Susceptibility of Group A Beta-Hemolytic *Streptococcus* Isolates to Penicillin and Erythromycin

GREG R. ISTRE,1 DAVID F. WELCH,1,∗ MELVIN I. MARKS,1 AND NELSON MOYER2

Oklahoma University Health Sciences Center, Division of Pediatric Infectious Disease,1 and Oklahoma State Department of Health, Microbiology Division,2 Oklahoma City, Oklahoma 73190

Received 21 January 1981/Accepted 15 May 1981

We have reevaluated the antibiotic susceptibilities of group A beta-hemolytic streptococci in view of recent reports of a high prevalence of erythromycin resistance in Japan and of an increase in penicillin treatment failures in the United States. A total of 474 isolates recovered during a 2- to 3-month period in 1980 were tested. All were susceptible by microtiter broth dilution to a penicillin concentration of $\leq 0.03 \mu g/ml$ (minimal inhibitory concentration), and 473 were killed by $\leq 0.06 \mu g/ml$ (minimal bactericidal concentration). Erythromycin minimal inhibitory concentrations showed a bimodal distribution: 95% were $\leq 0.06 \mu g/ml$, and 5% were $\geq 1 \mu g/ml$. Of the minimal bactericidal concentrations, 21% were $\geq 1 \mu g/ml$ and 3% were $\geq 16 \mu g/ml$. Group A beta-hemolytic streptococci remain susceptible to the inhibitory and bactericidal actions of penicillin, thus providing no in vitro explanation for the bacteriological relapses reported in some clinical studies. Unlike the Japanese experience, only 5% of our isolates were resistant to erythromycin (minimal inhibitory concentration, $\geq 1 \mu g/ml$; however, 22% were tolerant (ratio of minimal inhibitory/bactericidal concentrations, $\geq 32$).

Although in vitro resistance to penicillin has not been described for group A beta-hemolytic *Streptococcus*, a trend of increasing resistance to erythromycin has been reported (2–4, 9). Recently some clinicians have noted an increased incidence of treatment failure as compared with data relating to treatment results derived 2 decades ago (6, 7, 10–12, 14). In view of these findings, we have evaluated antibiotic susceptibilities of group A beta-hemolytic *Streptococcus* strains recently isolated from patients in Oklahoma.

MATERIALS AND METHODS

Group A beta-hemolytic *Streptococcus* isolates. Throat cultures submitted to the microbiology laboratories of Oklahoma Children's Memorial Hospital and the Oklahoma State Department of Health yielded 474 group A beta-hemolytic *Streptococcus* (S. pyogenes) isolates. Isolates originated, geographically, from a statewide region. Serogroup identification was based on the fluorescent-antibody technique, and hemolysis was determined by macroscopic examination of primary streak plate cultures containing 4 to 5% defibrinated sheep blood. Inocula were stabbed to facilitate detection of oxygen-labile hemolysin. Plates were incubated at 35 to 37°C in an atmosphere containing 6% CO2 and were examined at 24 and 48 h. Group A beta-hemolytic *Streptococcus* colonies were transferred to blood agar plates and maintained in pure culture at room temperature until susceptibility tests were performed.

Susceptibility testing. Growth from 18- to 24-h cultures on sheep blood agar plates was adjusted to a density of approximately $10^8$ colony-forming units per ml in Mueller-Hinton broth. A 1:10 dilution was then made in 5 ml of broth for micro-broth dilution testing, or a 1:20 dilution was made before agar dilution testing. Agar dilution tests were performed on antibiotic-supplemented Mueller-Hinton agar containing 5% sheep blood. Inoculation of plates was accomplished by use of a Steers replicator (13). Micro-broth dilution tests were performed using the Dynatech MIC 2000 system (Dynatech Laboratories, Inc., Alexandria, Va.) according to the manufacturer's directions. Serial twofold antibiotic dilutions in Mueller-Hinton broth supplemented with 5% sheep blood were contained in a total volume of 0.1 ml per well. Minimal bactericidal concentrations (MBC) were performed using the Dynatech inoculator pin assembly to subculture approximately 0.0015 ml from all wells of the microtiter plate onto a 150-mm petri plate containing 5% sheep blood agar. MBC endpoints were interpreted as wells containing the lowest concentration of antibiotic which yielded no growth after subculture. Quality control of susceptibility testing media was performed with *Staphylococcus aureus* ATCC 29213.

