Rapid and Economical Identification and Antimicrobial Susceptibility Test Methodology for Urinary Tract Pathogens

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To decrease the time and cost of processing urine cultures, we devised a critical pathway to identify and perform antibiotic susceptibility tests on commonly isolated microbial pathogens within 6 h of growth detection. The strategy was based on eliminating expensive kits and automated procedures when not required. A pathway utilizing a statistical matrix and three rapid biochemical tests required to identify the most common pathogen, Escherichia coli, was developed. This species, which represented 82% of urinary isolates, was identified in 1 h for less than 10% the cost of a commercial kit. The specificity of the 1-h E. coli identification battery was ≥99.9% with a sensitivity of 93%. In addition, this critical pathway, adapting published methods, permitted the identification of other enteric pathogens, the group D streptococci, and Pseudomonas aeruginosa within 4 to 6 h. Furthermore, it accounted for other microbes that required longer periods of incubation. The pathway also included a rapid disk diffusion sensitivity test. Utilizing the critical pathway strategy, 76% (E. coli frequency of 0.82 × E. coli sensitivity of 0.93) of all urinary pathogens were identified within 1 h, and 98% were identified within 4 h with an antibiotic sensitivity test available within 6 h after the observation of growth. Costs were reduced from 2.5 to 5.0 times. This methodology is applicable to other specimen types.

In the last decade we have witnessed a significant growth in the number of laboratory tests carried out per hospitalized patient and an increase in the sophistication and complexity of each individual test. Too often this growth was given impetus by the tendency to axiomatically presume that more was better than less. Costs have increased concomitantly (10). To address this situation, we devised a critical pathway strategy to reduce costs, increase accuracy, and decrease the time required for identification and antimicrobial susceptibility testing of microbes isolated from urinary tract infections. The method can also be applied to other specimen types. The strategy was based on developing a critical pathway of work flow that would permit the laboratory to specifically identify the most common isolates with the fewest number of tests and in the most rapid time span. Complex, time-consuming, and expensive procedures required for only a small percentage of laboratory isolates would not be routinely used for all microbes, as is now common practice. A requirement of the strategy was the generation of a completed report for over 95% of isolates within 6 h of the time significant growth was detected.

The urine specimen was chosen as the critical pathway strategy model because it represented the largest number of culture requests received by the Clinical Microbiology Laboratory of the Yale-New Haven Hospital. The distribution of microbiology specimens was as follows: urine cultures, 35.9%; blood cultures, 23.2%; stool specimens, 11.9%; respiratory specimens, 10.2%; wound specimens, 9.4%; mycology-tuberculosis specimens, 6.4%; and sterile body fluids (other than blood cultures), 3.0%. Although the ratios of some specimen types, particularly stool and respiratory, can vary by season, this division approximated that seen nationally at primary teaching hospitals.

At Yale-New Haven Hospital and nationally, 95% of urinary tract infections are caused by a single infecting microbe, with Escherichia coli being responsible for approximately 80% of all nosocomial and more than 90% of those community-acquired infections. After E. coli, other members of the family Enterobacteriaceae, the group D streptococci, and Pseudomonas aeruginosa comprise all but 2% of isolates. The majority of hospital microbiology laboratories devote a significant portion of their budgets to purchasing commercial products to identify E. coli and other enteric pathogens. Single kits range in price from $1.90 to $2.50 and can cost up to $4.00 if instrumentation is utilized. Be-
because these kits are designed to identify all enteric pathogens, they contain a large number of individual biochemical tests, many of which are extraneous to the identification of a particular species such as *E. coli*. Yet, we pay for the additional tests and must wait for their reactions to develop. Most commercial kits require 24 h of incubation after inoculation before an identification is established. The group D streptococci and *P. aeruginosa* can also be identified rapidly and with a much smaller number of tests than are required for other microbes in their respective families.

To decrease the cost and time of identification of *E. coli*, a statistical matrix was devised to determine the minimum number of tests required (1, 6, 15, 16). The tests were selected by their ability to separate *E. coli* from other microbes and to identify this species with 99.9% specificity. These tests were formulated to yield results within 1 h after inoculation and were integrated into the routine identification system for urinary tract pathogens. To achieve maximal benefit from this system, the work flow pathway was further designed to yield an identification and antimicrobial susceptibility test from 98% of human urinary isolates within 6 h.

