Original Article

Predictive Value of Tests in Screening Urine Samples for Bacterial Culture

Prem Anand Nagaraja, El Sayed M M El Aasar
Department of Microbiology, Farwaniya Hospital, Kuwait

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ABSTRACT

Objective: To evaluate the predictive values of screening parameters which would be useful in identifying urine samples that would yield positive results on culture.
Design: Prospective study
Setting: Out patients' clinic, Farwaniya Hospital, Kuwait
Subjects: A total of 7951 urine samples received in the department of microbiology over a period of three months were chosen for the study.
Main Outcome Measures: The predictive values of the screening parameters which would be useful in identifying urine samples that would yield significant growth in culture were analyzed.
Results: Mid-stream urine samples from all age groups analyzed with a combination of leucocyte esterase and nitrite test with either pH or protein assay had a negative predictive value of 100% and a specificity of 100% related to the outcome of bacterial culture. However, the analytical parameters taken singly or in combination showed good predictive values in some age groups only. Substantial savings were realized when unnecessary urine cultures were avoided.
Conclusions: Screening tests are not only useful in providing a rapid indication of presence of urinary pathogens, but also help cut costs of unnecessary cultures and processing of urine samples. A high negative predictive value with a high sensitivity of a combination of leucocytes and nitrite with either protein or pH assay is useful in identifying cases that are not UTIs. The predictive values of screening parameters also help in identifying those patients who may be placed on empiric antibiotic therapy.

KEY WORDS: negative predictive value, positive predictive value, sensitivity, significant bacteriuria, specificity, urinary tract infection

INTRODUCTION

Semi-quantitative urine cultures are done to conclusively diagnose urinary tract infections (UTIs), to isolate, identify bacterial pathogens and perform antimicrobial susceptibility testing. The time and the cost involved in such processing is substantial and it is imperative that truly representative mid-stream urine samples are collected from clinically identifiable cases in order to lower the financial burden of diagnostic laboratories. UTIs are among the most common bacterial infections encountered in clinical practice[1]. Urine analyses are by far the commonest and the most frequently requested tests by clinicians, as an aid to diagnosis of UTI[1]. As many as 60 to 80% of all urine samples received for culture in medical center laboratories contain no etiological agents of infection or contain only contaminants[1]. Microbiology laboratories apply screening assays to identify urine samples that are suitable for culture. These screening methods are insensitive when the bacterial counts are less than 105 CFU/ml and so are not used to screen out urine samples obtained by catheterization, suprapubic aspiration or by cystoscopy[1]. The objectives of this study were to evaluate the screening criteria applied to urine samples sent to the laboratory for routine examination and/or bacterial culture and assess the predictive values of combination of these criteria in various age groups like adults, pediatrics and neonates. Additionally, the utility of the predictive values of these screening criteria in suggesting empiric therapy for clinical cases of urinary tract infections (UTI) is dealt upon.

SUBJECTS AND METHODS

Patients attending the out-patient clinics and admitted patients undergoing investigation for urinary tract infections in Farwaniya Hospital are usually asked to submit mid-stream urine samples for urine analysis and culture. A total of 3821 urine samples were submitted to the microbiology department for routine examination while 3159 mid-stream urine samples were submitted for

Address correspondence to:
Dr. Prem Anand Nagaraja, Microbiologist, Farwaniya Hospital, Post Box 18373, Safat, State of Kuwait. Tel: +965 - 4888000, Ext. 3344, dr.prem@indiatimes.com

Table 1: Screening criteria for urine samples designated suitable for culture[1]

a. All mid-stream urine samples from pregnant women, irrespective of screen results
b. All samples from children less than three years of age
c. Urine samples obtained by catheterization, suprapubic aspiration or cystoscopy
d. Clinical cases of urinary tract obstruction
e. Follow-up after removal of an indwelling catheter
f. Follow-up after a previous therapy for proven UTI
g. Presence of one or more of these five parameters: Alkaline pH, leucocytes 100/µl (2+), positive nitrite test, protein 2+ or erythrocytes 500/µl (3+)

analysis and bacterial culture, both over a period of three months from August to October 2005. Requests for bacterial culture had been made to rule out cystitis or lower urinary tract infection (UTI), pyelonephritis, as a routine in pregnant women and from catheterized patients. Urine samples from pregnant women were sent for analysis during their ante-natal visits or if the patient had symptoms of UTI. All urine samples of pregnant women were processed for detection of significant bacteriuria irrespective of the screening results.

