Clinical Evaluation of Three Urine Screening Tests

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Evaluations of screening tests for bacteriuria have traditionally compared the test results with those of quantitative urine cultures. However, many patients with symptomatic urinary tract infections can have <10^5 CFU/ml in their urine. Therefore, the results of urine culture and three screening tests (Bac-T-Screen, Chemstrip LN [a test for leukocyte esterase and nitrate reductase], and Gram stain) were correlated with the clinical classification of urinary tract infection. The Bac-T-Screen test detected 98, 93, and 100% of the infections classified as probable, possible, and asymptomatic, respectively. In contrast, the Gram stain, leukocyte esterase, and nitrate reductase tests were all insensitive screening tests for infection. Additionally, only 45% of the patients with probable infections had ≥10^5 CFU/ml. Thus, the majority of infected patients would not have been detected if quantitative urine cultures were used alone.

Urine cultures represent a large portion of the specimens processed in most clinical microbiology laboratories. In the effort to provide relevant microbiology data rapidly, many screening tests for the detection of bacteriuria have been developed and evaluated. Three screening tests that have been the focus of considerable interest are the Bac-T-Screen (BTS; Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.), the Chemstrip LN (Boehringer Mannheim Diagnostics, Indianapolis, Ind.), and the Gram stain. A number of studies have compared these screening tests with quantitative urine cultures and have documented that the tests can detect bacteriuria reliably when ≥10^4 CFU/ml are present (2, 7, 12-14). From a clinical standpoint, however, this approach to evaluation of urine screening tests is problematic because patients with symptomatic urinary tract infections may have colony counts of <10^5 CFU/ml (5, 6, 8, 16, 18-20). The ability of these screening tests to detect low-level-significant bacteriuria and to differentiate these specimens from specimens contaminated with low numbers of uropathogens is unknown. To assess accurately the reliability of a screening test, the standard by which the patients are initially classified must be precise. A predetermined level of bacteriuria, as measured by quantitative culture, cannot be used as a "gold standard" for determining significant bacteriuria. This must be done by an analysis of the clinical presentation of the patient.

The purpose of this study was to correlate the clinical diagnosis of urinary tract infections with the results of quantitative culture and three screening tests: BTS, Chemstrip LN, and Gram stain.

MATERIALS AND METHODS

Specimens. A total of 500 randomly selected urine specimens were processed in the Barnes Hospital Clinical Microbiology Laboratory, St. Louis, Mo. Urine specimens were collected in sterile containers and were stored in the laboratory at 4°C until processed. All specimens in this study were processed within 2 h of collection.

Quantitative cultures. Cultures were performed by using a 0.01-ml-calibrated loop to inoculate a tryptic soy agar plate with 5% defibrinated sheep blood and a MacConkey agar plate. Colony counts were determined after incubation at 35°C for 24 and 48 h, and bacterial and fungal isolates were identified by conventional procedures. Growth was recorded as <10^2, 10^2 to <10^4, 10^4 to <10^6, or ≥10^6 CFU/ml.

Urine screening with BTS. The BTS model 402 instrument with Dynadeph test card reader was used for these studies. Specimens were processed as described in the instructions of the manufacturer. After the filter card was inserted into the instrument, a 1-ml sample of well-mixed urine was poured into the test reservoir. The instrument then automatically added acetic acid to dilute the urine and lyse some nonmicrobial cells present in the urine specimen. Safranin O dye was stained to stain the remaining cells, and acetic acid was decolorized in the nonspecific staining of the filter card. The color intensity retained on the filter test card was measured by the test card reader, and a difference of ≥4 U of relative absorbance between the sample and reagent blank was considered to be positive by the manufacturer. Specimens that could not be processed in the BTS instrument because the filter clogged with cellular material were considered to be positive, as recommended by the manufacturer.

Urine screening with Chemstrip LN. The Chemstrip LN test strips were used according to the instructions of the manufacturer. A trace or greater reaction for either leukocyte esterase or nitrate reductase was considered to be positive.

Urine screening with Gram stain. Each urine specimen was mixed with Gram stain, and examined under ×1,000 magnification. A minimum of 20 microscopic fields was examined, and the average number of organisms per field was recorded. The stain was considered to be positive if one or more organisms were seen per microscopic field.

