Detection of Bacteriuria: Manual Screening Test and Early Examination of Agar Plates

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We assessed two procedures for the initial processing of urine specimens: (i) manual screening of urine samples by inoculation into tryptic soy broth and incubation for 3 to 6 h, and (ii) examination of agar plates inoculated with urine and incubated for 4 to 6 h. Of the urine samples with >10⁵ colony-forming units per ml, 73.6 and 90.1% were detected with the broth cultures after 3 and 6 h of incubation, respectively. The organisms not detected at 6 h were Corynebacterium spp., Lactobacillus spp., and one of six isolates of yeasts. When inoculated plates were examined for growth, 53.3 and 80.0% of the specimens with >10⁵ colony-forming units per ml were detected after 4 and 6 h of incubation, respectively. Of the cultures with significant growth of Enterobacteriaceae or Pseudomonas spp., 95% were detected on the plates after 6 h of incubation.

Various techniques and instruments have been introduced into microbiology laboratories in an effort to reduce the processing time of clinical specimens or to eliminate unnecessary cultures. Urine specimens are particularly amenable to these new procedures because most laboratories process a large number of specimens, and generally more than 100,000 organisms per ml of urine are present in significant bacterial infections (12). Methods for screening urine specimens include microscopy (1, 11, 15), biochemical tests (14), electronic counting of bacterial particles (5, 8, 18), detection of endotoxin (11) or bacterial adenosine triphosphate (19), and measurement of bacterial growth by impedance (4, 20, 21), microrcolorimetry (2), and photometry (7, 9, 10, 17). However, none of these is the ideal screening method, either because the procedure is technically demanding, insensitive, or expensive.

In the present study, we evaluated two procedures for the initial processing of urine specimens: (i) manual screening of urines with broth cultures incubated for 3 to 6 h, and (ii) early examination (at 4 and 6 h) of agar plates inoculated with urine.

MATERIALS AND METHODS

Specimens and quantitative cultures. A total of 1,000 urine specimens, collected during a 6-week period, were processed in the Barnes Hospital clinical microbiology laboratory. Midstream (62%), catheterized (28%), or unspecified (12%) urine specimens were collected in sterile containers and were stored in the laboratory at 4°C until processed. All specimens were plated within 2 h of receipt. Urine specimens were initially inoculated with a 0.01-ml calibrated platinum loop onto tryptic soy agar with 5% defibrinated sheep blood and MacConkey agar plates. Colony counts were determined after incubation at 35°C for 24 and 48 h, and bacterial isolates were identified by conventional procedures. After the initial processing, the specimens were refrigerated at 4°C until screening tests were performed.

Preliminary urine screening with broth cultures. The first 500 urine specimens received in this study were cultured quantitatively as described above and were screened for significant bacteriuria by inoculating 100 μl of urine into 1 ml of tryptic soy broth. Each broth was incubated at 35°C and examined for turbidity after 3, 4, 5, and 6 h. Turbidity of the broth was compared with the results of the quantitative cultures.

Preliminary examination of inoculated plates. For the second part of this study, the plates inoculated for quantitative cultures were examined for growth after incubation for 4 and 6 h. Plates were examined under reflected light with the unaided eye. Growth was recorded as <10⁴, 10⁴ to 10⁵, or >10⁵ colony-forming units (CFU)/ml, and this was then compared with the results of the same plates incubated for an additional 24 and 48 h.

RESULTS

Preliminary urine screening with broth cultures. Of the 500 urine specimens processed in the first part of the study, 87 (17.4%) contained >10⁴ CFU/ml as determined by the calibrated loop method, 92 (18.4%) contained between 10⁴ and 10⁵ CFU/ml, and 321 (64.2%) contained fewer than 10⁴ CFU/ml. Table 1 is a summary of the urine specimens with detectable growth in the 3- to 6-h broth cultures. After 3 h of incubation, 64 (73.6%) of the urine specimens with >10⁵ CFU/ml produced turbidity in the
TABLE 1. Preliminary screening of urine specimens with broth cultures

<table>
<thead>
<tr>
<th>Colony counts (CFU/ml)</th>
<th>No. of urine specimens</th>
<th>No. of specimens with growth in broth after following time of incubation (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>&gt;10^5</td>
<td>87</td>
<td>64</td>
</tr>
<tr>
<td>10^4-10^5</td>
<td>92</td>
<td>11</td>
</tr>
<tr>
<td>&lt;10^4</td>
<td>321</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE 2. Detection of organism by screening with broth culture

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total no. of isolates</th>
<th>No. of isolates detected after following time of incubation (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Yeasts</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

* A total of 97 organisms (>10^5 CFU/ml) were isolated in 87 urine specimens.

TABLE 3. Preliminary detection of urine specimens with more than 10^5 CFU/ml

<table>
<thead>
<tr>
<th>Preliminary report (CFU/ml)</th>
<th>% Detected on plates after following time of incubation (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>&gt;10^5</td>
<td>53.3</td>
</tr>
<tr>
<td>10^4-10^5</td>
<td>9.5</td>
</tr>
<tr>
<td>&lt;10^4</td>
<td>37.2</td>
</tr>
</tbody>
</table>

* A total of 105 urine specimens contained >10^5 CFU/ml.

broths, and 85.3% of all turbid broths were from cultures with >10^5 CFU/ml. After 6 h of incubation, broths were turbid for 79 (90.1%) of the urine specimens with >10^5 CFU/ml. A total of 45.7% of all turbid broths were from cultures with >10^5 CFU/ml.