Urine samples were processed immediately on arrival by performing a semi-quantitative urinalysis using COMBUR^{10} TEST® (Roche Diagnostics GmbH, Germany), a 10-parameter assay on a MIDITRON® M (Roche Diagnostics GmbH, Germany), followed by microscopy of uncentrifuged urine for evaluation of leucocytes. Quality control of the instrument and strips was performed daily as part of the standard laboratory practices. Out of the 3821 urine samples received for routine examination only, samples which were found to conform to the screening criteria (Table 1) were processed further by culture; provided the urine sample was submitted in a sterile container. Urine samples submitted for culture conforming to any of the screening criteria (Table 1) were also processed for bacterial culture using standard methodology[1] and identification along with antimicrobial susceptibility was done once the standard growth criteria (Table 2) were fulfilled. All mid-stream urine samples that were screened and excluded from being processed for bacterial culture were reported as "Culture Not Recommended" (CNR). Clinicians were aware of this method of reporting CNR and this was applied to urine samples submitted for bacterial culture excepting those that fulfilled the screening criteria (Table 1). However, urine samples submitted for routine exam alone were processed as such without a CNR comment. Mid-stream urine samples from pregnant women were cultured irrespective of the screening results. Processed samples showing no growth after 24 hours incubation at 37° C were

Table 2: Standard growth and processing criteria for interpretation of culture plates[1]

Result	Specimen Type or Clinical Condition	Workup
10^4 CFU/ml of a single potential pathogen or for each of two potential pathogens	CCMS Urine/pyelonephritis, acute cystitis, asymptomatic bacteriuria or catheterized urines	CW
10^3 CFU/ml of a single potential pathogen	CCMS urine/symptomatic males or catheterized urine or acute urethral syndrome	CW
Three organism types with no predominating organism	CCMS or catheterized urine	Possible specimen contamination Repeat specimen requested
Two or three organism types with predominant growth of one type and < 10^4 CFU/ml of the other type(s)	CCMS Urine	CW for the predominant organism and description of the others.
10^2 CFU/ml of any number of organism types (Culture setup with a 0.001 or 0.01 ml calibrated loop)	Suprapubic aspirates or surgically obtained urine specimens (like ileal conduits and cystoscopy)	CW

CFU = Colony Forming Units, CCMS = Clean-Catch Mid Stream, CW = Complete Workup including bacterial identification and antibiotic sensitivity, Predominant growth = 10^4 - 10^5 CFU/ml

reported as "No growths" while samples showing mixture of organisms were reported as "contaminated" (Table 2). Significant growth in culture plates as determined by standard criteria[1] were processed on VITEK-2 (bio-Me_rieux SA, France) for both identification and antimicrobial susceptibility.

Data was collected for all the samples prospectively for three months and analyzed using Microsoft FoxPro® for MS-DOS® (Version 2.6).

RESULTS

A total of 7951 samples were submitted for routine examination and / or bacterial culture over a period of three months. Out of the 3821 samples submitted for routine examination, 400 urine samples (10.5%) received in sterile containers and conforming to the screening criteria were processed for identification of significant bacteriuria by culture. 2759 of the 4130 mid-stream samples (66.8%) submitted for bacterial culture were decided suitable for processing, while a total of 1371 samples (33.2%) were reported as CNR. Out of the total 3159 samples that were cultured, 905 samples (28.7%) were found positive for significant bacteriuria, while 1960 samples (62%) were reported as "no growths" and 294 samples (9.3%) were reported as "contaminated". Among the 400 samples cultured from the routine group, 312 (78%) showed no growth, 54 (13.5%) were positive for significant bacteriuria and 34 samples (8.5%)

