Facilitating Quality Control of the Antimicrobial Susceptibility Test

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Standard reference strains of Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa were suspended in sterile deionized water and tested daily via disk agar diffusion for their antimicrobial susceptibilities. Inocula of E. coli and P. aeruginosa yielded acceptable results for up to 32 days; however, results on S. aureus were unacceptable due to loss of viability of the organism in water. E. coli and P. aeruginosa inocula, in water, can be used daily for quality control of the disk agar diffusion test.

The standardized (3) disk agar diffusion test is one of the commonest methods for determining antimicrobial susceptibility patterns of various clinical isolates. Test results can be influenced by many variable factors including the type of medium, its thickness and pH, inoculum concentration, incubation time and atmosphere, and antimicrobial disk concentration. To ensure accurate testing and thus facilitate adequate patient therapy, quality control (QC) is performed. Susceptibility patterns of standard reference strains (Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853) should be determined daily (5). This is especially important in determining antimicrobial disk potency.

Occasionally, when test variables are not adequately controlled, these QC test results are not acceptable. In this instance, the microbiologist must determine whether or not to report results of tests run concurrently on patient isolates. This may not be an easy decision since QC results could be unacceptable, whereas results on patient isolates could be perfectly accurate. In addition, the decision to not report results on patient isolates can delay patient therapy and increase costs for the patient and hospital.

One common procedural error, which could result in unacceptable QC results, would be the inadvertent improper preparation of the inoculum. This could occur daily. In addition, the inoculum is usually discarded after plating on Mueller-Hinton agar. It would therefore be difficult to determine if the inoculum concentration was correct on the next day when the test is read.

An attempt was thus made to eliminate improper inoculum concentration as a source of QC error and to facilitate daily QC testing by eliminating daily inoculum preparation. Since many organisms remain viable in water for extended periods, this investigation was done to determine if standard reference strains could be suspended in water to a known turbidity and subsequently be used daily as inocula.

Standard reference strains of S. aureus ATCC 25923, E. coli ATCC 25922, and P. aeruginosa ATCC 27853 were inoculated onto 5% sheep blood agar plates and incubated overnight at 35°C. Colonies from the plates were then suspended in sterile deionized water to a turbidity equal to that of a 0.5 McFarland standard. Antimicrobial disk agar diffusion testing and acceptable zone-of-inhibition range testing were performed and interpreted as described elsewhere (5), except colonies were suspended in sterile deionized water instead of a broth medium. The same original inoculum, in water, was used daily. Antimicrobial disks were purchased from Difco Laboratories. The following antimicrobial agents were tested: E. coli—amikacin, ampicillin, carbenicillin, cefamandole, cefoxitin, cephalexin, chloramphenicol, colistin, erythromycin, gentamicin, nitrofurantoin, sulfamethoxazole-trimethoprim, tetracycline, and tobramycin; P. aeruginosa—amikacin, carbenicillin, colistin, gentamicin, and tobramycin; S. aureus—cefamandole, cephalothin, chloramphenicol, clindamycin, erythromycin, gentamicin, penicillin, sulfamethoxazole-trimethoprim, tetracycline, and vancomycin.

On four different occasions, susceptibility tests were performed daily for 12 to 32 days. The zone-of-inhibition sizes were within acceptable ranges for all antimicrobial agents on all occasions with E. coli and P. aeruginosa. However, on three of four occasions, results with S. aureus were unacceptable. Zone-of-inhibition sizes became too large by day 4. This suggested a loss of viability of S. aureus in water. Subsequent col-
mony counts on *S. aureus* water suspensions revealed a decrease in the number of colony-forming units per milliliter from $10^6$ on day 1 to $10^5$ on day 7.

The increased emphasis on quality control in clinical microbiology has undoubtedly resulted in improved accuracy and precision of some test results, along with an increase in cost to the patient and hospital. One test which must be controlled properly, the disk agar diffusion method for determining the antimicrobial susceptibilities of clinical isolates, has many variables which can alter the final test results. Therefore, several reports note that standard reference strains should be tested on each batch of tests performed (1) and on each batch of medium (2), daily or weekly (4). In effect this means that three organisms will be tested daily, in most instances, against many antimicrobial agents. This protocol therefore entails a tremendous number of man-hours daily when one considers all of the clinical microbiology laboratories in the United States alone.

I wish to make QC testing as efficient as possible. Data from this study indicate when *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 are suspended in water and tested daily from the original inocula, antimicrobial susceptibility results are consistent and fall within acceptable ranges for up to 32 days. This is not true for *S. aureus* ATCC 25923, however. Results with this organism, under these conditions, are unpredictable due to loss of viability of the organism in water.

It is therefore recommended that *E. coli* and *P. aeruginosa* QC strains be suspended in sterile deionized water at a standardized concentration and tested daily for up to 32 days. Therefore, one inoculum with each of these two organisms would suffice for several weeks. If unacceptable QC results occurred during the use of the original inoculum the possibility of improper inoculum concentration could be eliminated for all practical purposes. This protocol would determine if the antimicrobial disks are potent from day to day and would help the laboratory personnel decide whether or not to report results on patient isolates.

**LITERATURE CITED**