Clinical diagnosis of urinary tract infections. Clinical histories were reviewed for all patients who had an abnormal urinalysis, ≥10^2 CFU/ml, or a positive result with at least one of the following tests: BTS, leukocyte esterase, nitrate reductase, or Gram stain. The physician of the patient was also interviewed when appropriate. Information sought included the chief complaint and pertinent medical history of the patient. The presence of certain specific symptoms (including dysuria, frequency, urgency, flank pain, abdominal pain, foul-smelling urine, urethral discharge, hematuria, fever, and abnormal urinalysis) was noted, as were the presence of immunosuppression and prior or concomitant
antibiotic therapy. Patients were classified into one of four groups: probable infection, possible infection, asymptomatic infection, and no infection. Patients with probable infections included those with at least one symptom specific for urinary tract infection (e.g., dysuria, pyuria, and foul-smelling urine) in the absence of an alternative diagnosis. Patients classified with possible infections included those with at least one symptom specific for infection, but an alternative diagnosis could not be excluded. For example, a patient with pyuria and renal calculi or with dysuria and fever in the presence of an abdominal mass would be classified as having a possible infection. Patients with asymptomatic infections had no symptoms referable to the urinary tract, but $\geq 10^5$ CFU of uropathogens per ml (e.g., members of the family Enterobacteriaceae, Staphylococcus aureus, Staphylococcus saprophyticus, group D Streptococcus spp., and yeasts) were present in the urine specimen. Noninfected patients had no abnormal urinary tract signs or symptoms and normal urinalysis, and $<10^2$ CFU/ml were present by culture.

RESULTS

Specimens from 500 patients were analyzed. A total of 301 (61.8%) specimens were midstream collections and 186 (38.2%) were catheterized specimens (the method of collection was not specified for 13 specimens). Specimens were collected from 323 (64.6%) female patients and 177 (35.4%) male patients; 410 (82%) specimens were from inpatients, 50 (10%) specimens were from clinic patients, and 40 (8%) specimens were from emergency room patients. The reason that the specimens were collected could be determined for 419 specimens. A total of 231 (55.1%) specimens were collected as part of the routine clinical work-up of patients, 120 (28.6%) specimens were collected from symptomatic patients, and 68 (16.2%) specimens were part of the work-up of septic patients.

The results of the screening tests were initially compared with the quantitative culture results (Table 1). The BTS and Gram stain tests were sensitive indicators of high-grade bacteriuria, whereas only 80 and 58% of the infections of $\geq 10^5$ CFU/ml were detected with the leukocyte esterase and nitrate reductase tests, respectively. The combination of a positive leukocyte esterase test or nitrate reductase test did not significantly improve the results with the leukocyte esterase test alone. The results with the nitrate reductase tests were not surprising because no effort was made to collect first-voided morning specimens. This test is insensitive with randomly collected specimens (10). Each of the screening tests was less sensitive when fewer organisms were present in the urine specimen.

The data were reanalyzed after the patients were classified into the four clinical categories (Table 2). Of the patients with probable infections, only 45% had $\geq 10^5$ CFU/ml by quantitative culture, whereas 98% had $\geq 10^5$ CFU/ml. The BTS test detected 98, 93, and 100% of the infections classified as probable, possible, and asymptomatic, respectively. The Gram stain, leukocyte esterase, and nitrate reductase tests were all insensitive screening tests for infection. If the leukocyte esterase and nitrate reductase tests were combined (with a positive for either test considered to be a positive test result), only 84% of the probable infections, 52% of the possible infections, and 82% of the asymptomatic infections were detected. This was essentially the same as with the leukocyte esterase test alone. Of the 387 patients with no evidence of infection, 52% had $\geq 10^2$ CFU/ml by quantitative culture, and 45% had a positive BTS test. Thus, these tests were sensitive but nonspecific.

The BTS results were measured quantitatively by the BTS card reader. The individual test results were plotted to determine whether there was a significant difference between readings of specimens from noninfected patients and the other three groups (Fig. 1). Although the majority of readings with noninfected specimens were low (53%, $<3$ U; 27%, 4 to 10 U), there was significant overlap with the other specimens at the higher readings. Thus, the specificity of the BTS test could not be improved without compromising the test sensitivity.

Because the use of any screening test is influenced by the population that is tested, the frequency of disease in the

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**TABLE 1. Comparison of urine screening tests with quantitative cultures**

<table>
<thead>
<tr>
<th>Quantitative culture (CFU/ml)</th>
<th>No. of patients</th>
<th>% of patients with positive tests:</th>
<th>BTS</th>
<th>Leukocyte esterase</th>
<th>Nitrate reductase</th>
<th>LN test</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\geq 10^5$</td>
<td>60</td>
<td>97</td>
<td>80</td>
<td>58</td>
<td>85</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>$10^2$&lt; $10^5$</td>
<td>82</td>
<td>79</td>
<td>55</td>
<td>11</td>
<td>58</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>$10^2$&lt; $10^4$</td>
<td>167</td>
<td>44</td>
<td>29</td>
<td>2</td>
<td>29</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>$&lt;10^2$</td>
<td>191</td>
<td>47</td>
<td>24</td>
<td>2</td>
<td>25</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* Percent of patients with either a positive leukocyte esterase test or a positive nitrate reductase test.

