JOURNAL OF CLINICAL MICROBIOLOGY, June 1999, p. 1727–1731 Vol. 37, No. 6
0095-1137/99/$04.00+0

Characterization of Antimicrobial Resistance in *Streptococcus pyogenes* Isolates from the San Francisco Bay Area of Northern California

MARY K. YORK,[1]* LAUREL GIBBS,[1] FRANCOISE PERDREAU-REMINGTON,[2] AND G. F. BROOKS[1]

Department of Laboratory Medicine[1] and Department of Medicine, Infectious Diseases Division,[2] University of California, San Francisco, California

Received 21 August 1998/Returned for modification 17 October 1998/Accepted 23 February 1999

During 1994 and 1995, 157 isolates of *Streptococcus pyogenes* from patients with invasive disease were consecutively collected in the San Francisco Bay area to determine the frequency of antimicrobial resistance. Susceptibility testing was performed according to the guidelines of the National Committee for Clinical Laboratory Standards by the disk method and by broth microdilution. For comparison of susceptibility patterns, an additional 149 strains were randomly collected from patients with pharyngitis. For San Francisco County, 32% of the isolates from invasive-disease-related specimens but only 9% of the isolates from throat cultures from the same period were resistant to erythromycin ($P = 0.0007$). Alameda County and Contra Costa County had rates of resistance of ≤10% from isolates from all cultures. When the data were analyzed by hospital, the San Francisco County Hospital had a statistically higher rate of erythromycin resistance (39%) among the strains from serious infections compared to those from other counties ($P = <0.0003$). For tetracycline, high rates of resistance were observed in San Francisco County for both isolates from patients with invasive disease (34%) and pharyngitis (21%) in the same period. Using pulsed-field gel electrophoresis, two clones, one at the San Francisco County Hospital and a second in the entire area, were identified. The latter clone exhibited resistance to bacitracin. Of 146 strains that were tested by microdilution, all were susceptible to penicillin. Clindamycin resistance was not seen among the erythromycin-susceptible strains, but two of the 39 erythromycin-resistant strains were also resistant to clindamycin. An additional 34 strains showed resistance to clindamycin when exposed to an erythromycin disk in the double-disk diffusion test, suggesting that the mechanism of erythromycin resistance is due to an *erm* gene. This study demonstrates a high rate of resistance to macrolides and tetracycline among *S. pyogenes* isolates in San Francisco County and shows that macrolide resistance is more common in strains from patients with invasive disease than in strains from those with pharyngitis.

Antimicrobial resistance among group A beta-hemolytic streptococci (GABHS) is an emerging concern. Although penicillin is the first choice for treatment of pharyngeal and most other infections with this organism, erythromycin or one of the newer macrolides is the second-line drug of choice and is used in penicillin-hypersensitive patients (8, 9). Addition of clindamycin to the therapy of serious streptococcal infections is recommended to inhibit protein synthesis and, therefore, toxin production by the organism. Clindamycin is also the drug of choice for chronic, recurrent pharyngitis.

Erythromycin resistance ranges from as low as 1.3 to 5% at endemic levels (11) to >45% during outbreaks in Finland (24), Sweden (9), and Japan (7). High rates of resistance have also been reported sporadically in Australia (17%) (28), the United Kingdom (22.8%) (20), Taiwan (rate not specified) (10), and Italy (40%) (5). Documentation shows that changes in the prescribing patterns of physicians to reduce macrolide antibiotic use have often resulted in a decrease in resistance (5, 23). Outbreaks of resistant GABHS above 5% have almost never been reported in the United States (8, 11, 21), and those reports may have been flawed if disk testing methodology was used (3). In 1993, Rathore and Jenkins in Jacksonville, Fla., reported that 2 μg of erythromycin/ml was needed to inhibit growth of 99% of isolates by agar dilution (21). They concluded that while most isolates in their area remain susceptible to erythromycin, many no longer were fully susceptible, with MICs of ≤0.5 μg/ml.

