

## Rapid Method for Identification and Susceptibility Testing of *Escherichia coli* Bacteriuria

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Received 14 December 1982/Accepted 11 April 1983

This report describes a rapid method for the identification and susceptibility testing of *Escherichia coli* bacteriuria by use of the Autobac urine screen (AUS), a 5-h indole test, and the AutoMicrobic system *E. coli* Susceptibility Card (AMS-ECSC). All specimens that were AUS negative at 3 and 5 h were reported as negative. All specimens that were AUS positive at 3 or 5 h were removed from the refrigerator and streaked to a MacConkey-blood agar biplate with a 0.001-ml calibrated loop and incubated at 35°C. The standard method for identification and susceptibility testing consisted of inoculating isolated colonies into the AutoMicrobic system *Enterobacteriaceae*-Plus Biochemical Card and the AutoMicrobic system General Susceptibility Card. Of 915 specimens, 212 (23.2%) were AUS positive at 3 h, of which 112 (52.8%) were indole positive when tryptophan broth was tested at 5 h. The sensitivity and specificity of this screening method for *E. coli* bacteriuria were 78.8 and 72.2%, respectively. If contaminated cultures were excluded, specificity was 94.4%. When the indole test was positive, 10 µl of growth from tryptophan broth was used as inoculum for the AMS-ECSC. AMS-ECSC results were final at 12 h. The sensitivity and specificity of the AMS-ECSC for identification of *E. coli* were 90.4 and 55.0%, respectively. If contaminated cultures were excluded, specificity was 100%. AMS-ECSC susceptibility results demonstrated an overall agreement of 94.3% with the standard method, with 0.5% very major, 3.4% major, and 1.8% minor discrepancies. The biplate was examined after overnight incubation, and when colonies compatible in morphology with *E. coli* were present in significant numbers, AMS-ECSC results were reported. When discrepancies were found between biplate and AMS-ECSC results, the biplate was processed in the conventional manner. A rapid method for identifying and performing susceptibility tests for approximately 70% of the specimens with *E. coli* bacteriuria is described.

Urine specimens constitute a major portion of the workload in clinical microbiology laboratories. Rapid identification and antibiotic susceptibility testing of urinary tract pathogens are important, and there have been many reports describing techniques to achieve these objectives (6, 7, 9, 12, 16, 17). However, except for automated screening for significant bacteriuria, there has been little application of rapid methods to the performance of urine bacteriology. Since *Escherichia coli* is the principal urinary pathogen (1, 2, 11), the rapid identification and antibiotic susceptibility testing of this bacterium would have significant impact on both laboratory work flow and patient care.

We have evaluated a rapid method for the identification and susceptibility testing of *E. coli* bacteriuria by use of the Autobac urine screen (AUS; General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.), a 5-h indole test, and

the AutoMicrobic system *E. coli* Susceptibility Card (AMS-ECSC; Vitek Systems, Inc., Hazelwood, Mo.).

### MATERIALS AND METHODS

**Specimens.** A total of 915 midstream urine specimens were collected from the outpatient and inpatient services of Ben Taub General Hospital. The protocol called for the specimens to be processed or refrigerated within 2 h after collection. Refrigerated specimens were processed within 4 h.

**AUS.** The AUS procedure has been previously described (5, 10, 12). A decrease of at least 0.2 V at 3 or 5 h from the initial value of the light-scattering voltage indicated a positive ( $\geq 10^5$  CFU/ml) urine.

**Indole screen.** The indole screen was performed concurrently with the AUS. A 0.1-ml volume of urine was inoculated into 0.5 ml of 1% tryptophan broth (Difco Laboratories, Detroit, Mich.) and incubated at 35°C. When the AUS was negative at 3 h, the tryptophan broth was discarded. When the AUS was positive at 3 h, the tryptophan broth was incubated for an

additional 2 h, at which time 10  $\mu$ l of growth in tryptophan broth was inoculated to a tube containing 1.8 ml of 0.5% sodium chloride (AMS-ECSC inoculum). An indole test was performed on the remaining growth in the originally inoculated tryptophan broth. When the indole test was negative, the AMS-ECSC inoculum was discarded. When the indole test was positive, the AMS-ECSC inoculum was used to inoculate the AMS-ECSC.