**TABLE 2. Comparison of urine screening tests with clinical classification of infections**

<table>
<thead>
<tr>
<th>Clinical diagnosis (no. of patients)</th>
<th>Culture (CFU/ml)</th>
<th>BTS</th>
<th>Leukocyte esterase</th>
<th>Nitrate reductase</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probable infection (64)</td>
<td>$\geq 10^5$</td>
<td>45</td>
<td>95</td>
<td>98</td>
<td>81</td>
</tr>
<tr>
<td>Possible infection (27)</td>
<td>$\geq 10^5$</td>
<td>33</td>
<td>70</td>
<td>93</td>
<td>48</td>
</tr>
<tr>
<td>Asymptomatic infection (22)</td>
<td>$\geq 10^5$</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>82</td>
</tr>
<tr>
<td>No infection (387)</td>
<td>$\geq 10^2$</td>
<td>0</td>
<td>52</td>
<td>45</td>
<td>28</td>
</tr>
</tbody>
</table>

* Number of uropathogens isolated with quantitative culture.

* All specimens with $\geq 10^5$ CFU/ml and no evidence of infection were classified as asymptomatic infections.

**FIG. 1. BTS readings for urine samples collected from four patient populations.**
different patient groups was analyzed. Of the patients with specimens that were submitted as part of a routine culture work-up, 91% were not infected (Table 3). Similarly, 86% of the patients who had urine samples cultured as part of a work-up for sepsis were not infected. In contrast, two-thirds of the patients who had samples cultured because they initially presented with symptoms of genitourinary tract disease were considered to be infected. Of the urine samples that were cultured from inpatients, 82% were not infected, compared with 68% of the emergency room patients and 50% of the clinic patients (Table 4). The sensitivity and specificity of the BTS test was the same for each population of patients.

**DISCUSSION**

Marple in 1941 (9), Barr and Rantz in 1948 (1), and Sanford et al. in 1956 (15) introduced the concept that large numbers of organisms in urine were significant, whereas small numbers generally represented contaminants. These investigators reported that the presence of as few as 1,000 to 10,000 CFU/ml in a urine specimen could be clinically significant. However, Kass (3) and Kass and Finland (4) are attributed with establishing the statistical basis of quantitative urine cultures. They demonstrated that 95% of patients with pyelonephritis or with asymptomatic bacteriuria had at least 10^5 CFU/ml, with >10^6 CFU/ml present in 82% of the patients. Kass demonstrated in his studies (3, 4) that noninfected patients had either sterile urine or urine with fewer than 10^4 CFU/ml. Unfortunately, these studies were extended uncritically to include all patients with urinary tract infections. We now recognize that a variety of factors can reduce the number of organisms present in a urine specimen, including the site of infection, the infecting organism, the state of hydration of the patient, and the time the specimen was collected. Despite the realization that the number of organisms in urine may be low, most physicians and microbiologists have held sacrosanct the quantity 10^5 CFU/ml. This belief has only recently been critically questioned. Studies have now shown that a significant portion of women with symptoms suggestive of acute cystitis have infections that are caused by low numbers of uropathogens. Stamey and Pfau (17) reported that 33 to 44% of urinary tract infections are caused by fewer than 10^5 CFU/ml. Stamm and associates (8, 18, 19) have also documented that one-half of women with cystitis have infections caused by as few as 100 CFU/ml. Similar observations have also been reported for pediatric patients (16).

Despite the recognition that small numbers of uropathogens can be clinically significant when isolated in a well-collected urine specimen, virtually all evaluations of urine screening tests have used the presence of 10^5 CFU/ml as the definitive test result. Although some studies have also examined the sensitivity of the screening tests for detecting smaller numbers of organisms, there is generally no effort to differentiate between contaminants and significant isolates. Table 4. Relationship between location of patient in hospital and clinical condition of patient

<table>
<thead>
<tr>
<th>Location (no. of patients)</th>
<th>% of patients with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Probable infection</td>
</tr>
<tr>
<td>Inpatient (410)</td>
<td>8</td>
</tr>
<tr>
<td>Clinic (50)</td>
<td>36</td>
</tr>
<tr>
<td>Emergency room (40)</td>
<td>28</td>
</tr>
</tbody>
</table>

Thus, the value of these screening tests for identifying infected patients is unknown. To make this assessment, clinical parameters must be used to define whether the patient is infected. We used this approach to determine the value of three screening tests for bacteriuria: the Marion BTS, the Chemstrip LN, and the Gram stain.