Resistance to tetracycline has been reported to be high, making it virtually unusable as an alternative for the treatment of GABHS infections (8). All studies agree that there is a need for surveillance of antimicrobial susceptibility to detect emerging local patterns, since resistance varies widely in different parts of the world, as well as in different areas of the same country.

Because the level of erythromycin resistance in San Francisco has been persistently elevated, this study was undertaken to systematically survey resistance to erythromycin, tetracycline, and clindamycin among GABHS in the San Francisco Bay area of Northern California. To our knowledge, this work is the first to document high-level macrolide resistance in the United States. The results were confirmed by the current National Committee for Clinical Laboratory Standards (NCCLS) microdilution test method with lysed horse blood supplementation (14, 19).

(A preliminary report of this work was presented previously [30].)

MATERIALS AND METHODS

Strains. A total of 306 GABHS isolates were studied. The 157 isolates from patients with invasive infections were received sequentially between May 1994 and November 1995 from 32 institutions representing San Francisco Bay area counties (San Francisco, 50%; Alameda, 33%; Contra Costa, 15%; other or

* Corresponding author. Mailing address: Department of Laboratory Medicine, L 515, Box 0100, University of California, San Francisco, CA 94143. Phone: (415) 476-3233. Fax: (415) 502-0929. E-mail: york@labmed.ucsf.edu.

TABLE 1. Resistance patterns by county for San Francisco Bay area[a]

Resistance	Isolates from patients with invasive disease				Isolates from patients with pharyngitis			
	SF County Hospital	SF County other hospitals	Alameda County	Contra Costa County and other	SF County Hospital	SF County other hospitals	Alameda County	Contra Costa County and other
Total isolates	36 (26)	43 (30)	52 (44)	26 (21)	39	77	10	23
Tetracycline only	2 (1)	5 (4)	0	1 (1)	6	9	0	1
Erythromycin only	4 (4)	0	0	0	0	2[b]	1	1[b]
Erythromycin and tetracycline	5 (4)	2 (1)	2 (2)	0	5	3	0	1
Erythromycin, tetracycline, and clindamycin	1 (1)	0	0	0	0	1	0	0
Erythromycin, tetracycline, and bacitracin	4 (4)	5 (4)	1	1 (1)	0	0	0	0
Tetracycline (total)	12 (10)	12 (9)	3 (2)	2 (2)	11	13	0	2
Erythromycin (total)	14 (13)	7 (5)	3 (2)	1 (1)	5	6	1	2
Erythromycin (% of total)	39 (50)	16 (23)	6 (5)	4 (5)	13	8	10	9

[a] Numbers in parentheses are for a 12-month period in which throat isolates were collected; SF, San Francisco.
[b] Strains were not resistant to clindamycin even after induction.

unknown counties 1%) as part of the Centers for Disease Control and Prevention Active Surveillance Program (1). San Francisco County is separated from Alameda and Contra Costa counties by San Francisco Bay. Of the strains from patients with invasive disease, 118 were isolated from blood, 20 from joint specimens, 1 each from peritoneal fluid and pericardial fluid, and 2 each from lymph node and pleural fluid. The remaining 13 isolates were from wounds and tissues associated with necrotizing fasciitis or toxic shock.

Seven hospitals plus an Alameda County central laboratory that receives specimens from several counties in the area randomly collected 149 respiratory site isolates (San Francisco, 78%; Alameda, 7%; Contra Costa, 5%; and central laboratory, 10%) from November 1994 to November 1995 for comparison of the strains with those from the surveillance program. Statistical analysis was performed by the one-sided test on the difference between proportions.

Identification to species level. The isolates were identified as group A streptococci by the submitting institution. All identifications were confirmed upon receipt as *Streptococcus pyogenes* by colony morphology, catalase reaction, and beta-hemolysis. All isolates were pyrrolidonyl arylamidase (PYR) positive. The first 200 strains were also tested on the BactiCard Strep (Remel, Lenexa, Kans.) and were leucine aminopeptidase positive and esculin negative.

