selected based on certain inclusion and exclusion criteria.

Inclusion criteria:

Patients with burning micturition, dysuria, frequency, urgency, supra-pubic pain, fever with or without chills, haematuria, and loin pain for duration of 1week.

Pregnant women with or without symptoms were included in the present study.

NOTE: any one or more of the above symptom(s) was considered.

Exclusion criteria

All cases who had taken any type of antibiotics in the past five days.

Clinical specimens:

COLLECTION OF SAMPLE:

Mid-stream urine specimen were collected in sterile plastic containers and transported immediately to the laboratory and processed within 30 minutes.

In case of Women: they were instructed to wash perineum and perirectal region with soap followed by thorough rinse with water. Labia were held apart while voiding and mid-stream portion of urine was collected in a sterile container[3].

Men: In uncircumcised males, they were instructed to retract foreskin and clean glans penis with soap and water, and then mid-stream portion of urine was collected in a sterile container[4].

In catheterized patients: distal part of catheter was clamped and proximal part was disinfected with 70% alcohol and sample was collected from this part with the help of sterile syringe[5].

History of patients' prior antibiotic usage, catheterization, any instrumentation, recent major or minor surgery on kidney or urinary tract etc was recorded in a pre-designed pre-tested proforma.

Urine was processed soon after collection in order to minimize multiplication of any contaminating organisms, if present.

Sample was divided into two parts – one for culture and the other for screening tests.

Appearance and colour of the urine was observed and noted as clear or turbid.

Processing of samples:

First part of sample: was well mixed and using a standard loop, which delivers a volume of 0.001 ml of urine, was inoculated on to solid medium like Blood agar, MacConkey agar, CLED and HiChrome UTI agar.

Using **second part** the following screening tests were done

1. DIRECT WET MOUNT:

0.05ml of well mixed un-centrifuged urine sample was placed on a clean microscopic slide and a coverslip of dimension 22 x 22 mm was placed on the drop and was seen under microscope with 40x objective lens. Pus cells/high power field (hpf) was counted. About 20 fields were searched. Finding >1 pus cell/ 7 hpf indicates significant pyuria. Apart from pus cells, RBC, any casts, bacteria, yeast cells were also noted.

2. GRAM'S STAIN:

A drop of well mixed un-centrifuged urine sample was placed on a clean microscope slide. It is air dried, heat fixed and gram stained. This stained smear was viewed under oil immersion objective. 1 or >1 organism/oil immersion field (oif) was considered significant.

3. CATALASE TEST[6]:

1.5 to 2 ml of urine was placed in a test tube. Four drops of 10% hydrogen peroxide were added to the test tube, and the mixture was shaken gently for 5 seconds. A positive finding was defined as the formation of effervescence sufficient to form a complete ring or layer on the surface of the liquid within 1 to 2 minutes of the addition of the hydrogen peroxide. The test result was considered negative in the absence of effervescence or when the ring of effervescence was incomplete after 2 minutes.

- Positive control- *Staphylococcus aureus*
- Negative control - *Enterococci spp.*

Precaution taken: shaking the H_2O_2 reagent before use will help to expel any dissolved oxygen and false positives can be avoided.

4. TRIPHENYL TETRAZOLIUM CHLORIDE (TTC) TEST[7]:

To 2ml of well mixed uncentrifuged urine, 2-3 pinches of TTC salt was added and incubated for 2hrs.

- Positive test – red precipitate or red colour deposit.
- Positive control- overnight broth culture of *E.coli.*
- Negative control – normal saline.

In case of doubt, precipitate was examined by microscopy to rule out any red cell deposit.

5. GRIESS'S NITRITE TEST[8]:

To 2-3ml of well mixed uncentrifuged urine sample, 0.5-1g mixture of sulphanilic acid and alpha naphthylamine powder was added.
Positive – red colour production within seconds.
Precaution: when nitrite is not detected it is necessary to test whether the organism has reduced nitrate beyond nitrite to ammonia and nitrogen gas. This is done by adding zinc dust.

- If red colour developed – negative test.
- No red colour – positive test.

➢ Positive control – *E.coli*
➢ Negative control – *Enterococci*

6. MODIFIED GRIESS'S NITRITE TEST[9]:
We obtained about 8 ml. of urine in a test tube and centrifuged this for 15 minutes. The supernatant was decanted. To the precipitate, we added 0.5 ml. of a 10% solution of potassium nitrate. This was incubated for one half hour at room temperature. Then, we added 1 ml of the Griess reagent. The development of a pink or a red color in a matter of seconds was considered to be a positive test. Asepsis was strictly observed.

➢ Positive control – *E.coli*
➢ Negative control – *Enterococci spp*.