**AMS-ECSC.** The AMS-ECSC is a plastic card containing wells filled with lyophilized media. The selective enrichment medium employed in the AMS-ECSC is specific for growth of *E. coli*. The positive control well contains this medium with no antimicrobial agents added. The remaining wells contain the same medium with the addition of either 21  $\mu$ g of ampicillin, 30  $\mu$ g of carbenicillin, 14  $\mu$ g of cephalothin, 20  $\mu$ g of chloramphenicol, 5  $\mu$ g of colistin, 3  $\mu$ g of gentamicin, 8  $\mu$ g of kanamycin, 10  $\mu$ g of tetracycline, 20  $\mu$ g of nalidixic acid, 30  $\mu$ g of nitrofurantoin, or 10  $\mu$ g of trimethoprim-sulfamethoxazole per ml. Colistin and nalidixic acid were not evaluated in this study. AMS-ECSC inocula (see indole screen) were poured into AutoMicrobic system single-tube injectors attached to the AMS-ECSC and processed. The *E. coli* medium permitted selective growth of *E. coli*. As metabolic activity of *E. coli* occurred within a well, the optical characteristics of the medium changed. The change in medium color or opacity was then measured once each hour by the photometric reader in the reader/incubator module. In the event that other species of microorganisms were present in the inoculum, their metabolic activity was inhibited during the incubation cycle. The final status of the *E. coli* susceptibility results was available 12 h after the card was inoculated.

**Culture method.** Immediately after inoculation of the AUS and tryptophan broth, urine specimens were stored at 4 to 8°C. Urine specimens that were AUS positive at 3 or 5 h were streaked with a 0.001-ml calibrated loop to a MacConkey-blood agar biplate and incubated at 35°C overnight, and colony counts were performed. Probable pathogens were defined as one or two species of gram-positive cocci, gram-negative bacilli, or yeast at  $>10^5$  CFU/ml or as a single species of the above microorganisms at  $10^4$  to  $10^5$  CFU/ml, which are known to cause urinary tract infection. Cultures were considered contaminated if they grew viridans streptococci (other than *Enterococcus*), diphtheroids, lactobacilli, three species at  $\geq 10^5$  CFU/ml, or two or more species at  $<10^5$  CFU/ml.

As part of our method, MacConkey agar was examined after overnight incubation for the presence of significant numbers of colonies that were morphologically compatible with *E. coli*. These results were compared with those obtained with the AMS-ECSC. When discrepancies were found between biplate and AMS-ECSC results, AMS-ECSC results were not reported and the biplate was processed in the standard manner.

**Standard identification method.** The biplate was examined at 24 and 48 h for the presence of significant numbers of probable pathogens. Rapidly growing gram-negative bacilli were identified by the AutoMicrobic system *Enterobacteriaceae*-Plus Biochemical Card (3, 8). All other microorganisms were identified by conventional methods (14).

**Standard susceptibility method.** The standard sus-

ceptibility method, against which the AMS-ECSC results were compared, was the AutoMicrobic system General Susceptibility Card (AMS-GSC). The AMS-GSC consists of 30 wells containing 12 antimicrobial agents at two dilutions, one antimicrobial agent at three dilutions, one positive control broth, and two blank wells. Ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, nitrofurantoin, tetracycline, and trimethoprim-sulfamethoxazole were evaluated in this study. Three or four colonies from the MacConkey-blood agar biplate were inoculated into 0.5 ml of eugonic broth and incubated for 1 h at 35°C. The inoculum was prepared by adding 10  $\mu$ l of the eugonic broth to 1.8 ml of 0.5% sodium chloride. An interpretive call of susceptible, intermediate, or resistant and the bracket minimal inhibitory concentration value were printed by the data terminal for each card in the reader/incubator, usually within 4 to 5 h.

**Sensitivity and specificity.** Sensitivity and specificity were calculated according to the method of Ransohoff and Feinstein (18).