Erythromycin and tetracycline disk susceptibility testing. Initially, disk susceptibility testing was performed according to the guidelines in NCCLS document M2-A5 (15). Mueller-Hinton sheep blood agar (PML, Tualatin, Oreg.) was inoculated with a suspension of each organism equivalent to a 0.5 MacFarland turbidity standard. Disks with 15 μg of erythromycin or 30 μg of tetracycline (BBL, Cockeysville, Md.) were dispensed on the agar, and zone diameters were measured after 20 to 24 h of incubation in ambient air at 35°C. Breakpoints were determined according to NCCLS standard M100-S5 (17), using 14 to 22 mm for the "intermediate" range. *Staphylococcus aureus* ATCC 25923 was used as a control. For strains isolated in 1995, the tests were performed according to the NCCLS standards found in M100-S6 (18), which are identical to those of the previous standard, except that incubation is in 5% CO_2, breakpoints are revised, with a range of 16 to 20 mm for the intermediate category, and *Streptococcus pneumoniae* ATCC 49619 is used as a control. Bacitracin susceptibility was measured by the same method, with bacteria producing no zone being considered resistant. The disks contained 0.04 U of bacitracin (6).

Erythromycin and clindamycin double-disk susceptibility testing. To test the effect of erythromycin on the expression of clindamycin resistance, a disk containing 15 μg of erythromycin was placed 20 mm from the center of a disk containing 2 μg of clindamycin. Inhibition of the circular zone around the clindamycin disk was considered positive for inducible resistance (25).

Dilution susceptibility test. MICs were determined by the NCCLS microdilution method (14, 18, 19). Antimicrobial agents were diluted in cation-adjusted Mueller-Hinton broth containing 2% lysed horse blood, 100 μl of each solution was dispensed into each well of microdilution plates, and the plates were kept frozen at −70°C until use. Inoculations were performed with a Dynatech 2000 inoculator to a final concentration of 3×10^5 to 7×10^5 CFU/ml, and the inoculated trays were incubated in ambient air at 35°C for 20 to 24 h and read visually by transmitted light with a reflective viewing device. *S. pneumoniae* ATCC 49619 was used as a control organism. A control plate was inoculated from each growth control well with a calibrated loop to confirm the purity of the culture and the initial inoculum density. MICs were interpreted by using the NCCLS breakpoints for streptococci (19).

PFGE. Preparation of chromosomal DNA for pulsed-field gel electrophoresis (PFGE) was performed as outlined by Bert et al. (2). PFGE was performed as described by Maslow et al. (12). Restriction fragments of chromosomal DNA after digestion with the enzymes *Sma*I and *Apa*I (Boehringer Mannheim, Indianapolis, Ind.) were analyzed as described by Tenover et al. (29). Isolates were considered identical if they had exactly the same electrophoretic pattern, and they were considered to be clonally related if they showed differences of three or fewer bands between strains (29).

RESULTS

Of the 157 strains from patients with invasive disease, 25 were resistant to erythromycin. The frequency was 27% for San Francisco County, 6% for Alameda County, and 4% for Contra Costa County (Table 1). MICs for erythromycin-resistant isolates ranged from 2 to >8 μg/ml. No MIC results with an interpretation in the intermediate category (0.5 μg/ml) were detected. An unusually high rate of resistance (39%) was seen at one hospital, the San Francisco County Hospital, compared to those of the counties ($P = <0.0003$).

Because of the high rate of erythromycin resistance in isolates from patients with invasive disease, 149 strains of GABHS were randomly collected from patients with pharyngitis from several hospitals in the three counties between November 1994 and November 1995. Results of erythromycin testing of these specimens were compared to results for all the strains from patients with invasive disease submitted during the same period (Table 1). For San Francisco County, 32% of the invasive-disease-related specimens, but only 9% of the isolates from patients with pharyngitis from the same period, were resistant to erythromycin ($P = 0.0007$). For the San Francisco County Hospital, 50% of 26 strains from patients with invasive disease were erythromycin resistant, compared to 13% of 39 strains from patients with pharyngitis. The San Francisco County Hospital had statistically more resistant strains from pharyngitis compared to those from Alameda County ($P = 0.0004$).

