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Repeat Antimicrobial Susceptibility Testing of Identical Isolates

RICHARD B. THOMSON, JR.,[1,2]* THOMAS M. FILE, JR.,[2,3] AND REBECCA A. BURGOON[1]

Departments of Pathology and Laboratory Medicine[1] and Medicine,[3] Akron City Hospital, Akron, Ohio 44309, and Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272[2]

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Duplicate antimicrobial susceptibility test results were reviewed over a 1-year period to determine whether repeat testing of sequential isolates with the same identification from the same patient and specimen site was necessary. In our institution, repeat testing is always needed for coagulase-negative staphylococci and *Pseudomonas aeruginosa* and is needed after 3 days for members of the family *Enterobacteriaceae*, but it is not routinely necessary for *Staphylococcus aureus*.

It is recommended (2) that clinical microbiology laboratories save time and money by eliminating duplicate antimicrobial susceptibility testing of sequential bacterial isolates with the same identification from the same patient and specimen site. It is not clear how many days should elapse before testing is needed again. Recommendations vary from 3 to 7 days (1).

Repeat testing may not be necessary for several days or weeks, since the usual mechanisms by which bacteria develop resistance (12), including conjugation, transduction, and random chromosomal mutation, do not appear to contribute to the rapid appearance of resistance during the treatment of disease. On the other hand, resistance may be detected during treatment if multiple strains were originally present but not recognized (10), if derepression of a β-lactamase-producing gene occurs (8), or if an error was made during the original antimicrobial susceptibility test (3).

We present here a review of 690 bacterial stains for which antimicrobial tests were repeated up to 28 days after the original test in order to determine whether repeat testing could be eliminated or postponed and, if so, for how long.

Repeat antimicrobial susceptibility tests were performed as part of the routine bacteriology laboratory workup during 1986 on patient isolates obtained from specimens other than blood. A repeat susceptibility test was defined as one that was performed on an isolate from the same patient and specimen site with the same identification as a previous isolate but that was cultured from a subsequent specimen obtained on the same day or up to 28 days after the original specimen was cultured. Antimicrobial susceptibility tests were performed by standardized disk diffusion susceptibility methods (6). Only susceptible to resistant changes, which were very major errors, were counted (13). We extended the original meaning of very major error to include any report of susceptibility which, in truth, was resistant. Isolates used for data collection included *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and coagulase-negative staphylococci. The batteries of antimicrobial agents that were tested included ampicillin, mezlocillin, piperacillin, cefazolin, cefoxitin, cefoperazone, gentamicin, tobramycin, amikacin, tetracycline, and trimethoprim-sulfamethoxazole for members of the family *Enterobacteriaceae*; mezlocillin, piperacillin, cefoperazone, gentamicin, tobramycin, and amikacin for *P. aeruginosa*; and penicillin, oxacillin, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, and vancomycin for the staphylococci.

Susceptibility test batteries were reviewed retrospectively for 692 microorganisms. This included 5,575 organism-antimicrobial agent combinations. The number of these combinations which showed susceptible to resistant changes, the total number of organism-antimicrobial agent tests performed, and the percentage of the total which changed are given in Table 1. Because only susceptible strains could show a susceptible to resistant change, relevant antibiograms representing the period of data collection are summarized in Table 2. The most common organisms to show a susceptible to resistant change were the coagulase-negative staphylococci and *P. aeruginosa*. Changes were much less common for members of the family *Enterobacteriaceae* and *Staphylococcus aureus*. All antimicrobial agents in the coagulase-negative staphylococcal test battery, with the exception of vancomycin, showed a greater than 10% susceptible to resistant change for all strains retested during the first 7 days. The most common antimicrobial agents to change, when tested with *P. aeruginosa*, were mezlocillin, piperacillin, and cefoperazone, which showed 5, 3, and 3% susceptible to resistant changes, respectively, for all strains that were retested during the first 7 days. When penicillin was tested with *Staphylococcus aureus*, there was a 3% change for strains that were retested during the first week. No other microorganism-antimicrobial agent combination showed a 1.5% or greater susceptible to resistant change during the first 7 days. Bacteria which showed susceptible to resistant changes were isolated from the respiratory and urinary tracts and wounds. Of the strains that were retested during the first week, 0.7% of the organism-antimicrobial agent combinations for the respiratory tract, 0.7% for the urinary tract, and 0.4% for wounds showed susceptible to resistant changes.

