BRIEF COMMUNICATION

Evaluation of Screening Methods for the Detection of Urinary Tract Infection

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ABSTRACT

Background: *Urinary tract infection occurs in all populations, but with particular impact in females of all ages, male at the two extremes of their life, renal transplant patients, and anyone with functional or structural abnormalities of the urinary system.*
Method: *Evaluation of reagent strips, unspun gram stain urine and wet mount as a screening tests for urinary tract infection was done from March 20 to May 30, 2000. A cross sectional study was done on 300 patients attending Jimma hospital. On a morning midstream urine collected from these patients, semi-quantitative culture,a biochemical tests for nitrite, leucocyte esterase, blood and protein using a commercial reagent strip, unspun gram stain and wet mount were done. The data obtained from this study was analysed using EPI-Info statistical package for sensitivity , specificity and predictive values.*
Result: *Using the culture result as a gold standard, the unspun gram stain urine demonstrated the highest sensitivity and negative predictive value (95% and 99% respectively). A single chemical test has not been found to be efficient in detecting significant bacteriuria; however, accuracy increased as the number of combination increased. When a positive result from any one of the above mentioned screening tests were used to indicate the presence of infection, a sensitivity of 88%, specificity of 75% , a positive predictive value of 47% and an impressive negative predictive value of 96% were achieved. In agreement with the result elsewhere this study doesn't support the diagnosis of urinary tract infection on the basis of pyuria alone. The minimum number of bacteria per oil immerssion field that correlate with $\geq 10^5$ CFU / ml is found to be about 20 not ≥ 1 as stated in previous studies.*
Conclusion: *There is no single test for bacteriuria which gives an immediate and infallible result from the above screening tests unspun gram stain urine and combination of chemical tests are simple and reliable screening tests in detecting urinary tract infection. This study recommends the application of these tests as routine test in screening UTI.*

Key words: UTI Screening, Significant bacteriuria, Nitrite, Leucocyte esterase, Proteinuria.

INTRODUCTION

Urinary tract infection (UTI) is the presence of significant number of micro-organisms in a properly collected urine specimen. Although lower counts (as few

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as 10^2 CFU/ml) can be of clinical significance under some circumstances (for instance, infection with gram positive cocci, women with acute urethral syndrome, patient receiving antibacterial drugs); for the majority of patients the presence or absence of $\geq 10^5$ CFU of a probable pathogen/ml may be used as an appropriate indicator of infection (1). UTI occurs in all populations, but with particular impact in females of all ages, male at the two extremes of their life, renal transplant patients, and anyone with functional or structural abnormalities of the urinary system (2).

Among infections, those affecting the urinary tract are second only to respiratory tract infections in frequency, however, the number of specimen requests far exceed the number of upper respiratory tract specimens. As a result urine culture represent a major portion of microbiology work load. The majority of urine specimens (70-80%) submitted to clinical laboratories for investigation are negative or have a bacterial count below levels to be considered to be significant (3, 4).

Although semiquantitative culture is the best method for defining UTI, it consumes considerable time, labour and materials which is a problem in Ethiopia where resources are limited and the method isn't widely available. Therefore, a screening test has dual advantages of providing an approximate result in an area where culture isn't available and saving resources in an area where culture can be performed (4-6).

Before direct application of culture technique many screening tests have been developing in recent years, in an attempt to increase recovery rate and decrease detection time of bacteria. These include: culture techniques such as filter paper and dip slide agar coated -vehicle. Even though these methods are not time consuming, there is a minimum of 12hrs between sampling and reporting of result whatever their accuracy. In laboratories with large work load there is a requirement for an automated system, but they are not affordable for developing countries like Ethiopia. Different chemical tests that are used as a screening test including Leucocyte Esterase (LE), Nitrite, Blood, Protien and catalase are unsatisfactory if used independently because of a substantial number of false negative results and some lack specificity; even if their combination is found to be very sensitive as different evaluations shows. For instance, one study has achieved a sensitivity of 97%, specificity of 70%, a positive predictive value of 40% and an impressive negative predictive value of 99% by considering a positive result from anyone of four chemical tests: LE, Nitrite, Blood and Protien (6-9).