**MATERIALS AND METHODS**

**Analysis of microbial growth.** All specimens were human urine samples submitted for analysis of microbial content and antibiotic susceptibility to the Clinical Microbiology Laboratory of the Yale-New Haven Hospital. Microbes were quantified by means of a standard calibrated loop technique (3). A total of 1,200 patient samples were tested, of which 465 showed significant growth. *E. coli* was the single pathogen in 381 (82%) of these positive cultures; *E. coli* with another microbe was present in an additional 5%. A non-*E. coli* enteric pathogen, a group D streptococcus, or *P. aeruginosa* represented 16% of the remaining isolates encountered in this study. Therefore, microbes other than members of the family *Enterobacteriaceae*, group D streptococci, or *P. aeruginosa* were encountered in 2% of cases during the course of this study.

**Identification of *E. coli*.** By applying an analytical matrix (4, 9), the number of tests required to identify *E. coli* specifically were reduced to the following: growth and lactose fermentation on MacConkey agar, indole positivity, cytochrome c oxidase negativity, and β-glucuronidase positivity.

The indole test was carried out as a spot procedure on filter paper and required 30 s (17). Kovacs reagent was added to standard laboratory filter paper in one half of a petri plate; a portion of a bacterial colony was rubbed onto the filter paper, and if within 30 s the colony became red, the test result was indole positive. If the reagent remained unchanged, it was negative.

The oxidase test was likewise a spot test, with 1% N,N,N′,N′-tetramethyl-p-phenylenediamine dihydrochloride reagent (Sigma Chemical Co., St. Louis, Mo.) added to filter paper in the other half of the petri plate (5, 12). Sodid ascorbate (0.1% final concentration) was added to this reagent to prevent autoxidation. A commercial reagent (Marion Scientific, Kansas City, Mo.) was also found to be satisfactory. Like the indole test, a portion of the colony was rubbed onto the filter paper; if the reagent turned purple in 30 s, the test result was positive; if it remained unchanged, it was negative. At least 50 tests can be carried out on each half of the filter paper.

β-Glucuronidase reagent consisted of 0.5 mg of p-nitrophenyl-β-D-glucuronide (Sigma) per ml in 0.05 M Sorenson phosphate buffer (pH 7.5). Portions (0.25 ml) were transferred into test tubes (12 by 75 mm). Several colonies from MacConkey agar were inoculated into a tube and incubated for 1 h at 35°C. An unchanged, colorless solution was regarded as a negative result. A yellow hue indicated a positive result as color development when the p-nitrophenyl was released from p-nitrophenyl-β-D-glucuronide by β-glucosidase.

The group D streptococci and *P. aeruginosa* were also rapidly identified (2, 7, 8). *Candida albicans* was identified by the germ tube test (3). Other pathogenic microbes were identified as directed by the critical pathway by conventional means.

**Processing urine cultures.** Agar plates inoculated with patient urine were examined after 18 to 24 h of incubation. If growth was not observed, plates were reincubated and examined again at 48 h. All plates showing significant growth (≥10⁴ microbes per ml) were batch processed. Identification and antibiotic susceptibility proceeded in the following sequence (Fig. 1): (i) The rapid spot indole and oxidase tests were performed and recorded. (ii) The test tubes containing p-nitrophenyl-β-D-glucuronide reagent were inoculated and placed in the incubator. (iii) A 6-h plate antibiotic disk susceptibility test (13) was inoculated and placed in the incubator. (iv) The β-glucuronidase test was read 1 h after inoculation, and the results were recorded. Those microbes that were indole positive, oxidase negative, lactose positive, and β-glucuronidase positive were identified as *E. coli*. Those organisms that were oxidase negative and negative on any other test were considered an enteric pathogen and inoculated into a commercial, 4-h identification system (Micro-ID; General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.). Those organisms that were oxidase positive were considered nonfermenters and were identified as *P. aeruginosa* by a rapid test (2) or as another species by conventional means (3, 6). Group D streptococci were identified by rapid tests (7, 8), and other gram-positive bacteria were identified by conventional tests (7). *C. albicans* was identified by the 2-h germ tube test, and other yeasts were identified by a commercial product (Analytab Products, Plainview, N.Y.). (v) After 4 h, the Micro-ID tests were read and the enteric pathogens were identified. (vi) The antibiotic susceptibility plates were examined and recorded. If quality control criteria were fulfilled, final reports containing an identification and antibiotic sensitivity were available for 98% of isolates 6 h after initiation of batch processing.