CULTURE BY - SEMIQUANTITATIVE METHOD:

Standard loop method[10]**:**
Culture was done by inoculating a loop full of well mixed urine on the surface of blood agar, MacConkey, CLED agar and HiCrome UTI agar plates.
A calibrated standard loop was used which delivers a volume of 0.001 ml of urine.

Method of inoculation:

- A calibrated loop was flamed and allowed to cool without touching any surface
- Urine was mixed thoroughly and the lid of the container removed.
- The loop was inserted vertically into the urine.
- Then the loopful of urine was touched over the surface of the agar plate at the center of the plate, from which the inoculums was spread in a line along the diameter of the plate.
- Without flaming or reentering urine, the inoculum was spread across the entire plate in a zig zag fashion, each time touching the central line, to produce isolated colonies.
- Similar steps were carried out for each plate.
- The inoculated plates were aerobically incubated at 37°C for 24 to 48 hrs.

Accordingly, 100 or >100 colonies correspond to 10^5 or $>10^5$ cfu/ml respectively. (Colony count is multiplied with 1000 in order to give estimate of the organisms per ml of urine.)

Ethical considerations[11]**:**
The protocol for this study was approved by the Institutional Ethical Committee (IEC). The approval was on the agreement that patient anonymity must be maintained, good laboratory practice, quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. All work was performed according to the International guidelines for Human Experimentation in Biomedical Research. Approval was obtained from the subjects by taking the informed consent.

Data management and statistical analysis:
During data collection completed questionnaires were checked regularly to check to rectify any discrepancy, logical errors or missing information. The data entry was carried using Microsoft Office Excel worksheet and then exported to statistical software and analysed using appropriate statistical tests by using Statistical Package for Social Services (SPSS vs 15).

RESULTS:

A total of 500 patients were included in the study.

Table 1: Distribution of Cases Based on Sex in our Study

SEX	NUMBER OF CULTURE POSITIVE CASES (X)	NUMBER OF CULTURE NEGATIVE CASES (X)
FEMALE (246) X/246	148	98
MALE (254) X/254	108	146

Table 2: Showing Incidence of UTI With Regard to Age

AGE(YRS)	NUMBER OF CASES(X)	NUMBER OF POSITIVE CASE (Y)	PERCENT OF AGE-ADJUSTED INCIDENCE (Y/X)
20-40	236	164	(69.49%)
41-60	146	62	(42.47%)
61-70	92	14	(15.22%)
>70	16	10	(62.5%)

From the above tables it is evident that the highest incidence of UTI i.e 69.49% is found in age group of 20 -40 yrs. Among these cases females were affected more than males. Next highest incidence is seen in the age group of >70yrs (62.5%) and most of these patients were males.

Table 3: Statistical Values of Various Screening Tests

Tests	True Positives (culture positive, screening test positive)	False Negatives (culture positive, screening test negative)	False Positives (culture negative, screening test positive)	True Negatives (culture negative, screening test negative)
Wet mount examination	196	60	112	132
Gram's stain	220	36	40	204
Catalase test	236	20	50	194
TTC test	222	34	08	236
Griess nitrate test	180	76	14	230
Modified Griess nitrate test	224	32	04	240

Table 4: Sensitivity, Specificity and Predictive Values of Various Screening Tests

Tests	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Wet mount examination	76.56	54.10	63.64	68.75
Gram's stain	85.94	83.61	84.62	85.00
Catalase test	92.19	79.51	85.52	90.65
TTC test	86.72	96.72	96.52	87.41
Griess nitrate test	70.31	94.26	92.78	75.16
Modified Griess nitrate test	87.50	98.36	98.25	88.24

Out of 500 samples include in the study, 256 samples (51.2%) were identified by urine culture to have significant bacteriuria. Urine culture was taken as gold standard against which various screening tests were evaluated for their diagnostic accuracy. Catalase with a sensitivity and positive predictive value (PPV) of 92.19% and 85.52% was found to be the most useful screening in diagnosing UTI followed by modified Griess nitrate test with sensitivity and PPV of 87.50% and 98.25% respectively. Modified Griess nitrate test with specificity and negative predictive value (NPV) of 98.36% and 88.24% respectively was found to be the most useful test in ruling out UTI followed by TTC test with a specificity and NPV of 96.72% and 87.41% respectively. Wet mount examination with sensitivity, specificity, PPV and NPV values of 76.56%, 54.10%, 63.64% and 68.75% respectively was found to be the least useful test in either diagnosing or ruling out UTI. The overall statistical values and their accuracies of various screening test for detection of UTI are shown in Table 3 and Table 4.