Table 3: Results of the urine cultures - group-wise breakup (%)

	Culture positives n (%)	No growths n (%)	Mixed growths n (%)	Total n (%)
Adult males	73 (17.3)	339 (80.5)	9 (2.1)	421 (13.3)
*Adult females	334 (28.1)	686 (57.8)	167 (14.1)	1187 (37.6)
Pregnant women	155 (27.3)	361 (63.7)	51 (9)	567 (17.9)
Pediatrics (males)	58 (29)	134 (67)	8 (4)	200 (6.3)
Pediatrics (females)	242 (39.9)	324 (53.4)	40 (6.6)	606 (19.2)
Neonates (males)	24 (28.2)	56 (65.9)	5 (5.9)	85 (2.7)
Neonates (females)	19 (20.4)	60 (64.5)	14 (15)	93 (2.9)
Total	905 (28.7)	1960 (62)	294 (9.3)	3159

*The category "Adult Females" include only non-pregnant women

showed mixed growth. A total of 567 samples of urine (17.9% of all cultures) were from pregnant women attending the antenatal clinic or admitted in the hospital. Among these, 155 samples (27.3%) showed significant bacteriuria while 350 samples (61.7%) had one or more of the screening parameters positive.

The group-wise breakup of the results of culture is depicted in Table 3. Results of samples from adults have been categorized to pregnant women and a group comprising of adult males and non-pregnant women (henceforth referred to comprehensively as "adults"). A total of five parameters and 25 combinations of these were evaluated against a positive culture separately in samples obtained from adults, pregnant women, pediatric and neonatal cases. The positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of the various parameters and their combinations with respect to positive culture results are depicted in Table 4. Taking only the five parameters into consideration, the PPV ranged from zero for protein in neonates to 83.3% for nitrite in pediatric cases, while the NPV ranged from 28% for leucocytes in neonates to 96% for leucocytes in pediatric cases. The various combinations analyzed showed a diverse range of values and consensus among the combinations or parameters taken alone was difficult to show between the four groups. However, the combination of pH and nitrite showed a PPV of 100% in all groups except pregnant women and specificity of 100% for all the four groups. Considering only the results of urine samples in pregnant women, the PPV of the screening parameters was 45% with a specificity of 96%.

Out of the 905 positive cultures, members of the family *Enterobacteriaceae* accounted for 76.35% of all isolates with 550 isolates of *Escherichia coli* and 105 isolates of *Klebsiella pneumoniae*. A total of 36 samples showed growth of a second organism. A total of 23 strains (4.2%) of extended spectrum - lactamase (ESBL) producing *E. coli* were isolated. ESBL production was confirmed by testing the isolates with Etest TZ/TZLand CT/CTLstrips (AB BIODISK, Solna, Sweden) according to standardized procedures. The details are depicted in Table 5.

Table 4: Positive and negative predictive values of the screening parameters in relation to bacterial culture results (%)

Patients Category	PPV	NPV	Sensitivity	Specificity
Samples from adult patients	**PPV**	**NPV**	**Sensitivity**	**Specificity**
pH	11.9	87	11.4	87.5
Leucocytes	31.4	62	65	62
Nitrite	74.1	87	24.4	98.3
pH + Nitrite	100	87	1.6	100
pH + Leucocytes + Nitrite + Protein + Erythrocytes		87		99.5
Leucocytes + Erythrocytes + Nitrite	90.6	87	31.9	99.3
Samples from pediatric patients				
Nitrite	83.3	74.5	13.5	98.9
Leucocytes	57.1	96	42.9	96
Protein	50	74.5	1.03	99.6
pH + Nitrite	100	74.5	2.04	100
Leucocytes + Erythrocytes + Nitrite	92.3	74.5	11.1	99.6
Leucocytes + Erythrocytes + pH + Protein	100	74.5	1.03	100
Erythrocytes + Leucocytes + Protein	80	74.5	7.7	99.3
Samples from neonates				
Leucocytes	55.6	28	15.2	28
Nitrite		75.4		100
Leucocytes + Nitrite	100	24.6	3.5	32.6
Leucocytes + Nitrite + Erythrocytes + pH	100	75.4	3.5	100
pH + Nitrite	100	75.4	3.5	100
Leucocytes + Nitrite + Erythrocytes + Protein	100	75.4	3.5	100
Samples from pregnant women				
pH	11.8	82.7	5.6	91.6
Leucocytes	32.2	34	59	34
Nitrite	85.7	82.7	26	98.8
pH + Nitrite		82.7		100
Leucocytes + Nitrite	76.9	17.3	22.7	20.5
pH + Leucocytes + Nitrite + Erythrocytes + Protein		82.7		100