Direct Microscopy of urine is also used as one method in detecting significant bacteriuria. The most easiest approach of direct microscopy with minimum material requirement is that of unspun gram stain urine smear combined with pyuria determination. Studies shows that the presence of ≥1 bacteria /100x objective in unspun urine have a sensitivity of 94% and specificity 90% respectively in reflecting a colony count of $\geq 10^5$/ml (9).

A traditional method used to indicate UTI is wet mount preparation for the detection of abnormal number of leukocytes (i.e., Pyuria). There is one published study in Ethiopia that deals with correlation of pyuria with significant bacteriuria in UTI. This study doesn't support the common practice in Ethiopia of diagnosing UTI on the basis of pyuria alone, since their study reveals that in using pyuria as the sole laboratory

criterion for the diagnosis of UTI 25% of the cases may be missed. On the other hand, over 60% of cases clinically suspected to have UTI may be falsely diagnosed as such on the basis of pyuria (10). The objective of this study is to assess the efficacy of microscopy and chemical tests of urine as screening tests for UTI by using semiquantitative culture as a standard.

MATERIALS AND METHODS

Patient population: From 20 March - 30 May 2000, 300 first morning midstream urine samples from inpatient and out patient department of Jimma hospital on which urinalysis had been requested were randomly selected without taking into consideration of their clinical background.

Specimen collection and processing- *Pa*tients were instructed to bring midstream urine samples to hospital laboratory. All specimens were processed as soon as possible but with not more than an hour. Samples that shows any contamination through investigation were excluded. Inoculation of media, reagent strip tests and microscopy were done in Jimma hospital laboratory and the remaining work is performed in School of Medical Laboratory Technology. In both sites technicians and technologist were involved in the sample processing.

Laboratory technique:

1) ***Validating procedure-*** To quantify the unspun gram stain urine, surface viable count were done based on Miles and Misra method (5). A serially diluted (1:10-10^9) *S.aureus and E.coli* suspension was deposited in separate number of sectors as drops from a pasteur pipette that deliver 0.02 ml/drop on Nutrient agar and MacConkey agar respectively. Counts are made in drop are showing colonies without confluence growth after overnight of incubation aerobically at 37°. Simultaneously, from each and every dilution 0.02ml of bacterial suspension was placed and allowed to dry without spreading, heat fixed and gram stained. After the number of CFU is calculated , the number of bacteria that could be found on slides per average ten oil immersion field is determined for bacterial suspension that contains 10^3-10^6CFU/ml.

2) ***Semi-quantitative culture*** - were done by inoculating, from a standard loop, 0.002ml of uncentrifuged urine on to Nutrient agar and MacConkey agar plates and incubating aerobically at 37°C overnight. Growth of $\geq 10^5$ CFU was considered positive. Mixed cultures with one predominant organisms of $\geq 10^5$ uropathogens /ml were considered positive.

3) *Urine dipstick testing* -(BM Line^{10} test strips -Boehringer Mannheim-Germany) were used according to Manufacturer's instructions. Significant bacteriuria was defined as a dipstick test that give positive result either for the presence of leucocyte esterase activity or for the presence of nitrite, blood or protein. For purposes of comparing screening approaches various combinations of test components were assessed as positive tests.

4) *Unspun gram stain urine smear:* One drop of well mixed unspun urine were put on slide using a pasteur pipette that deliver and allowed to dry without spreading ,heat fixed and stained. The slide was examined for bacteria: a positive smear was defined as more than 20 bacteria per oil immersion field as determined by validating procedure.

5) *Wet mount*- To determine pyuria a drop of thoroughly mixed urine were put on slide and covered by a cover slip of 18x18 mm. The number of WBCs / ten average high power field (HPF) were counted.

Significant pyuria was inferred by the presence of ≥1 WBCs /HPF.
All test results were interpreted without the knowledge of the other test results
6)*Data analysis*-Dipstick . unspun gram stain and wet mount test results were evaluated for sensitivity. specificity and predictive values by EPI-Info statistical package with semi-quantitative culture used as a reference test.