Susceptible to resistant changes were also calculated for the antimicrobial agent test battery for each microorganism, as opposed to each antimicrobial agent. The number of repeat test batteries containing at least one susceptible to resistant change, the total number of repeat test batteries performed, and the percentage of repeat test batteries containing one or more changes are given in Table 3.

To determine whether a technical error in the susceptibility testing procedure contributed significantly to the number of repeat results which changed, 1,218 repeat *P. aeruginosa* disk susceptibility tests were also reviewed for resistant to susceptible changes. Eight (0.4%) such changes were found.

There are two solutions to the problem of when to repeat

* Corresponding author.

TABLE 1. Antimicrobial agent disk tests that changed from susceptible to resistant following repeat testing of identical isolates[a]

Organism	No. of strains tested	No. of repeat disk results that changed/total no. of repeat disk tests performed (% of repeat disk tests that changed) on day(s)[b]:									Total for all days
		1	2	3	4	5	6–7	8–10	11–14	15–28	
Coagulase-negative staphylococci	88	42/264 (16)	15/60 (25)	3/48 (6)	1/36 (3)	2/36 (6)	9/48 (18)	3/30 (10)	0/6 (0)	0/0 (0)	75/528 (14)
Pseudomonas aeruginosa	203	6/258 (2)	0/108 (0)	3/102 (3)	4/84 (5)	0/84 (0)	15/168 (9)	11/138 (8)	17/138 (12)	15/138 (11)	71/1,218 (6)
Members of the family *Enterobacteriaceae*[c]	287	5/1,155 (0.4)	1/264 (0.4)	2/253 (0.8)	2/176 (1.1)	4/154 (2.6)	1/187 (0.5)	3/330 (0.9)	1/176 (0.6)	20/462 (4.3)	39/3,157 (1.2)
Staphylococcus aureus	112	1/348 (0.3)	0/48 (0)	1/66 (1.5)	0/42 (0)	1/18 (5.6)	0/42 (0)	0/36 (0)	0/36 (0)	0/36 (0)	3/672 (0.4)
Total	690										188/5,575 (3.4)

[a] Susceptibility testing was performed by standard disk diffusion techniques. Identical isolates were the original and subsequent isolate from the same patient and the same specimen site and with the same identification.

[b] The staphylococci, *Pseudomonas aeruginosa*, and members of the family *Enterobacteriaceae* were tested against 6, 6, and 11 antimicrobial agents, respectively.

[c] Includes 125 *Escherichia coli*, 52 *Enterobacter aerogenes*, 48 *Serratia marcescens*, 32 *Klebsiella pneumoniae*, and 30 *Enterobacter cloacae* isolates.

an antimicrobial agent susceptibility test of a sequential, presumably identical, isolate. First, the laboratory should not reculture duplicate specimens unless it is indicated clinically. Second, the laboratory should determine, for those situations in which reculturing is necessary, how many days can elapse before repeat antimicrobial susceptibility testing is needed again. Reculturing is necessary when the clinical response of the patient is less than expected (5), when intubated patients become colonized with gram-negative bacilli and require microbiologic monitoring of respiratory tract secretions (7), or when a duplicate specimen is needed to substantiate the significance of a potentially pathogenic microorganism isolated previously (4). Although we discourage, through ongoing house staff educational programs, the collection of duplicate specimens for reasons other than those mentioned above, we did not attempt to eliminate unnecessary duplicate specimens from our study. Unnecessary duplicate specimens included in our data represent specimens that we and those in other clinical microbiology laboratories process unknowingly. One approach which decreases the resources of a laboratory devoted to the processing of duplicates is to eliminate repeat antimicrobial susceptibility testing of identical isolates.