Tetracycline resistance was found in 31 of the 39 erythromycin-resistant strains. In addition, resistance was found in 24 erythromycin-susceptible strains. The rate of tetracycline resistance was 25% in San Francisco County, with no significant difference between strains from patients with invasive infections and those from throat cultures. Tetracycline resistance was uncommon (<10%) in isolates from the other counties.

Broth microdilution susceptibility testing was performed on all of the strains that were classified as resistant or intermediate to erythromycin by disk test plus approximately 40% of the strains that were classified as susceptible to erythromycin. All 146 strains tested were susceptible to penicillin, with MICs of ≤0.015 μg/ml. There was no resistance to clindamycin among

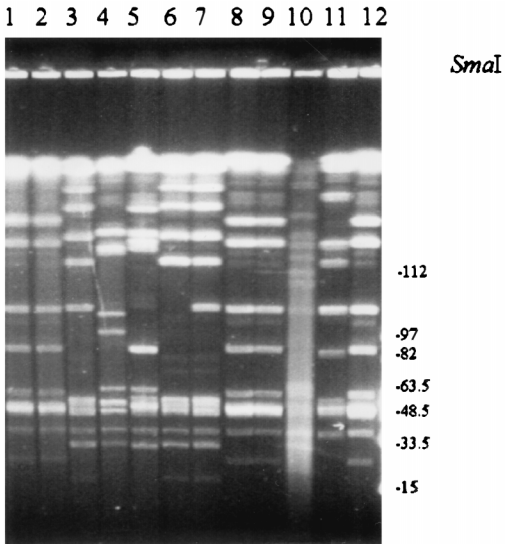

FIG. 1. PFGE of 12 erythromycin-resistant strains digested with *Sma*I. Lanes 1 to 12 contain strains SF1818, SF3413, SF1608, SF1544, SF1611, SF1612, SF1613, SF1810, SF1615, SF1618, SF1617, and SF1923, respectively; a molecular weight ladder was in well 13. The PFGE patterns (from left to right) are designated A, A, B, C, D, B2, B, A, A, E, F, and A, respectively.

the erythromycin-susceptible strains. Of the erythromycin-resistant strains, only two strains (one from a blood culture and one from a throat culture) were resistant to clindamycin by both disk and microdilution methods, with MICs of >1 μg/ml. Of the 37 remaining erythromycin-resistant strains, all except three strains isolated from throat cultures demonstrated inhibition of the zone of susceptibility to clindamycin in the presence of an erythromycin disk. Eleven strains isolated from invasive specimens were resistant to erythromycin, tetracycline, and bacitracin. Bacitracin resistance was not seen among the throat isolates.

In an effort to determine if there was a clonal strain resistant to erythromycin within San Francisco County, PFGE was performed on the 12 strains collected between May 1994 and March 1995. The results identified two clones (five strains with pattern A and three strains with pattern B) by restriction with either *Apa*I or *Sma*I (Fig. 1 and Table 2). All strains of clone A had identical restriction patterns with both enzymes. One isolate of clone A was from the San Francisco County Hospital; three strains of clone A were collected from patients at three different hospitals in San Francisco within a 5-week period. All five strains of clone A were resistant to bacitracin. Clone B was isolated only at the San Francisco County Hospital. Strain SF1612 differed from the other strains of clone B by one restriction fragment.

DISCUSSION

No resistance to penicillin was seen in 146 GABHS isolates that were studied, reinforcing the fact that penicillin resistance has not developed in this organism, despite the presence of resistance in other species of streptococci. High levels of erythromycin and tetracycline resistance were found by surveillance of GABHS in the San Francisco Bay Area. San Francisco County contributed 82% of the erythromycin-resistant strains and 87% of the tetracycline-resistant strains. Resistance has been prevalent at the San Francisco County Hospital for at least 20 years and continues to be problematic, with a documented rate of resistance in 1998 of 25% (personal communication). The cause of this resistance has not been determined. Macrolide resistance usually results from overuse of these drugs for treatment of pharyngitis (5, 23), but such usage has not been identified in San Francisco. The higher number of macrolide-resistant strains among the strains from patients with serious infections suggests that erythromycin resistance has an association with strains that are more invasive. This association could be a result of the unsuccessful treatment of infections with macrolide antibiotics, resulting in more invasive infections. Bacitracin resistance was also associated with the more invasive strains. Possibly resistance to these agents is merely a marker for a clone with pathogenic factors.