RESULTS

The accuracy of the broth dilution methodology was verified by performing simultaneous susceptibility determinations by agar dilution on the first 31 isolates. Identical results were obtained for 24/31 and 27/31 strains for penicillin and erythromycin, respectively. Agar and broth

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dilution minimal inhibitory concentrations (MICs) for both antibiotics were within one dilution for all isolates tested. When single dilution step differences for penicillin (7/31) or erythromycin (4/31) occurred, the micro-broth result was consistently greater than the agar dilution result.

All 474 isolates were susceptible to penicillin at an MIC of \( \leq 0.03 \, \mu g/ml \), and more than three-fourths had an MIC of \( \leq 0.007 \, \mu g/ml \). None showed tolerance (arbitrarily defined as MBC/MIC \( \geq 32 \)) to penicillin (Fig. 1). Only one isolate required an MBC as high as 0.25 \( \mu g/ml \); the remaining 473 required MBCs of \( \leq 0.06 \, \mu g/ml \).

Erythromycin MICs, however, showed a bimodal distribution. As seen in Fig. 2, 95\% of isolates required erythromycin MICs of \( \leq 0.06 \, \mu g/ml \); the remaining 5\% required \( \geq 1 \, \mu g/ml \) for inhibition. Cumulative erythromycin MBCs were \( \leq 0.125 \, \mu g/ml \) for 62\%, \( \leq 1 \, \mu g/ml \) for 79\%, and \( \leq 16 \, \mu g/ml \) for 97\% of strains (Fig. 1). Tolerance (as defined above) to erythromycin was found in 22\% of isolates, and erythromycin MICs and MBCs in general showed a much greater disparity than those of penicillin (Fig. 1).

**DISCUSSION**

Despite recent reports from Japan of 62 to 72\% resistance of group A beta-hemolytic *Streptococcus* to erythromycin (5, 9), our study showed a comparatively low (5\%) prevalence of erythromycin resistance in Oklahoma. It does, however, reflect a trend of gradually increasing resistance seen in the United States since 1963. Eickhoff and Finland (3) reported no resistance to erythromycin among over 300 strains in 1963; Dixon and Lipinski (2) showed 0.05\% resistance in over 18,000 strains in 1968-70; Finland et al. (4) showed a slightly higher average MIC in 1972; and by 1980 Christopher and Stuart (J. C. Christopher and J. G. Stuart, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, A55, p. 10) found a 6\% resistance in over 200 strains. These percentages are all based on a definition of resistance comparable to that of the present study (\( \geq 1 \, \mu g/ml \)).

Although erythromycin is generally described as a bacteriostatic antibiotic, some reports have suggested that it is bactericidal against streptococci (6; J. Fonteyne, R. Vanhoof, D. Dekkel, R. Dierickx, and J. P. Butzler, Program Abstr. Int. Cong. Chemother. 11 and Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 520, 1979). Our study, using 474 isolates from patients with pharyngitis, showed a significant portion (22\%) with MIC and MBC disparity for erythromycin, indicating that erythromycin does exhibit more apparent bacteriostatic effects than penicillin against streptococci. This could be clinically significant since concentrations of erythromycin in tonsils and serum (8) may not be adequate for killing of streptococci with higher MICs and MBCs.

As has been the case with previous studies, we found that group A beta-hemolytic streptococci continue to be susceptible to penicillin, and we did not observe the MIC/MBC disparity for penicillin that was previously observed by Allen and Sprunt (1).

In summary, although penicillin clearly remains an effective drug in vitro against group A beta-hemolytic *Streptococcus*, it should be recognized that an increasing proportion of these organisms have developed resistance to eryth-
romycin, thus limiting the potential usefulness of this drug in the therapy of group A beta-hemolytic Streptococcus disease. Although the actual clinical significance of this resistance is yet to be determined, physicians in the United States should be alert to the possibility that we may be experiencing the early phase of streptococcal resistance patterns now seen in other parts of the world.

ACKNOWLEDGMENTS

We thank the staff of the Oklahoma State Department of Health Microbiology Division and the Oklahoma Children's Memorial Hospital Microbiology Laboratory.

LITERATURE CITED