We found that 3.4% of all antimicrobial disk susceptibility tests repeated on sequential isolates with the same identification from the same patient and specimen site changed from susceptible to resistant (Table 1). Only susceptible to resistant changes were counted, since reporting of an isolate as being susceptible to an antimicrobial agent when it is actually resistant is considered a very major error, according to the interpretation of Thornsberry et al. (13). Very major errors contribute to the selection of an antimicrobial agent that is inactive against the patient isolate. Other errors occurred, but they were less important. It is not clear when repeat testing should be eliminated. According to Sherris and Ryan (11), very major errors attributable to a new antimicrobial agent testing procedure should not exceed 1.5%. Although the elimination of repeat testing does not constitute a new procedure, it does represent a modification which potentially introduces error. If the 1.5% very major error rate is used as a guide, then repeat results of coagulase-negative staphylococci and *P. aeruginosa* should always be retested, since susceptible to resistant errors consistently exceeded 1.5% (Table 1). Similar errors for members of the family *Enterobacteriaceae* and *Staphylococcus aureus* rarely exceeded 1.5%, suggesting that repeat testing may not be necessary. On the other hand, if one examines the percentage of repeat antimicrobial agent test batteries containing at least one susceptible to resistant change (Table 3), repeat testing of coagulase-negative staphylococci is always found to be necessary. Repeat testing of *P. aeruginosa* and members of the family *Enterobacteriaceae* may not be necessary until 4 days after the original test, and repeat testing of *Staphylococcus aureus* is not needed. An examination of the number of repeat batteries that change from susceptible to resistant may be more relevant to the clinical microbiology laboratory than an examination of the number of individual antimicrobial agent tests that change. Laboratories commonly report microorganism susceptibility results as a battery of antimicrobial agents. It was surprising to us that such a high percentage of antimicrobial agent test batteries contained at least one susceptible to resistant change (Table 3). A practical interpretation of these data (Tables 1 and 3) suggests that, in our institution, repeat testing is always needed for coagulase-negative staphylococci and *P. aeruginosa*, is needed after 3 days for members

TABLE 2. Percentage of microorganisms susceptible in vitro during 1986[a]

Organism	% Susceptible to the following antimicrobial agent:															
	PN	OX	AM	MEZ	PIP	CZ	FOX	CEP	GM	TB	AK	CM	EM	SXT	TE	VM
Coagulase-negative staphylococci	19	53	NA[b]	NA	NA	NA	NA	NA	NA	NA	NA	70	49	66	NA	100
Pseudomonas aeruginosa	NA	NA	NA	80	95	NA	NA	86	84	94	96	NA	NA	NA	NA	NA
Members of the family *Enterobacteriaceae*[c]	NA	NA	44	88	87	77	79	94	99	99	99	NA	NA	93	61	NA
Staphylococcus aureus	12	99	NA	NA	NA	NA	NA	NA	NA	NA	NA	97	89	99	NA	100

[a] Abbreviations: PN, penicillin; OX, oxacillin; AM, ampicillin; MEZ, mezlocillin; PIP, piperacillin; CZ, cefazolin; FOX, cefoxitin; CEP, cefoperazone; GM, gentamicin; TB, tobramycin; AK, amikacin; CM, clindamycin; EM, erythromycin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline; VM, vancomycin.
[b] NA, Not applicable.
[c] Includes *Escherichia coli*, *Enterobacter aerogenes*, *Serratia marcescens*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*.

of the family *Enterobacteriaceae*, but is not routinely necessary for *Staphylococcus aureus*.