The tests were done on routinely collected urine specimens. Patients were not subjected to non-indicated testing and were not subjected to additional cost. In addition the screening and the culture results were reported to their physicians as they were available.

RESULTS

The participants of the study includes age range of 9 months to 70 years old. These patients consists of 172 females and 128 males where 75% of them were found with the age range of 15-49 years old of both sexes.

The validating procedure was done to verify the unspun gram stain urine quantification. This demonstrate the presence of 20 bacteria/field correlates with significant bacteriuria (10^5 organisms/ml). as shown in table 1. This does not agree with previous studies which states the presence of ≥1 bacteria per oil immersion field is equivalent to $\geq 10^5$ bacteria/ml (9) . Accordingly. this finding was considered to evaluate the accuracy of unspun gram stain urine in detecting UTI.

Table 1. Correlation of semi-quantitative culture and microscopy of unspun suspension of gram positive and gram negative bacteria. Jimma hospital. March 20- May 2000.

Bacterial species tested	No of CFU/ml	No of bacteria in average 10 oil immersion field
S. aureus	3.5×10^6	Full field
	3.5×10^5	40-80
	1.0×10^5	20
	3.5×10^4	7
	3.5×10^3	Nill
E. coli	6.0×10^6	Full field
	6.0×10^5	> 90
	1.0×10^5	20
	6.0×10^4	12
	6.0×10^3	Nill

Among the 300 samples collected. 60 (20%) had significant bacteriuria where 2/3 of these patients were females. The unspun gram stain identifies 95% (55/60) of samples that have significant bacteriuria and nitrite test identifies 100% (240/240) specimens that do not have significant bacteriuria. A summary of the results obtained when the chemical multistix. unspun gram stain and wet mount compared with the gold standard semi-quantitative culture is shown in table 2.

Table 2. Comparison of Chemical Multistix, unspun gram stain and wet mount with semiquantitative culture in detecting significant bacteriuria, Jimma hospital, March 20- May 2000.

Screening tests		Significant growth	No growth or not significant
Nitrite	Positive	14	0
	Negative	46	240
LE	Positive	32	15
	Negative	28	225
Protein	Positive	18	15
	Negative	42	225
Blood	Positive	31	40
	Negative	29	200
Gram stain	Positive	55	55
	Negative	5	185
Wet mount	Positive	30	30
	Negative	30	210

Table 3. Performance of screening methods in comparison with semi-quantitative culture results. Jimma hospital. March 20- May 2000.

Screening tests	sensitivity	specificity	PPV	NPV
Nitrite (N)	24	100	100	84
Leucocyte Esterase (LE)	53	94	68	89
Blood (B)	52	83	44	87
Protein (P)	30	94	55	84
N or LE	63	94	72	91
N or B	65	83	49	91
N or P	47	94	65	88
LE or P	62	88	56	90
LE or B	75	78	46	93
B or P	65	80	45	90
N or LE or B	80	78	47	94
LE or B or P	80	76	45	94
N or LE or P	73	88	61	93
N or B or P	72	80	47	93
N or LE or B or P	88	75	47	96
Gram stain	95	86	66	99
Wet mount	50	77	43	86

From the screening tests involved in this study, unspun gram stain shows highest sensitivity and negative predictive value where as chemical tests- Nitrite, LE, Protein and Blood- ave lowest performance when they are used separately and their performance increases as the number of combination increases. Pyuria determination is also poor in reflecting UTI. A summary of the performance of these screening tests are shown in table 3.

DISCUSSION

For the detection of bacteriuria, the diagnostic sensitivity of nitrite was 23% for $\geq 10^5$ CFU/ml which is comparable with similar studies (8, 11). This poor correlation between the culture and the nitrite test could be due to short incubation time in the bladder (i.e., < 4hrs) or dilution of urine by large volume of intravenous infusions. Beside this, false negative results may also be encountered as result of non-nitrite reducing bacteria such as Enterococcus, Acinetobacter species and some Pseudomonas species or if the patient was in a vegetable free diet which is important source of nitrite. On the other hand, there is no a single false positive result, all specimens without significant bacteriuria did not have positive nitrite test (7, 11).