Note:
a. The category "adult patients" includes all adult males and non-pregnant adult women.
b. Results of other parameters and their combinations are not depicted for lack of space.
c. PPV = Positive Predictive Value, NPV = Negative Predictive Value

DISCUSSION

Screening assays in urinalysis play a very important role in preliminary identification of cases of lower urinary tract infection or of pyelonephritis in both adults and children so that empiric antimicrobial therapy is started early in the course of disease thereby limiting morbidity. Even a brief delay in instituting therapy in children below two years is known to cause permanent renal scarring and thus a reliable indicator of urinary tract infection is needed[2]. However, the concept of screening using rapid assays is controversial in

Table 5: Isolates in bacterial culture (%)

	Adults	Pregnant	Pediatric women	Neonates cases	Total
	n	n	n	n	n (%)
Escherichia coli	229	71	232	18	550 (60.8)
Klebsiella pneumoniae	58	22	10	15	105 (11.6)
Enterobacter spp.	7	3	8	0	18 (2.0)
Pseudomonas aeruginosa	17	1	8	2	28 (3.1)
Enterococcus fecalis	11	3	13	3	30 (3.3)
Acinetobacter baumanii	9	2	0	0	11 (1.2)
Staphylococcus aureus	8	3	1	2	14 (1.5)
Streptococcus agalactiae	29	37	6	0	72 (8.0)
Others	39	13	22	3	77 (8.5)
Total - (%)	407 (45)	155 (17.1)	300 (33.1)	43 (4.8)	905

pregnant women. The American Family Physicians, Canadian Task Force on Preventive Health Care and the National Health Services (NHS), UK do not recommend use of screening tests. On the other hand, the American Academy of Pediatrics (AAP) and the American College of Obstetrics and Gynecologists (ACOG) strongly recommend use of screening assays for urinalysis in pregnant women.

Detection of cases of pyuria helps to avoid extensive invasive diagnostic procedures. Microscopic examination of urine is the standard method used to detect pyuria. The dipstick test used widely as a rapid measure of urinary leukocyte esterase (LE) activity is quick, inexpensive, and does not require technical expertise. This test is commonly used to identify pyuria in accident and emergency departments and in out-patient clinics in which a urine microscopy service is not available. Clinics which can avail the assistance of a well-equipped laboratory can utilize the service of complete urinalysis than just depend on a two-parameter dipstick. These laboratories would have facilities to perform screening tests, bacterial culture and identification of microbes on mid-stream urine samples. It is important to note that the quality of urine specimens would determine the outcome of such testing. It is hence ideal to have an early morning mid-stream urine specimen submitted in a sterile container. Single use sterile urine bags are employed for collecting urine samples for neonates and infants. Urine samples are stored at 4°C in case they are not tested at the earliest, to avoid false positive test results.