The high percentage of coagulase-negative staphylococci which had repeat tests showing susceptible to resistant changes, the relatively even distribution of these changes over the 28 days of data collection, and the lack of evidence suggesting that in vivo development of resistance for this group of microorganisms commonly occurs suggest that different strains were isolated for the repeat test (Table 1). Although 6% of *P. aeruginosa* repeat tests changed from susceptible to resistant, most changes occurred after day 5. We speculate that most of the changes which occurred after day 5 represented repeat tests on newly acquired strains. Those that occurred during the first 5 days may have represented the development of resistance by the original isolate or the presence of multiple *P. aeruginosa* strains in either the initial or repeat culture which went undetected. Strain dissociation is common when *P. aeruginosa* is isolated from patients with cystic fibrosis. Each strain that is dissociated may have a different antibiogram (9). This phenomenon may occur to a lesser extent in all infected patients (10). The usual method of touching four to five colonies for disk diffusion susceptibility testing would not ensure that all strains are sampled. The enteric gram-negative bacilli had relatively few susceptible to resistant changes. The development of resistance, the acquisition of a new strain, or the inability to recognize multiple strains in a culture could explain these changes. The three susceptible to resistant changes for *Staphylococcus aureus* all involved penicillin. We checked all penicillin-susceptible *Staphylococcus aureus* isolates for penicillinase production following enzyme induction around an oxacillin disk. All were negative. The *Staphylococcus aureus* isolates which changed were most likely different strains.

Similar percentages of susceptible to resistant changes were detected in specimens from the respiratory and urinary tracts and from wounds. The source of the specimen did not predict whether repeat testing of the isolate was necessary.

Technical errors in the susceptibility testing procedure were most likely not the cause of the susceptible to resistant changes, since the opposite change for *P. aeruginosa*, resistance to susceptibility, was very uncommon (0.4%). We assume that inconsistent test procedures would give roughly equal errors in both directions.

The most common antimicrobial agents, other than those tested with the coagulase-negative staphylococci, to show susceptible to resistant change following repeat testing were mezlocillin, piperacillin, and cefoperazone when they were tested with *P. aeruginosa*. Since resistance to the ureidopenicillins and cephalosporins in our institution is common (Table 2), we would expect to see susceptible to resistant changes resulting from the development of resistance or the testing of a second strain. Aminoglycoside resistance, on the other hand, is unusual; therefore, we would not expect to see such changes for these antimicrobial agents.

In summary, repeat testing in our institution is always needed for coagulase-negative staphylococci and *P. aeruginosa* and is needed after 3 days for members of the family *Enterobacteriaceae*, but it is not routinely necessary for *Staphylococcus aureus*. Since resistance to antimicrobial agents varies from one institution to another, these recommendations may not apply to all laboratories. Susceptible to resistant changes were most common with the ureidopenicillins and the cephalosporins that were tested. The source of the specimen did not predict whether repeat testing of the isolate was necessary. The reasons for the susceptible to resistant changes are not known for sure. Development of resistance or testing of a new strain are two probable causes.

TABLE 3. Antimicrobial agent batteries containing susceptible to resistant change(s) following repeat testing of identical isolates[a]

Organism	No. of repeat batteries containing change(s)/total number of repeat batteries [% of repeat batteries containing change(s)] on day(s)[b]:									
	1	2	3	4	5	6–7	8–10	11–14	15–28	Total for all days
Coagulase-negative staphylococci	26/44 (59)	7/10 (70)	1/8 (13)	1/6 (17)	1/6 (17)	5/8 (63)	1/5 (20)	0/1 (0)	0/0 (0)	42/88 (48)
Pseudomonas aeruginosa	3/43 (7)	0/18 (0)	1/17 (6)	4/14 (29)	0/14 (0)	8/28 (29)	6/23 (26)	9/23 (39)	7/23 (30)	38/203 (19)
Members of the family *Enterobacteriaceae*[c]	3/105 (3)	1/24 (4)	1/23 (4)	2/16 (13)	4/14 (29)	1/17 (6)	2/30 (7)	2/16 (13)	8/42 (19)	24/287 (8)
Staphylococcus aureus	1/58 (1.7)	0/8 (0)	1/11 (9)	0/7 (0)	1/3 (33)	0/7 (0)	0/6 (0)	0/6 (0)	0/6 (0)	3/112 (3)