**RESULTS**

**Specificity and sensitivity of the identification of *E. coli*.** By comparison with the methods statistically the same as the methods used by the
Centers for Disease Control (1, 6), 354 of 354 microbes that grew on MacConkey agar were β-glucuronidase, indole, and lactose positive, and oxidase negative and were identified as *E. coli* (specificity of ≥99.9%). We found that *E. coli* was 100% positive for growth on MacConkey agar, 95% indole positive, 94% β-glucuronidase positive, 88% lactose positive, and 0% oxidase positive. All of the 12% lactose-negative isolates that were indole and β-glucuronidase positive and oxidase negative were also identified by conventional means as *E. coli*. Theoretically, 8% of *Shigella* sp. may be β-glucuronidase, indole, and lactose positive; however, none were encountered from urine during the course of this study, and only two had been identified from the previous 200,000 urine cultures at this hospital.

Seven percent (27 of 381) of *E. coli* showed either a negative indole or negative β-glucuronidase.
The group D streptococci and *P. aeruginosa* were identified by a small number of rapid tests, with the remaining microbes identified by a large series of biochemical tests. A critical pathway for processing specimens automatically directed the workflow to higher levels of complexity when required.

First, we have demonstrated that by choosing the tests to be used with a statistical matrix, *E. coli* can be identified specifically by the following characteristics: growth and lactose fermentation on MacConkey agar, production of β-glucuronidase, production of indole, and lack of production of oxidase. By using these characteristics, there is less than a 0.1% chance that it may be confused with another organism (99.9% specificity). Approximately 7% of *E. coli* will demonstrate an atypical reaction in any one of these tests (93% sensitivity). However, these isolates are captured by inoculation into the 4-h commercial system, as are all other enteric bacilli (4). Rapid systems have been reported that utilize only growth on MacConkey agar, indole positivity, and oxidase negativity (14) for identification. Without the β-glucuronidase test, however, there is a sacrifice of specificity for sensitivity. For example, *Citrobacter diversus* and *Klebsiella oxytoca* may be confused with *E. coli* (11).

Second, after designing the minimum number of tests required to identify *E. coli* and other commonly isolated pathogens, we analyzed cost parameters. Our thesis was that by determining whether an organism was or was not *E. coli*, a critical pathway could be developed that would obviate the need for using large numbers of commercial, expensive kits. Our data indicated that we eliminated 93% of *E. coli* kit identifications and reduced kit usage in the laboratory overall by 65%. Because the difference in cost between a commercial or homemade identification system is from 2.5 to 5.0 times more than the 1-h test, substantial savings occurred.

After establishing a specific and inexpensive means of identifying *E. coli* and other frequent urinary isolates, our third and final goal was to
perform it in a time frame that would be rapid and useful for the patient. Initiation of therapy is based on two factors: the name of the organism and the antibiotic susceptibility pattern. There is an association between the species name and its antibiotic susceptibility pattern, and therapy is often started on the basis of microbiological identification. Therefore, we felt that to identify an organism specifically and rapidly would aid in the proper therapy and management of the patient. Our system identified *E. coli* in 1 h, other enteric pathogens in 4 h, and common nonenteric pathogens in 6 h. In addition, our pathway decreased the antibiotic susceptibility test time from 24 to 6 h. It should be mentioned that this scheme, although presented in reference to urine culture, can be used for the identification of *E. coli* and members of the family *Enterobacteriaceae* from other body sites.

The 1-h identification method described here allows the specific identification of the most common human pathogen from the human urinary tract, *E. coli*, in 1 h and the identification of other pathogens that account for 98% of urinary isolates in 4 h. It permits specific treatment based on the name of the organism 24 h before other commonly employed systems. In addition, it is between 2.5 and 5.0 times less expensive than other commonly utilized identification methods.

LITERATURE CITED