Letters to the Editor

Emergence of Erythromycin-Resistant, Clindamycin-Susceptible *Streptococcus pyogenes* Isolates in Madrid, Spain

In Spain, the frequency of resistance of group A streptococci (GAS) to erythromycin was very low, increasing from 0 to 0.7% in 1989 and 1990 (1, 7) to approximately 3% in 1991 to 1994 (2). The purpose of the present study was to determine the susceptibilities of recent isolates of GAS to macrolides and, in the case of resistant isolates, to ascertain their patterns of erythromycin resistance. We also studied the in vitro activities of penicillin G and clindamycin.

A total of 222 isolates of GAS were collected from April to August 1996 from outpatient samples in Madrid, Spain. A total of 203 isolates were throat swab samples and 19 were from other sources. Identification was done by biochemical tests according to standard criteria (8).

Penicillin G, erythromycin, azithromycin, miocamycin, and clindamycin were tested. The MICs were determined by the agar dilution method with Mueller-Hinton agar supplemented with 5% defibrinated horse blood. The plates were incubated overnight at 35°C in a 5% carbon dioxide atmosphere. The procedures for susceptibility testing were those recommended by the National Committee for Clinical Laboratory Standards (6). The MIC breakpoint for miocamycin resistance was >4 μg/ml, as defined by the Comité de l'Antibiogramme de la Société Française de Microbiologie (10).

Erythromycin resistance was classified according to the results of disk diffusion, with a double-disk test with erythromycin and clindamycin disks, as previously described (9), to test for inducible resistance. The MIC ranges and the MICs at which 50 and 90% of isolates were inhibited (MIC_{50}s and MIC_{90}s, respectively) for the 222 isolates of *Streptococcus pyogenes* are given in Table 1. Penicillin remained exquisitely active, with an MIC_{90} of 0.03 μg/ml.

Thirty-nine (17.6%) of the isolates tested were resistant to erythromycin (MIC breakpoint, ≥1 μg/ml). In contrast, only 0.5% of the *S. pyogenes* isolates were resistant to clindamycin (MIC_{90}, ≤0.12 μg/ml). The resistance to both 14- and 15-membered macrolides tested was 17.6%, whereas the resistance to miocamycin, a 16-membered macrolide, was 0.5%. Thirty-seven (95%) of 39 erythromycin-resistant GAS were susceptible to clindamycin (MICs, ≤0.12 μg/ml) and miocamycin (MICs, 0.5 to 1 μg/ml). One isolate was resistant to erythromycin (MIC, >64 μg/ml), azithromycin (MIC, >16 μg/ml), miocamycin (MIC, >64 μg/ml), and clindamycin (MIC, >64 μg/ml), which indicates cross-resistance to macrolides, lincosamides, and streptogramin B-type antibiotics (MLS_B phenotype). Another erythromycin-resistant strain was susceptible to clindamycin but showed an inducible type of erythromycin resistance.

All erythromycin-resistant isolates were also resistant to the 15-membered macrolide azithromycin. However, the 16-membered macrolide miocamycin showed good activity against these isolates. The MICs for 37 of 39 erythromycin-resistant isolates were moderate (8 to 32 μg/ml), but the MICs of clindamycin were the same as those for the erythromycin-susceptible isolates (≤0.12 μg/ml); this pattern has been described with the M phenotype (11) and "novel resistance" (9). With this phenotype, susceptibility to clindamycin and streptogramin B antibiotics but resistance to 14- and 15-membered

TABLE 1. In vitro susceptibilities of 222 recent GAS strains to five antimicrobial agents

Antibiotic	MIC (μg/ml)[a]			
	Range	50%	90%	S (%)
Penicillin G	≤0.008–0.06	0.015	0.03	100.0
Erythromycin	≤0.12–>64	≤0.12	16	82.4
Azithromycin	≤0.25–>16	≤0.25	>16	82.4
Miocamycin	≤0.25–>64	1	1	99.5
Clindamycin	≤0.12–>64	≤0.12	≤0.12	99.5

[a] 50 and 90%, MIC_{50} and MIC_{90}, respectively. S, percent of strains susceptible.

macrolides is seen (11). The mechanism of erythromycin resistance involves active efflux. The rate of isolation of strains with this novel phenotype among the erythromycin-resistant GAS of our work is even higher than that recently reported in the United States (75%) (11), although the incidence of erythromycin-resistant GAS is rare in this country (3). However, 50% of erythromycin-resistant GAS isolated in Japan (5) and in Taiwan (4) were also resistant to clindamycin and tetracycline.

In conclusion, our report documents the emergence of high rates of resistance to erythromycin in *S. pyogenes* in Spain. Strains with the M phenotype account for the majority of those isolates, which in a sample of this size represents a significant finding. Clindamycin remained active against these strains and might be useful for treatment of patients with clinical failure after phenoxymethyl penicillin treatment and for penicillin-allergic patients. We suggest that clinical laboratories review their local susceptibility data. If erythromycin resistance rates are high, we recommend that susceptibilities to both macrolides and clindamycin in *S. pyogenes* isolates be routinely tested.