[a] Susceptibility tests were performed by standard disk or diffusion techniques. Identical isolates were the original and subsequent isolate from the same patient and the same specimen site and with the same identification. The batteries of antimicrobial agents tested for the staphylococci (6 drugs), *Pseudomonas aeruginosa* (6 drugs), and members of the family *Enterobacteriaceae* (11 drugs) are given in the text.
[b] One or more susceptible to resistant changes within an antimicrobial agent test battery, follwoing repeat testing of an identical isolate, constituted a changed battery.
[c] Includes 125 *Escherichia coli*, 52 *Enterobacter aerogenes*, 48 *Serratia marcescens*, 32 *Klebsiella pneumoniae*, and 30 *Enterobacter cloacae* isolates.

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LITERATURE CITED

1. **Bartlett, R. C.** 1974. Medical microbiology: quality, cost, and clinical relevance, p. 13. John Wiley & Sons, Inc., New York.
2. **Bartlett, R. C.** 1982. Making optimum use of the microbiology laboratory. III. Aids of antimicrobial therapy. J. Am. Med. Assoc. **247**:1868–1871.
3. **Jones, R. N., D. C. Edson, and the CAP Microbiology Resource Committee.** 1982. Interlaboratory performance of disk agar diffusion and dilution antimicrobial susceptibility tests, 1979–1981. Am. J. Clin. Pathol. **78**(Suppl.):651–658.
4. **Lowy, F. D., and S. M. Hammer.** 1983. *Staphylococcus epidermidis* infections. Ann. Intern. Med. **99**:834–839.
5. **Milatovic, D., and I. Braveny.** 1987. Development of resistance during antibiotic therapy. Eur. J. Clin. Microbiol. **6**:234–244.
6. **National Committee for Clinical Laboratory Standards, Subcommittee on Disk Diffusion Susceptibility Testing.** 1984. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A3; December. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **Salata, R. A., M. M. Lederman, D. M. Shlaes, M. R. Jacobs, E. Eckstein, D. Tweardy, Z. Toosi, R. Chmielewski, J. Marino, C. H. King, R. C. Graham, and J. J. Ellner.** 1987. Diagnosis of nosocomial pneumonia in intubated, intensive care unit patients. Am. Rev. Respir. Dis. **135**:426–432.
8. **Sanders, C. C., and W. E. Sanders, Jr.** 1983. Emergence of resistance during therapy with the newer beta-lactam antibiotics: role of inducible beta-lactamases and implications for the future. Rev. Infect. Dis. **5**:639–648.
9. **Seale, T. W., H. Thirkill, M. Tarpay, M. Flux, and O. M. Rennert.** 1979. Serotypes and antibiotic susceptibilities of *Pseudomonas aeruginosa* isolates from single sputa of cystic fibrosis patients. J. Clin. Microbiol. **9**:72–78.
10. **Sheehan, D. J., M. Janda, and E. J. Bottone.** 1982. *Pseudomonas aeruginosa*: changes in antibiotic susceptibility, enzymatic activity, and antigenicity among colonial morphotypes. J. Clin. Microbiol. **15**:926–930.
11. **Sherris, J. C., and K. J. Ryan.** 1981. Evaluation of automated and rapid methods, p. 1–5. *In* R. C. Tilton (ed.), Rapid methods and automation in microbiology. Proceedings of the Third International Symposium on Rapid Methods and Automation in Microbiology. American Society for Microbiology, Washington, D.C.
12. **Sugarman, B., and E. Pesanti.** 1980. Treatment failures secondary to in vivo development of drug resistance by microorganism. Rev. Infect. Dis. **2**:153–168.
13. **Thornsberry, C., T. L. Gavan, J. C. Sherris, A. Balows, J. M. Matsen, L. D. Sabath, F. Schoenknecht, L. D. Thrupp, and J. A. Washington II.** 1975. Laboratory evaluation of a rapid, automated susceptibility testing system: report of a collaborative study. Antimicrob. Agents Chemother. **7**:466–480.