## RESULTS

Of 915 specimens, 212 (23.2%) were AUS positive at 3 h, of which 112 (52.8%) were indole positive when tryptophan broth was tested at 5 h (Table 1). Contaminated specimens were not identified. The three indole-positive, gram-negative bacilli other than *E. coli* were *Citrobacter diversus*, *Enterobacter cloacae*, and *Klebsiella oxytoca*. The sensitivity and specificity of this screening method for detection of *E. coli* bacteriuria were 78.8 and 72.2%, respectively. When contaminated cultures were excluded, specificity was 94.4%.

There were 93 AMS-ECSCs inoculated. The AMS-ECSC contained a control well that selectively allowed the growth of *E. coli*. Therefore, both identification accuracy and antibiotic susceptibility test results were evaluated. Table 2 summarizes the results of the control well evaluation for identification of *E. coli*. The sensitivity of the AMS-ECSC for identification of *E. coli* in AMS-ECSC inoculum was 90.4% and the speci-

TABLE 1. Indole results (5 h) for microorganisms that were AUS positive (3 h)

Microorganism	Indole production (5 h)	
	Positive	Negative
<i>E. coli</i> <sup>a</sup>	82	22
Contaminated <sup>b</sup>	27	27
Gram-negative rod	3	32
Gram-positive coccus	0	18
Yeast	0	1

<sup>a</sup> Present at  $>10^5$  CFU/ml.

<sup>b</sup> Contamination was defined as three species at  $>10^5$  CFU/ml or two or more species at  $<10^5$  CFU/ml, suggesting an improperly collected specimen. Contaminated specimens were not identified.

TABLE 2. AMS-ECSC control well results

Microorganism	AMS-ECSC control well	
	Positive	Negative
<i>E. coli</i> <sup>a</sup>	66	7
Contaminated <sup>b</sup>	9	9
<i>E. cloacae</i>	0	1
<i>K. pneumoniae</i>	0	1

<sup>a</sup> Present as a probable pathogen at  $>10^5$  CFU/ml.

<sup>b</sup> Contamination was defined as three species at  $>10^5$  CFU/ml or two or more species at  $<10^5$  CFU/ml, suggesting an improperly collected specimen. Contaminated specimens were not identified.

ficity was 55.0%. When contaminated cultures were excluded, specificity was 100%.

A total of 66 AMS-ECSC susceptibility results were compared with the standard method. The results are summarized in Table 3. In a total of 594 antimicrobial agent-organism test pairs, there were 0.5% very major, 3.4% major, and 1.8% minor discrepancies. The overall correlation was 94.3%; when minor discrepancies were disregarded the correlation was 96.1%. Cephalothin and tetracycline were responsible for 61.8% of the discrepancies.

#### DISCUSSION

This report describes a rapid method for identification and susceptibility testing of *E. coli* bacteriuria by use of the AUS, a 5-h indole test, and the AMS-ECSC. The sensitivity of the indole screen for detecting *E. coli* bacteriuria was 78.8%. Although the proportion of false-negative results for the indole screen was approximately 20%, these false-negative specimens were plated to MacConkey-blood agar biplates since the AUS results were positive. The next day, isolated colonies were identified and susceptibility tests were performed; thus, patient care was not compromised. The 1% tryptophan broth used in our study is used in the standard indole test (15) that requires up to 48 h of incubation. Vracko and Sherris (19) described a 2-h indole test that used peptone water heavily inoculated with isolated colonies and found that 57 of 57 *E. coli* were indole positive. Although the inoculum in that study was heavier than that used in our study, use of peptone water or other tryptophan-containing broth might enhance the sensitivity of the indole screen.

The specificity of the indole screen for *E. coli* bacteriuria was 72.2%. Contaminated urine specimens sometimes contain insignificant numbers of indole-positive organisms. The percentage of contaminated specimens that yielded a positive indole result was 24.1%. When contaminated specimens were excluded, the specificity of the indole test for *E. coli* was 94.4%.