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Synergistic Activity of Trovafloxacin and Ceftriaxone or Vancomycin against *Streptococcus pneumoniae* with Various Penicillin Susceptibilities

While interest in *Streptococcus pneumoniae* has always been high because of its role as a frequent pathogen, the increasing prevalence of penicillin-resistant isolates is of growing concern (2, 9). Rising resistance rate coupled with the failure of monotherapy for meningitis due to these isolates now warrant that empirical therapy consist of ceftriaxone and vancomycin (5, 7, 8). Trovafloxacin, a new fluoroquinolone, has displayed exceptional activity against pneumococci and penetrates well into the cerebrospinal fluid (CSF) (1, 4). Although its role in treat-

calculated for each, and an overall mean FIC index was determined. Synergy was defined as an FIC of ≤0.5, indifference was defined as an FIC of >0.5 to ≤4, and antagonism was defined as an FIC of >4.

The median and range of MICs of each agent are displayed in Table 1. MICs of trovafloxacin and vancomycin were unaffected by penicillin susceptibility, whereas ceftriaxone MICs increased with increasing penicillin resistance. The in vitro interactions between trovafloxacin and ceftriaxone or trova-

TABLE 1. MICs of individual agents against penicillin-susceptible, -intermediate, and -resistant *S. pneumoniae*

S. pneumoniae penicillin susceptibility (*n*)	Median MIC (range) (μg/ml)			
	Penicillin	Trovafloxacin	Vancomycin	Ceftriaxone
Susceptible (15)	0.045 (0.03–0.06)	0.06 (0.015–0.125)	0.5 (0.5–1.0)	0.03 (0.03–0.125)
Intermediate (16)	0.25 (0.125–0.5)	0.06 (0.015–0.25)	0.5 (0.5–1.0)	0.06 (0.03–2.0)[a]
Resistant (16)	2.0 (2.0–4.0)	0.06 (0.015–0.5)	0.5 (0.5–1.0)	2.0 (0.06–8.0)[b]

[a] Includes three isolates resistant to ceftriaxone (two isolates with intermediate susceptibility [MIC of >0.5 to 1 μg/ml] and one resistant isolate [MIC of ≥2 μg/ml]).
[b] Includes 14 isolates resistant to ceftriaxone (1 isolate with intermediate susceptibility [MIC of >0.5 to 1 μg/ml] and 13 resistant isolates [MIC of ≥2 μg/ml]).

ment of pneumococcal meningitis is currently under investigation, its synergistic potential with currently utilized therapies is unknown.

Forty-seven clinical isolates of *S. pneumoniae* with various susceptibilities to penicillin were studied (penicillin-susceptible, MIC of ≤0.06 μg/ml [*n* = 15]; intermediate, MIC of 0.1 to 1 μg/ml [*n* = 16]; resistant, MIC of ≥2 μg/ml [*n* = 16]). All tests were conducted with cation-adjusted Mueller-Hinton broth (Becton Dickinson Microbiology Systems, Cockeysville, Md.) plus 5% lysed horse blood (Remel Inc., Lenexa, Kans.) and the following agents: trovafloxcin (CP-99,219; Pfizer Laboratories, New York, N.Y.), ceftriaxone (Hoffman-LaRoche Pharmaceuticals, Nutley, N.J.), and vancomycin (Sigma Chemical Company, St. Louis, Mo.).

The MIC for each drug-organism combination was determined in duplicate by using standard microdilution methods in ambient air. Synergy studies were undertaken by using the microdilution checkerboard technique. Bacterial dilutions in the logarithmic growth phase were prepared and pipetted into microtiter trays containing concentrations of each antibiotic which ranged from four to five times below the MIC to two times above the MIC. The final inoculum size, confirmed by colony count determinations, for all experiments was 5×10^5 CFU/ml. Prepared microtiter trays were incubated at 37°C for 24 h, after which the trays were read for inhibition of bacterial growth. Fractional inhibitory concentration (FIC) indices were calculated according to the method of Eliopoulos and Moellering (3). Individual checkerboard runs were repeated five times for each isolate-drug combination, an FIC index was

floxacin and vancomycin against the isolates are characterized in Table 2. Synergy ws observed in 55 and 15% of the isolates treated with the trovafloxacin and ceftriaxone or trovafloxacin and vancomycin, respectively. Antagonism was not observed. In addition, synergy was observed for 13 of 17 isolates in the trovafloxacin and ceftriaxone studies when organisms were intermediate or resistant (i.e., MIC of >0.5 μg/ml) to ceftriaxone.

Trovafloxacin has been shown to penetrate well into the CSF of healthy subjects, and concentrations that exceed the MICs for the most common organisms implicated in bacterial meningitis are maintained at this site (1). In addition, trovafloxacin has displayed excellent penetration and bactericidal activity within the CSF of rabbits with experimental meningitis due to a highly penicillin-resistant *S. pneumoniae* isolate (6). Al-

TABLE 2. In vitro interactions between trovafloxacin and ceftriaxone or vancomycin against penicillin-susceptible, -intermediate, and -resistant *S. pneumoniae*

S. pneumoniae penicillin susceptibility (*n*)	No. (%) of isolates			
	Trovafloxacin and Ceftriaxone		Trovafloxacin and Vancomycin	
	Synergy	Indifference	Synergy	Indifference
Susceptible (15)	7 (47)	8 (53)	3 (20)	12 (80)
Intermediate (16)	7 (44)	9 (56)	3 (19)	13 (81)
Resistant (16)	12 (75)	4 (25)	1 (6)	15 (94)

though the role of trovafloxacin in the treatment of meningitis is currently under study, our data may be of particular clinical relevance in the treatment of meningitis caused by penicillin-resistant *S. pneumoniae*, since treatment options for this life-threatening disease remain limited.

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