When the results of the screening tests with 500 specimens from patients were analyzed by the conventional comparison to results of quantitative culture, they were similar to what has been published from this laboratory and others (11–14). The BTS was the most sensitive test for detecting large numbers of urinary tract pathogens. The BTS test also was the most reliable screening test for detecting pathogens in patients defined as infected. A total of 110 of 113 infections defined clinically as probable, possible, or asymptomatic were detected by the BTS. In contrast, less than one-half of the patients with these infections had >10^5 CFU/ml cultured from their urine. This observation is consistent with previous studies by Stamm and associates (8, 18, 19). It is obvious from the study reported here that quantitative urine cultures cannot be used to determine urinary tract infections unless small numbers of organisms are routinely identified. However, the costs associated with this approach are difficult to justify. Latham et al. (8) suggested that low-level-significant bacteriuria can be accurately identified if the specimens are screened for pyuria in combination with quantitative culture. With this approach, however, it would be necessary to culture all specimens initially. The BTS test offers an attractive alternative for the initial screening of urine specimens because it identified virtually all specimens from infected patients and eliminated 65% of the specimens collected from noninfected patients. The Gram stain, leukocyte esterase test, and nitrate reductase test were too insensitive to be used to identify infected patients, particularly those patients with small numbers of pathogens.

One troublesome aspect of screening urine specimens with the BTS was the large number of false-positive tests. A total of 174 (45%) of 387 specimens from noninfected patients had positive BTS readings. When this population of 174 specimens was examined in detail, 109 (63%) specimens had evidence of either pyuria (determined by microscopic evidence or a positive leukocyte esterase test) or colony counts of 10^6 to 10^7 CFU/ml. Previous studies of the BTS instrument confirmed that the presence of leukocytes or moderately high numbers of organisms can give a positive reading (11). Thus, these false-positive results are not surprising. The other 65 of 174 specimens had >10^4 CFU/ml and negative Gram stain, leukocyte esterase, and nitrate reductase tests. All but one of these 65 specimens had a BTS reading between 4 and 20 U. Although these 65 false-positive tests could be eliminated from further processing by altering the criteria for defining a positive BTS test, this would result in a significant number of false-negative tests (Fig. 1). Thus, it is unlikely that the BTS test could be altered in a way that would improve the predictive value of a positive test.

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**Tables**

Table 3. Relationship between reason for submitting urine specimen and clinical condition of patient

<table>
<thead>
<tr>
<th>Reason* for submitting specimen (no. of patients)</th>
<th>% of patients with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Probable infection</td>
</tr>
<tr>
<td>Routine culture (231)</td>
<td>0</td>
</tr>
<tr>
<td>Symptomatic (120)</td>
<td>52</td>
</tr>
<tr>
<td>Septic work-up (68)</td>
<td>3</td>
</tr>
</tbody>
</table>

* This information was not available for 81 patients.
The population for which a sensitive screening test would be most useful is one in which the incidence of disease is low. In this situation the infected patients would be correctly classified, and many of the specimens from noninfected patients would be eliminated from further processing. The majority of specimens received during this study was from hospitalized patients and was collected as part of the routine work-up of ill patients. It could be argued that routine urine specimens should not be submitted for culture because 91% of these specimens were from noninfected patients. However, 6% of the specimens were from patients with asymptomatic infections. With the use of the BTS test, more than one-half of the routine cultures from noninfected patients could be eliminated from further processing. This would include specimens from the majority of hospitalized patients, including severely immunocompromised patients. The BTS test would be less useful in areas such as urology clinics or emergency rooms, because in our experience a significant portion of those patients were infected.

In conclusion, we found the BTS test to be a very sensitive screening test for clinically significant bacteriuria. Virtually all patients with urinary tract diseases, as defined by clinical parameters, had positive BTS readings. We use this system to screen urine specimens received from both hospitalized and ambulatory patients. BTS-negative specimens are not processed further. BTS-positive specimens are cultured in a conventional fashion. If a small number of uropathogens is present in the specimen (e.g., $<10^8$ CFU/ml), the Gram stain morphology of the isolate and the colony counts are reported to the physician with a note that further identification will be performed if requested. In this way, the physician has the opportunity to determine whether the isolate appears to be clinically significant for the specific patient. If large numbers of uropathogens are present in the specimen (e.g., $\geq10^4$ CFU/ml), the identification of the isolate is reported to the physician.

LITERATURE CITED