TABLE 3. Comparison of antibiotic sensitivities between the AMS-ECSC and the AMS-GSC

Antibiotic	No. of tests	No. of discrepancies (%)		
		Very major	Major	Minor
Ampicillin	66	1 (1.5)	2 (3.0)	0
Carbenicillin	66	1 (1.5)	2 (3.0)	0
Cephalothin	66	0	5 (7.6)	8 (12.1)
Chloramphenicol	66	0	0	0
Gentamicin	66	0	0	0
Kanamycin	66	0	2 (3.0)	0
Nitrofurantoin	66	0	0	2 (3.0)
Tetracycline	66	1 (1.5)	6 (9.0)	1 (1.5)
Trimethoprim-sulfamethoxazole	66	0	3 (4.5)	0

The sensitivity of the AMS-ECSC for identification of *E. coli* bacteriuria was 90.4%. The false-negative specimens on the AMS-ECSC were ultimately identified and susceptibility tests were performed, since significant numbers of colonies were detected on the biplates after overnight incubation.

The specificity of the AMS-ECSC was 55.0%. This low specificity was due to inoculation of the AMS-ECSC with mixed cultures which contained small numbers of *E. coli* or *E. coli* alone at levels judged to be insignificant by the reference colony count procedure. These false-positive results were never reported because of the required confirmation of significant colony counts after overnight incubation.

The AMS-GSC has been shown to be an accurate method for *E. coli* susceptibility testing (4, 13). In addition, data for *E. coli* susceptibility testing with the AMS-GSC have been obtained from Vitek Systems, Inc. (personal communication). AMS-GSC versus microtiter minimal inhibitory concentration results were obtained for 451 *E. coli* tested against the nine antimicrobial agents evaluated in our study. Percentages for complete agreement and agreement if minor discrepancies are disregarded (in parentheses) for each antimicrobial agent were: ampicillin, 96.9 (98.2); carbenicillin, 97.1 (97.6); cephalothin, 91.1 (98.2); chloramphenicol, 97.6 (99.3); gentamicin, 97.6 (98.4); kanamycin, 98.4 (99.8); tetracycline, 97.1 (98.4); trimethoprim-sulfamethoxazole, 99.6 (100); and nitrofurantoin, 96.0 (97.8). Complete agreement for all antimicrobial agents was 96.8%, and agreement if minor discrepancies were disregarded was 98.6%. Since the correlation of the AMS-GSC versus reference methods was excellent, we used the AMS-GSC as the standard for comparison in our study.

There were only 0.5% very major discrepancies for susceptibility tests with the AMS-ECSC. There were 3.3% major discrepancies,

with 55% of these discrepancies occurring with cephalothin and tetracycline. A standardized inoculum was not used, and it is likely that major discrepancies were caused by too heavy an inoculum. The use of a McFarland turbidity standard to standardize inoculum size would probably decrease major discrepancies. There were 1.8% minor discrepancies, with 72.7% of these discrepancies produced by cephalothin. Of the minor discrepancies with cephalothin, 85% were due to an indeterminate result by the standard method.

An analysis of the total direct cost, which included amortization of equipment, determined that use of the rapid method resulted in an average increased cost of \$0.32 per specimen. When the AMS-ECSC was inoculated, 90% of the specimens with *E. coli* bacteriuria were properly identified (Table 2) and susceptibilities were printed. Based on a workload of 1,000 urine cultures per month in our laboratory, approximately 74 (90% of 82; Table 1) patients would have *E. coli* bacteriuria identified and susceptibilities performed, with results available to the physician within 1 day. These 74 patients would represent 35% of all patients each month with bacteriuria. Although this method is somewhat labor intensive and increases the cost per specimen by \$0.32, the decrease of approximately 1 working day in turnaround time for 35% of the patients with bacteriuria seems well worth the expense.

In conclusion, this report describes a rapid method for the identification and susceptibility testing of approximately 70% of specimens with *E. coli* bacteriuria by use of the AUS, a 5-h indole test, and the AMS-ECSC.

#### ACKNOWLEDGMENTS

We thank J. Vernon Knight and Robert P. Williams for critical review of the manuscript and Janice Edwards for secretarial assistance.

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