The sensitivity, specificity, and predictive values of LE is much better than wet mount . The advantage of the former test is that leucocyte need not be viable for LE activity to be detected that is why it has better performance than does microscopic enumeration of neutrophils in settings where time of collection and processing of samples can not be controlled. However, the test is affected by various interferences. This finding supports the previous observation that both pyuria and LE correlate poorly with bacteriuria, i.e., they have a sensitivity of 50 and 53% respectively (12).

Unfortunately , in Ethiopia because of lack of culture facilities in most clinics and hospitals, many physicians diagnose UTI on the bases of pyuria alone on the erroneous belief that the presence of proteinuria is diagnostic. However: this investigation and others show the inferiority of these test in detecting bacteriuria. This is because these tests are non- specific since they can be demonstrated in various clinical conditions (10).

The presence of pus cells in urine may indicate bacterial infection , but some cases of UTI do not exhibit bacteriuria. In addition some cases of infection display pyuria subsequent to antibacterial therapy. Symptomatic patient with pyuria but without bacteriuria suggest urethritis. One should consider obtaining urethral exudate for smears and culture for *Neisseria gonorrhoea*, and urethral scrapings for *Chlamydia trachomatis*. Proteinuria occurs as a result of pre-renal, renal or post renal problem. The only advantage of proteinuria in the presence of bacteriuria will help to differentiate upper urinary tract infection from lower urinary tract infection (10, 11). This discussion shows that, these two determinations are unsatisfactory for differentiating UTI patients.

Although single chemical tests are not efficient in detecting significant bacteriuria their combination found to be very sensitive. If a positive result from protein blood, nitrite and LE were used to indicate the presence of infection a sensitivity of 88%, a specificity of 75% and a PPV of 47% and an impressive negative predictive value of 96% were achieved. This is in agreement with similar studies.

These evaluations revealed high NPV figures, allowing unnecessary work on negative specimens to be eliminated with confidence. In addition to this reagent stip can be done even by paramedical person in office (7, 11).

The unspun gram stain method demonstrated the highest sensitivity and NPV (95% and 99% respectively); with only 5 false negative screens at $\geq 10^5$ CFU/ml level . This value is in close agreement with similar studies that show NPV of 90-95% and a sensitivity of > 90%. This false negative can result if the concentration of bacteria in the urine is in the marginal at the time of detection and frequently occurs with specimens containing gram positive cocci. The predictive values for positive culture and its specificity at significant bacteriuria was 86 % and 66% respectively. The proportion of false positive screens of unspun gram stain were 14%, which may be due to a variety of causes. Organisms such as anaerobes, lactobacilli, diphtheriods or slow growing bacteria in the urine which grow poorly or not at all on conventional media. Bacterial growth may also be inhibited in patient who have been taking treatment for various reasons. In both instances ;therefore, bacteria may be seen in the smear but fail to grow (3, 9, 13).

Stained smears yield useful information about the Gram staining properties and morphology of the probable pathogens which may be useful as a guide to initial antimicrobial therapy, and when several different organisms or many gram positive bacilli were seen, indicates contamination of specimens. The disadvantage using of gram stains for routine screening is the amount of time required before results are obtained especially when a great number of specimens are being processed .

The elimination of routine laboratory culture of most urine samples by simple screening methods is a significant cost saving . Besides this treating positives with appropriate antibacterial agents based on information obtained from local or regional drug sensitivity pattern particularly in areas where culture facilities are unavailable has additional benefit .

In general, the result of these evaluative study, i.e., combination of selected chemical tests and unspun gram stain urine agrees with acceptability criteria of a screening test which states that the screening test should be able to demonstrate a sensitivity and a NPV of at least 95% and specificity should be around 70% and a positive predictive value above 40%(7).

There is no simple test for detection of significant bacteriuria that gives an immediate and infallible result. From the above screening tests, unspun gram stain urine smear and combination of chemical tests are reliable and simple screening methods which do not require any especial equipment and technician in the assessment of the procedures. It is recommended that these screening tests can be applied in local laboratories.

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