The LE test detects esterases released from degraded white blood cells (WBCs). It is therefore, an indirect measure of WBCs whose presence is induced by urinary bacteria. The nitrite test detects nitrites produced by urinary bacteria - usually limited to Gram-negative organisms. Nitrites normally are not found in urine but result when bacteria reduce urinary nitrates to nitrites. Many Gram-negative and some Gram-positive organisms are capable of this conversion, and a positive dipstick nitrite test indicates that these organisms are present in significant numbers (*i.e.* more than 10,000 per ml)[3]. This test is specific but not highly sensitive[3]. The nitrite dipstick reagent is sensitive to air exposure, so containers should be closed immediately after removing a strip. After one week of exposure, one third of these strips give false-positive results, and after two weeks, three fourths give false-positive results[4]. Non-nitrate-reducing organisms also may cause false-negative results, and patients who consume a low-nitrate diet may have false-negative results[4]. Both these reactions require concentrated urine and an early morning urine specimen is well-suited to these tests.

Studies have investigated the efficacy of the dipstick LE test in detecting pyuria in adults[5-7]. The sensitivity of the test ranges from 78.0 to 99.3% whereas the specificity ranges from 69.0 to 99.3%[8]. Studies involving children have suggested that dipstick tests (the LE test with or without the nitrite test) are as accurate as microscopic examination in predicting bacteriuria[9-11]. The accuracy of using the dipstick LE test to detect pyuria in children, however, remains uncertain[11].

A negative dipstick test alone and a negative microscopy alone have NPV of 95.8 and 96.8%, respectively. This may be adequate in the asymptomatic older child, but in ill febrile children, the combined negative dipstick and microscopy, which has a NPV of 98.1%, virtually excludes urinary tract infection[12]. A combination of a negative dipstick test for nitrite and LE has shown a NPV for UTI of 96.9% and a specificity of 98.7%. In children less than a year old these values were 96.7 and 99.2% respectively[13]. Studies in adults have shown the sensitivity of the leucocyte esterase method with or without nitrite detection to be 75 to 90%, while nitrite alone had a sensitivity of 35 to 85%[14]. We observed the sensitivity of leucocytes to be 65% in adults which dropped down to 49.2% in combination with nitrite, whereas nitrite alone has shown a low sensitivity (Table 4). Nitrite detection, in our study has shown a reasonably good PPV, NPV and a good specificity. However, 70.5% of adult samples and 85% of pediatric samples showed false negativity to nitrite alone. The false positives in the same groups were 25.9 and 17% respectively. False-positive and false-negative results are not unusual in dipstick urinalysis[15].

Patel *et al* [14] found that a NPV of 98% and a sensitivity of 98.3% with a specificity of 19.2% were observed with leucocytes, protein, erythrocytes and nitrite taken together. We report a NPV of 87% in adults, 82.7% in pregnant women, 74.5% in pediatric cases and 75.4% in neonates for the same combination. The sensitivity was observed to be 15.1% in adults and 17% in pregnant women, but

very low in the other two categories. However, the specificity was high in all the four groups (Table 4) and this is an indication that this combination could be used for exclusion of UTI.

Use of the screening parameters in children has been shown to have a PPV of 69.4%, an NPV of 98.6%, and a sensitivity of 97.1% and specificity of 82.5%[16]. The same study had also evaluated a commercial catalase test as a screening procedure and reported it to have a high rate of false-positive results. The NPV of all the five parameters together in our study was 87% in adults, 82.7% in pregnant women, 74.5% in pediatrics and 75.4% in neonates. However, the PPV has been seen to be undeterminable since no culture positive sample has had all the five parameters in the positive range. It is suggested to use the combination of all the five more for exclusion of UTI than for inclusion. We report a NPV of 87% with a specificity of 99.5% and a false negativity of 65% among adult samples when the combination of leucocyte esterase, nitrite and protein is considered. The NPV of the same combination in pediatric cases was 75.5% with a specificity of 100%. A study reported from France showed the predictive value of negative test (NPV) for the above combination to be 99.4% with no difference between boys and girls[17]. We have not seen any difference of NPV or specificity between both the sexes in pediatric cases.

Dipstick testing has been found to be no better than urine microscopy and both techniques have only modest sensitivities and specificities (around 80%) when compared to quantitative culture[2]. One consistent result reported in most studies is the high negative predictive value (> 95%). This may reflect the low prevalence of UTIs (4 -14.8%) in these studies (which included some symptomatic individuals). In the asymptomatic population it is likely that the negative predictive value would exceed 99% for both sexes[2]. A high negative predictive value is extremely useful as it helps to decide which urine samples to culture and which to discard. We have also encountered high NPVs for most of the parameters and their various combinations in all the age groups analyzed.