By PFGE, two distinct clones were identified among the erythromycin-resistant strains from patients with invasive disease. Initially, an attempt was made to separate clones by M and T typing (1); however, these methods do not have the discriminatory power of PFGE (27). The PFGE results were reinforced by the observation that all 5 strains of clone A, and none of the other erythromycin-resistant strains, were resistant to bacitracin. Historically, susceptibility to bacitracin has been used to identify GABHS and to separate them from group B streptococci. Because the test lacks specificity, most laborato-

TABLE 2. Characterization of 12 erythromycin-resistant strains of *S. pyogenes* from patients with invasive infections

Strain	Hospital of Origin[c]	Collection date (mo/day/yr)	Bacitracin[d]	PFGE pattern	
				*Apa*I	*Sma*I
SF1818	Hospital A (SF)	5/31/94	R	A	A
SF3413	Hospital B (Alameda)	8/11/94	R	A	A
SF1923	Hospital C (SF)	3/31/95	R	A	A
SF1810	Hospital A (SF)	2/21/95	R	A	A
SF1615	SF County Hospital	2/21/95	R	A	A
SF1608	SF County Hospital	9/27/94	S	B	B
SF1612	SF County Hospital	11/22/94	S	B2	B2
SF1613	SF County Hospital	1/7/95	S	B	B
SF1544	Hospital D (SF)	10/20/94	S	C	C
SF1611[a]	SF County Hospital	11/17/94	S	D	D
SF1618	SF County Hospital	3/22/95	S	E	E
SF1617[b]	SF County Hospital	3/10/95	S	F	F

[a] Strain is constitutively resistant to clindamycin; all other strains were inducibly resistant to clindamycin.
[b] Only strain susceptible to tetracycline.
[c] SF, San Francisco County.
[d] R, resistant; S, susceptible.

ries currently use the presence of PYR as a better test to identify GABHS (6). Our results confirm the importance of using an alternative to bacitracin susceptibility testing.

Erythromycin resistance in GABHS has been reported to be of three types. The most common is a target site modification, which involves dimethylation of adenine in 23S rRNA. This leads to reduced binding of macrolide, lincosamide, and streptogramin B antibiotics to their shared 50S rRNA target site (called the MLS phenotype). At least eight classes of these *erm* (erythromycin resistance methylase) genes have been identified in various species, including staphylococci (22), streptococci (4), and *S. pneumoniae* (26). The phenotype for this type of resistance is demonstrated by the double-disk diffusion test (25), in which resistance to clindamycin is demonstrated after induction with erythromycin. Most (34 of 39) of the erythromycin-resistant strains in this study demonstrated inducible clindamycin resistance, suggesting that the mechanism of resistance is an *erm* gene.

Uncommonly, another type of macrolide resistance is seen when the *erm* gene mutates and is constitutively expressed. Then the isolate demonstrates in vitro resistance to clindamycin without induction. Constitutive resistance to clindamycin was seen in two strains in this study, one from a throat culture and one from a blood culture (SF1611).

Three strains from throat cultures did not show inducible or constitutive resistance to clindamycin and probably had a different type of erythromycin resistance. Other known mechanisms of resistance involve an active efflux mechanism (*msr* genes) in staphylococci, an erythromycin esterase (*ere* genes), an undefined mechanism in *S. pneumoniae* (26), and an energy-dependent efflux pump in *S. pyogenes* called *mefA* (4). *msr* resistance confers inducible coresistance to macrolides and type B streptogramins but not to lincosamides, such as clindamycin. *mefA* confers resistance only to the macrolides but not to 16-membered macrolides, lincosamides, or streptogramin B. It is possible that three of our strains carry *mefA*, since this gene has been found in GABHS. Alternatively, an uncharacterized mechanism of resistance that does not confer resistance to clindamycin may be present in these strains.

