Procedure for Expediting Determinations of Antibiotic Susceptibility of Gram-Negative, Urinary Tract Pathogens

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Received for publication 30 July 1976

Standardized direct disk diffusion antibiotic susceptibility testing on monomicrobial urine specimens is compared with the Food and Drug Administration method. The direct procedure yields acceptable data and may conserve 24 h in reporting results.

Disk diffusion antibiograms of bacterial urinary tract pathogens are most appropriately determined by the Food and Drug Administration (FDA) recommended procedure (3, 5, 6, 7), which requires a single isolate to prepare inocula for susceptibility testing. The necessity to await pure cultures causes 36 to 48 h to elapse before antibiotic susceptibility data become available. This period has been shortened in clinically urgent situations by using the urine specimen itself as inoculum for disk diffusion testing (1, 4). Because the urine sometimes contains inhibitory factors and varying quantities of organisms, sensitivity data so obtained may differ from information collected by the accepted method.

Previous studies in this laboratory (9) showed that when bacteremia is caused by a single, rapidly growing pathogen, 24 h may be conserved by inoculating a small volume of blood culture fluid into a relatively large volume of nutrient broth and growing it to standard density. This results in an inoculum for disk diffusion testing that is standardized and in which toxic factors are eliminated by dilution. The present study shows that the principle is also applicable to the susceptibility testing of urine specimens containing a single gram-negative organism.

Urine specimens from patients with suspected urinary tract infection were inoculated onto MacConkey agar plates, colistin-nalidixic acid sheep blood agar, and 10% sheep blood agar plates. One drop of each urine specimen (ca. 0.01 ml) was inoculated into 2 ml of Columbia broth with a Pasteur capillary pipette.

The Columbia broth suspensions were incubated for 4 to 6 h at 35°C. Those showing turbidity were Gram stained and subsequently adjusted with 0.9% NaCl to match a 0.5 McFarland BaSO₄ standard. Mueller-Hinton agar plates were inoculated evenly, and antibiotic disks (BBL) were applied. After 18 to 24 h of incubation at 35°C, inhibition zone diameters were measured to the nearest whole millimeter with a linear scale. Urine specimens shown to be polymicrobial by stain or culture, those containing gram-positive organisms, and specimens containing slow-growing organisms were not considered.

Isolated colonies from the blood agar plates were used to prepare inocula for the FDA standardized Bauer-Kirby procedure. Zone sizes for each isolate and drug tested by the direct and standard procedure were recorded and compared to interpretive standards established by the FDA method (5).

Seventy-two urine specimens, containing single, rapidly growing, gram-negative pathogens, were evaluated. Bacterial titers of the specimens ranged from 30,000 organisms per ml to greater than 500,000 organisms per ml. Antibiograms of 26 Escherichia coli, 12 Proteus mirabilis, 11 Klebsiella pneumoniae, 9 Serratia marcescens, 9 Enterobacter cloacae, 3 Enterobacter aerogenes, and 2 Proteus vulgaris were obtained by both methods.

Data (Table 1) for the E. coli isolates are representative of the group of organisms. For each urine isolate and each antibiotic tested, discrepancies between zones obtained by both methods averaged less than 2 mm. Results obtained by the two methods necessitated a change in interpretive susceptibility category in only seven unrelated instances (1.0%). Since polymicrobial suspensions yield unreliable antibiotic susceptibility data (8), this technique is only applicable to the testing of monomicrobial urine specimens. Fortunately, the majority of bacterial urinary tract infections are caused by single gram-negative organisms.

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Because of the mixed flora at the terminal end of the urinary tract, extreme care must be exercised in specimen collection. Bacteria from a few urine specimens from patients receiving antibiotics grew on solid isolation media but did not grow in direct susceptibility nutrient broth. Therefore, if clinically feasible, it is also recommended that urine specimens for direct susceptibility testing be collected before initiation of antimicrobial therapy.

Because of the chance of polymicrobial urinary tract infection or infection by slow-growing organisms, direct susceptibility testing on all urine specimens is not recommended as a replacement for the FDA procedure. However, in clinically urgent situations, such as bacteremia secondary to bacteriuria, it is felt that this technique is a valid means to conserve 24 h.

**LITERATURE CITED**

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**Table 1. Comparison of experimental direct and indirect methods for obtaining antibiograms of E. coli isolates from urine specimens**

| Antibiotic (concn) | Mean difference (mm) | Range (mm) |
|--------------------|----------------------|------------|
| Ampicillin (10 µg) | 1.2                  | 0–3        |
| Tetracycline (30 µg) | 1.5                  | 0–3        |
| Cephalothin (30 µg) | 1.6                  | 0–4        |
| Colistin (10 µg)   | 0.2                  | 0–1        |
| Gentamicin (10 µg) | 1.2                  | 0–2        |
| Carbenicillin (100 µg) | 1.0               | 0–3        |
| Nalidixic acid (30 µg) | 1.0                 | 0–3        |
| Furadantin (300 µg) | 1.1                  | 0–3        |
| Gantrisin (2 µg)   | 1.1                  | 0–4        |

* Twenty-six urine specimens containing E. coli as the sole bacterial pathogen were evaluated.

* For each urine isolate, the antibiotic zones of inhibition were obtained by both the experimental direct and indirect Bauer-Kirby methods. Averages and ranges of the individual differences were then computed.