However, dipstick analysis also has been reported to be disappointing for screening hospitalized patients, in a study reported from Belgium. This study reported a false negative rate of 77% and a false positive rate of 6% when both LE and nitrite were combined[18]. We have also noted a high rate of false negatives when both leucocytes and nitrite were considered together, in adults (76.6%), among pediatric cases (75%) or in neonates (97%). The false positive rate was 20% in adults, 6.2% in pediatrics and 0% in neonates for the same combination. This appears to be consistent with findings reported by the Belgian study[18]. It is a wasteful exercise to culture all urine samples without screening for presence of infection.

The pH and nitrite combination showed a PPV of 100% with a specificity of 100% in all the study groups except among pregnant women. Prompt institution of empirical therapy in patients with a positive urine screen result for both pH and nitrite would therefore mean earlier remission rates and a reduction in the incidence of complications. Follow up of culture results in these cases can help document a cure or change the therapeutic regimen if indicated.

Screening of pregnant women for asymptomatic bacteriuria by an enzymatic urine-screening test has been reported to have 100% sensitivity and a NPV of 100%[19]. However, the authors conclude that the UriscreenTM used in the study could not replace the urine culture, but say that a policy change of performing cultures in samples with a positive test could save 80% of unnecessary cultures[20]. In our study, the NPV of the screening parameters except for leucocytes, taken singly or in combination in samples from pregnant women was 82.7% with a high specificity (Table 4). Even though, as a policy, we culture all the urine samples from pregnant women, the overall positivity rate in our study was 27.3% with *Streptococcus agalactiae* being the second commonest isolate (23.8% of positives). This probably reflects genital colonization. Abalos[21] in a review of screening tests for pregnant women concludes that although combined tests seem to be quite promising in detection of bacteriuria in asymptomatic patients there is insufficient evidence to reassure clinicians that a negative result is a truly negative one. Our results in samples from pregnant women also suggest the same conclusions. A total of 28 samples (4.9%) which had a negative screen showed significant bacteriuria while 197 samples (34.7%) had a positive screen but showed no growth. The practice of mandatory cultures of urine samples from pregnant women may be justified in the light of inconclusiveness of the screening parameters.

A study done in the UK[22] reports that an additional 1.7% samples that were negative by screening were positive for bacteriuria in culture. In our study, 54 (13.5%) of the 400 urine samples requested for routine examination were positive by culture after having been processed following inclusion criteria fulfillment. Using the screening criteria set by our laboratory (Table 1), a total of 1371 samples were not processed for culture over the period of three months, saving approximately an amount of US $ 20,565, averaging about US $ 6800 per month.

However, the 400 samples that were processed for culture following urinalysis in the period of study yielded 54 extra culture positives and contributed to detection of UTIs. Among these, 13.1% were adult cases, 17.07% pediatric and 12.5% neonate urine samples that showed inclusion criteria by urinalysis. The overall 13.5% extra positives detected by culture, which would otherwise have been missed have helped make an additional impact on patient care, more so in pediatrics. This can be considered good justification for screening urine samples prior to bacterial culture, in terms of the clinical impact that can be achieved.

CONCLUSION

Screening urine samples is a necessary step in the laboratory diagnosis of UTI. A combination of leucocyte esterase and nitrite test along with either protein positivity or an alkaline pH has been found to have both high specificity and high NPV and could hence be a valuable indicator for exclusion diagnosis of UTI in all age groups excluding pregnant women. A urine screen positive for both alkaline pH and nitrite could be a good guideline for instituting empirical therapy in all age groups except pregnant women. The inconclusiveness of urine screening justifies mandatory cultures for samples from pregnant women. Urine samples that do not fit into inclusion criteria after analysis can be excluded from being cultured to save valuable resources, time and money.

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