DISCUSSION:

Urinary tract infection constitutes a major problem for clinicians in diagnosis, management and treatment. The methods available for assessing the presence of UTI are clinical symptoms as fever, dysuria and presence of burning micturition, microscopic examination such as wet mount, Grams stain, chemical methods and isolation of pathogenic organisms by standard loop method. The diagnosis of UTI is currently based on concept of quantitative bacteriuria. Standard loop method was considered as an adequate substitution

for cumbersome pour plate method. For mass detection of UTI especially in risk groups like diabetics, antenatal cases and children in health surveys, reliable screening method is essential. The purpose of urine screening is to eliminate those specimens that do not contain significant number of bacteria. Whichever method is practiced, the screening test can give satisfactory results only if specimens are properly collected and transported and adequate time for incubation in the bladder is allowed[12].

At one time it was thought that presence of pus cells in urine was a definite hall mark in the diagnosis of UTI. But according to Kass in 1956 the test was only 70% reliable[12]. In the present study 196 (39.2%) cases showed significant pyuria and these cases are culture positive revealing the sensitivity of the test to be 76.56% while 60 (12%) cases showed no pus cells but culture positive. And 112 (22.4%) cases which showed pus cells were found to be culture negative. Only 132 cases showed both no pus as well as were culture negative, showing the specificity to be 54.10%. Carroll KC[13], et al 1994 showed similar sensitivity and specificity. Although the pus cell count of uncentrifuged urine using a Neubauer's counting chamber is a very accurate method, it is very cumbersome and in our study gave low sensitivity and NPV, therefore not useful for screening for UTI. The low sensitivity for wet mount examination observed suggests most probably but not always due to bladder colonization rather than actual infection. However, this is controversial[14]. Besides, hypotonic urine or alkaline urine due to the presenceof *Proteus*, *Klebsiella* and *Pseudomonas* can cause disintegration of the pus cells. The prevalence of 4.1% sterile pyuria may be attributed to infections due to organisms like *Chlamydiae*, which fail to grow in the media used for isolation[15].

All the samples which were taken for study were stained for the presence of bacteria by Gram's stain. Out of 260 Grams stain positive cases, 220(44%) cases showed significant bacteriuria i.e. $>10^5$cfu/ml on culture, and 40(8%) cases were Grams stain positive and culture negative (false positive). Thus it was observed that there is a fair approximation between the presence of bacteria in direct smear and bacteriological counts as said by Kass[12] in 1957.

The higher the count greater is the chance of demonstrating bacteria in direct smears. This constitutes presumptive evidence of bacteriuria

In case of Catalase test the sensitivity and specificity was 92.19% and 79.51% respectively which paralleled to the findings of Yehezkel Waisman[16] et al 1999, where his detection rate was 93.6% and specificity was 81.4%.

By Triphenyl Tetrazolium chloride test we obtained 86.72% sensitivity and 96.72% specificity. Other workers like Neelam Wagle etal[17] (1989) showed positivity of 86.9%[71]. While SV Lavanya et.al[18] (2002**)** in their study of asymptomatic bacteriuria in antenatal women, reported the sensitivity and specificity of TTC test as 76.1% and 85.1% respectively.

It was not well standardized because different bacteria respire at different rates. The only disadvantage of this test is that it requires 2 hrs incubation and cannot be performed for rapid diagnosis on large scale.

We obtained 34(13.28%) false negatives cases owing to enterococcal infection and mixed infections, when compared to 15% obtained by Shannon D Smith et al 1998[19].

The results of **Griess nitrite** test in comparison to culture showed a sensitivity of 70.3% and specificity of 94.26% which correlated well with Bachman JW et al[20] in 1993.

Modification of Griess nitrite was done in order to eliminate these false negatives. By this test we could detect 87.5% of cases as against Srihari et.al[9], who reported 76.4% of detection rate. The specificity of the test 98.36% makes it an useful screening test in ruling out UTI in the absence of facilities for culture.

CONCLUSIONS:

A combination of screening tests have to be performed as there is no single test with 100% sensitivity and specificity and in turn selection of screening tests depends on the laboratory and the patient population being served by the laboratory. Our study suggests that the catalase reagent offers a simple and sensitive test for the rapid screening of urine. Samples exhibiting positive reactions will require routine urinalysis as well as culturing, since the reagent detects both bacterial and somatic cells. Wet mount examination for presence of pus alone as screening test is not an ideal test for detection of urinary tract infections. . Also it is stressed from the study undertaken that a preliminary examination of Gram's stain smear of urine sample is of greater importance in those cases where bacteriological procedure like culture cannot be under taken for want of laboratory facilities.

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