The time of initial detection in the broth culture of 97 organisms (>10^5 CFU/ml) isolated in 87 urine specimens is summarized in Table 2. All staphylococci and Enterobacteriaceae were detected at 3 h, and all streptococci and Pseudomonas isolates were detected by 6 h. Six of seven Corynebacterium isolates, one of two lactobacilli, and one of six yeasts were not detected by 6 h. An additional four urine specimens contained between 10^5 and 10^6 yeasts per ml. One of these urine specimens was detected at 3 h, one was detected at 6 h, and two were not detected.

Preliminary examination of inoculated plates. Of the 500 urine specimens processed in the second part of the study, 105 (21.0%) contained more than 10^5 CFU/ml, 89 (17.8%) contained between 10^4 and 10^5 CFU/ml, and 306 (61.2%) contained fewer than 10^4 CFU/ml. The percent of urines with >10^5 CFU/ml detected after 4 or 6 h of incubation is summarized in Table 3. Of the 105 urine specimens, 56 (53.3%) and 84 (80.0%) were preliminarily reported as having >10^5 CFU/ml after 4 and 6 h of incubation, respectively. None of the urine specimens with less than 10^5 CFU/ml was preliminarily reported as >10^5 CFU/ml. Only 5 of the 13 urine specimens with more than one organism present at >10^5 CFU/ml were detected as mixtures after 6 h of incubation.

The detection of 118 organisms that were present at a concentration of >10^5 CFU/ml is summarized in Table 4. Whereas 80% or more of the Staphylococcus, Streptococcus, Enterobacteriaceae, and Pseudomonas isolates were accurately detected on the 6-h plates, only five of seven (71%) yeasts and none of the Corynebacterium or Lactobacillus isolates were detected.

DISCUSSION

Although screening of urine specimens for significant growth is desirable, most methods which have been developed are either technically demanding, insensitive, or expensive. The Gram stain is a rapid method and provides specific information (i.e., Gram stain morphology) that can be used to select appropriate antimicrobial therapy. However, the accuracy of the method depends on the technical proficiency of the laboratory personnel, with a sensitivity reported
from 70 to 99% (1, 11, 15). In addition, unless the preparation and interpretation of the Gram stain are part of the routine urine examination in the microbiology laboratory, this method would significantly increase the time required to process urine specimens.

Biochemical tests for significant bacteriuria (e.g., nitrate reduction, glucose utilization, detection of bacterial adenosine triphosphate or endotoxin) are either insensitive (14) or only useful for detecting certain organisms, e.g., gram-negative bacilli that produce endotoxin (11).

Detection of bacterial growth with various instruments is both technically simple and sensitive; however, the disposable reagents and instrumentation are frequently prohibitively expensive for even large laboratories. Recently, instruments originally developed for susceptibility tests have been adapted for screening urine specimens [10; L. McCarthy, C. Corlett, and J. Robson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C(H)95, p. 362; S. Hansen and W. Pope, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C251, p. 316]. In these systems, urine is inoculated into a broth, and bacterial growth is measured with a spectrophotometer. In the first part of this study, we screened urine specimens in the same way except the broths were manually examined for turbidity. Of the 87 specimens with >10^6 CFU/ml, we detected 73.6% by 3 h and 90.1% by 6 h (Table 1), which was comparable with the results obtained by Jenkins et al. (10) with the Autobac, i.e., they detected 75.4 and 95.4% of the specimens with significant growth at 3 and 6 h, respectively. After 5 h of incubation, Hansen and Pope detected 85% of the significant urine specimens with the Abbott MS-2 urine screening broth without Tween 80, which is similar to the 83% detected in this study. In addition, Hansen and Pope reported that 94% of the significant specimens were detected within 5 h with Tween 80-supplemented broth. In the studies described herein, we did not supplement the tryptic soy broth with Tween 80.

Some slow-growing organisms (diphtheroids, lactobacilli, and yeasts) were not detected at 6 h (Table 2). The diphtheroids and lactobacilli were present in mixed cultures, and their clinical significance was indeterminable. One specimen with a pure growth of yeast (>10^6 CFU/ml) was not detected in the broth cultures. This was a catheterized specimen from a patient receiving antibiotics, both of which are predisposing factors for fungal urinary tract infections (16). Although >10^6 CFU/ml is the widely accepted criterion for significant bacteriuria, Goldberg and co-workers (6) presented evidence that >10^5 yeast per ml of urine required further evaluation.

In the present study, only two of four urine specimens with between 10^4 and 10^5 yeasts per ml were detected by 6 h. The undetected urine specimens were catheterized.

In addition to evaluating broth as a screening test for urine specimens, we assessed the value of examining inoculated plates after incubation for 4 and 6 h. Chadwick and Avila (3) detected 89.8% of specimens with >10^6 CFU/ml by microscopically examining plates after 4 h of incubation. We considered the feasibility of examining plates without magnification after our experience and that of others (13; W. L. Drew, personal communication) by reading Kirby-Bauer tests after 4 to 6 h of incubation. In the present study, 85 of 105 (80%) cultures with >10^6 CFU/ml were detected by 6 h, and an additional 5.7% were reported as between 10^4 to 10^5 (Table 3). Most isolates which were undetected by 6 h were slow growers such as diphtheroids, lactobacilli, and yeasts. Although not all significant infections would be detected by 6 h and the presence of undetected mixtures would invalidate early bacterial identifications or susceptibility tests, the early examination of selected cultures (e.g., culture plated at night) would be reliable.

In summary, we have demonstrated a simple broth culture screen for significant bacteriuria that is as sensitive as semiautomated procedures and does not require expensive instrumentation. This procedure would be useful for screening voided urine specimens from patients not receiving antibiotics. Antibiotic therapy or catheterization could reduce the number of organisms in urine or predispose the patient to infections with fastidious organisms and, thus, reduce the sensitivity of this procedure. In addition, most cultures with significant growth can be detected after incubation of the inoculated media for 6 h.

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LITERATURE CITED