Prior to 1994, the NCCLS did not separately address breakpoints in susceptibility to erythromycin for different species (13, 15). The documents did indicate that for streptococci testing should be modified to use Mueller-Hinton sheep blood agar for disk testing without increased CO_2. Zone sizes in CO_2 tend to be smaller than those in air. In our initial study, more than 50% of the GABHS isolates were classified as intermediate in susceptibility to erythromycin compared to being classified as susceptible by microdilution testing (30). Using the current NCCLS standards (16, 19), this problem has been alleviated. It is imperative that laboratories use the current NCCLS standards to avoid reporting results with intermediate interpretations. In addition, we found that it was often difficult to determine the zone size for erythromycin. Because the drug is bacteriostatic, it can produce an uneven demarcation of the zone of inhibition. Care must be taken to avoid measuring the zone of hemolysis or the slight haze of growth within the zone of inhibition. Based on our results with disk and microdilution testing, we recommend that disk test results with interpretations in the intermediate category be confirmed by an MIC methodology.

Conclusions. This study indicates that, for GABHS isolates from patients with invasive disease, San Francisco County has a higher rate of erythromycin resistance (27%) than those found in two neighboring counties. Only two strains with erythromycin resistance also demonstrated constitutive resistance to clindamycin. Tetracycline resistance was also high in San Francisco County but was rarely found in the other counties. Erythromycin resistance, but not tetracycline resistance, was more common in isolates from patients with invasive disease than in isolates from patients with pharyngitis. No resistance to penicillin was found; however, a clone showing bacitracin resistance was identified among the erythromycin-resistant isolates from patients with invasive disease.

ACKNOWLEDGMENTS

We thank Stuart Beal for statistical analysis, R. Facklam for review of the manuscript, and Nhung Huynh for her technical assistance with the PFGE.

REFERENCES

1. **Beall, B., R. Facklam, T. Hoenes, and B. Schwartz.** 1997. Survey of *emm* gene sequences and T-antigen types from systemic *Streptococcus pyogenes* infection isolates collected in San Francisco, California; Atlanta, Georgia, and Connecticut in 1994 and 1995. J. Clin. Microbiol. **35:**1231–1235.
2. **Bert F., C. Branger, and N. Lambert-Zechovsky.** 1997. Pulsed-field gel electrophoresis is more discriminating than multilocus enzyme electrophoresis and random amplified polymorphic DNA analysis for typing pyogenic streptococci. Curr. Microbiol. **34:**226–229.
3. **Brorson, J., and P. Larsson.** 1987. The regression line for erythromycin is not valid for beta-hemolytic streptococci group A. Scand. J. Infect. Dis. **19:**243–246.
4. **Clancy, J., J. Petitpas, F. Dib-Hajj, W. Yuan, M. Cronan, A. V. Kamath, J. Bergeron, and J. A. Retsema.** 1996. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, *mefA*, from *Streptococcus pyogenes*. Mol. Microbiol. **22:**867–879.
5. **Cornaglia, G., M. Ligozzi, A. Mazzariol, M. Valentini, G. Orefici, the Italian Surveillance Group for Antimicrobial Resistance, and R. Fontana.** 1996. Rapid increase of resistance to erythromycin and clindamycin in *Streptococcus pyogenes* in Italy, 1993–1995. Emerg. Infect. Dis. **2:**339–342.
6. **Facklam, R. R., and J. A. Washington II.** 1991. *Streptococcus* and related catalase-negative gram-positive cocci, p. 238–257. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
7. **Fujita, K., K. Murono, M. Yoshikawa, and T. Murai.** 1994. Decline of erythromycin resistance of group A streptococci in Japan. Pediatr. Infect. Dis. J. **13:**1075–1078.
8. **Gerber, M. A.** Antibiotic resistance in group A streptococci. 1995. Pediatr. Clin. N. Am. **42:**539–551.
9. **Hölmström, L., B. Nyman, M. Rosengren, S. Wallander, and T. Ripa.** 1990. Outbreaks of infections with erythromycin-resistant group A streptococci in child day care centres. Scand. J. Infect. Dis. **22:**179–185.
10. **Hsueh, P. R., H. M. Chen, A. H. Huang, and J. J. Wu.** 1995. Decreased activity of erythromycin against *Streptococcus pyogenes* in Taiwan. Antimicrob. Agents Chemother. **39:**2239–2242.
11. **Kaplan, E. L.** Recent evaluation of antimicrobial resistance in β-hemolytic streptococci. 1997. Clin. Infect. Dis. **24**(Suppl 1)**:**S89–S92.
12. **Maslow, J. N., A. M. Slutsky, and R. D. Arbeit.** 1993. Applications of pulsed-field gel electrophoresis to molecular epidemiology, p. 563–572. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
13. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd ed. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
15. **National Committee for Clinical Laboratory Standards.** 1993. Performance standards for antimicrobial disk susceptibility tests, 5th ed. Approved standard M2-A5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests, 6th ed. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
17. **National Committee for Clinical Laboratory Standards.** 1994. Performance standards for antimicrobial susceptibility testing. Fifth information supplement M100-S5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
18. **National Committee for Clinical Laboratory Standards.** 1995. Performance standards for antimicrobial susceptibility testing. Sixth information supple-

ment M100-S6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
19. **National Committee for Clinical Laboratory Standards.** 1998. Performance standards for antimicrobial susceptibility testing. Eighth information supplement M100-S8. National Committee for Clinical Laboratory Standards, Wayne, Pa.
20. **Phillips, G., D. Parratt, G. V. Orange, I. Harper, H. McEwan, and N. Young.** 1990. Erythromycin-resistant *Streptococcus pyogenes*. J. Antimicrob. Chemother. **25:**723–724.
21. **Rathore, M. H., and S. G. Jenkins.** 1993. Group A beta-hemolytic *Streptococcus*: issue of resistance. Pediatr. Infect. Dis. J. **12:**354–355.
22. **Sanchez, M. L., K. K. Flint, and R. N. Jones.** 1993. Occurrence of macrolide-lincosamide-streptogramin resistances among staphylococcal clinical isolates at a university medical center. Diagn. Microbiol. Infect. Dis. **16:**205–213.
23. **Seppälä, H., T. Klaukka, J. Vuopio-Varkila, A. Muotiala, H. Helenius, K. Lager, P. Huovinen, and the Finnish Study Group for Antimicrobial Resistance.** 1997. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. N. Engl. J. Med. **337:**441–446.
24. **Seppälä, H., A. Nissinen, H. Järvinen, S. Huovinen, T. Henriksson, E. Herva, S. E. Holm, M. Jahkola, M.-L. Katila, T. Klaukka, S. Kontiainen, O. Liimatainen, S. Oinonen, L. Passi-Metsomaa, and P. Huovinen.** 1992. Resistance to erythromycin in group A streptococci. N. Engl. J. Med. **326:**292–297.
25. **Seppälä, H., A. Nissinen, Q. Yu, and P. Huovinen.** 1993. Three different phenotypes of erythromycin-resistant *Streptococcus pyogenes* in Finland. J. Antimicrob. Chemother. **32:**885–891.
26. **Shortridge, V. D., R. K. Flamm, N. Ramer, J. Beyer, and S. K. Tanaka.** 1996. Novel mechanism of macrolide resistance in *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. **26:**73–78.
27. **Stanley, J., M. Desai, J. Xerry, A. Tanna, A. Efstratiou, and R. George.** 1996. High-resolution genotyping elucidates the epidemiology of group A streptococcus outbreaks. J. Infect. Dis. **174:**500–506.
28. **Stingemore, N., G. R. Francis, M. Toohey, and D. B. McGechie.** 1989. The emergence of erythromycin resistance in *Streptococcus pyogenes* in Fremantle, Western Australia. Med. J. Aust. **150:**626–627, 630–631.
29. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
30. **York, M. K., L. Gibbs, and G. F. Brooks.** 1996. Comparison of antimicrobial susceptibility of group A streptococci from sterile site and respiratory site specimens, abstr. E56, p. 95. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.