ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1999, p. 1310–1313 Vol. 43, No. 5
0066-4804/99/$04.00+0

Emergence of Fluoroquinolone Resistance among Multiply Resistant Strains of *Streptococcus pneumoniae* in Hong Kong

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Received 8 December 1998/Returned for modification 29 January 1999/Accepted 3 March 1999

The MICs of 17 antimicrobial agents for 181 *Streptococcus pneumoniae* strains were determined by the E-test. Overall, 69.1% were penicillin resistant (MIC > 0.06 μg/ml). Resistance to ciprofloxacin (MIC > 2 μg/ml), levofloxacin (MIC > 2 μg/ml), or trovafloxacin (MIC > 1 μg/ml) was found in 12.1, 5.5, or 2.2% of the strains, respectively. These high rates of resistance raise concerns for the future.

The resistance of *Streptococcus pneumoniae* to β-lactams, macrolides, tetracyclines, and chloramphenicol has been increasing rapidly in southeast Asia (1). Some of the newer fluoroquinolones, by virtue of their excellent in vitro activity against *S. pneumoniae*, oral bioavailability, and tissue penetration, hold promise as the drugs of choice for therapy of respiratory tract infections due to multiple drug-resistant *S. pneumoniae* in this region (6). We have therefore conducted a study on the in vitro activities of 3 fluoroquinolones and 13 other antimicrobial agents.

A total of 181 consecutive, nonduplicate isolates of *S. pneumoniae* were obtained from four regional laboratories (61 isolates from laboratory A, 41 isolates from laboratory B, 31 isolates from laboratory C, and 49 isolates from laboratory D) during the second half of 1998. These four laboratories provide microbiology service to seven public hospitals, serving a population of about 3 million in the Hong Kong island (south and west), Kowloon (central), and the New Territory (south and north) regions of Hong Kong. The strains were isolated from throat (5), nose (4), eye (5), sputum (143), tracheal aspirate (4), and blood (21). Strains were identified as *S. pneumoniae* by Gram stain, colony morphology, optochin susceptibility, and bile solubility.

The MICs of ciprofloxacin, levofloxacin, trovafloxacin, penicillin, ampicillin, ticarcillin-clavulanate, piperacillin-tazobactam, cefuroxime, cefpodoxime, ceftibuten, ceftriaxone, cefepime, meropenem, erythromycin, azithromycin, clindamycin, and vancomycin were determined by the E-test (AB Biodisk, Solna, Sweden) following the manufacturer's instructions. All susceptibility testings were done in the University of Hong Kong by one technician. Susceptibility tests were performed from a bacterial inoculum whose turbidity was equivalent to that of a McFarland standard of 0.5. From this suspension, E-tests were performed on Mueller-Hinton agar with 5% sheep blood (Micro Diagnostics Incorporated, Lombard, Ill.). The plates were incubated at 35°C in 5% CO_2 for 20 to 24 h (10). MICs falling between two marks on the E-test strip were rounded up to the next higher twofold dilution, as recommended in the instructions. Quality control strains (*S. pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922) were included with each run. Interpretation of results was performed according to recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (8). Because the NCCLS breakpoints for microbroth dilution appear to have limited applicability to E-test results for macrolides versus *S. pneumoniae*, modified breakpoints as recommended by the manufacturer were used (2). For erythromycin, the breakpoints were as follows: susceptible, ≤0.5 μg/ml; intermediate, 1 μg/ml; and resistant, ≥2 μg/ml. For azithromycin, the following breakpoints were used: susceptible, ≤4 μg/ml; intermediate, 8 μg/ml; and resistant, ≥16 μg/ml. No reference breakpoints for cefpodoxime, ceftibuten, ticarcillin-clavulanate, piperacillin-tazobactam, and ciprofloxacin are available. Multiple drug resistance is defined as resistance to at least one member from each of the three classes of antimicrobial agents including the β-lactams, macrolides, and fluoroquinolones. The chi-square, Fisher exact, or Kruskal-Wallis test was used for statistical analysis.

The susceptibilities of the 181 pneumococcal isolates to 17 antimicrobial agents are summarized in Table 1. Rates of resistance to penicillin (MIC > 0.06 μg/ml) were 60.7% (37 of 61) for laboratory A, 80.5% (33 of 41) for laboratory B, 54.8% (17 of 31) for laboratory C, and 79.2% (38 of 48) for laboratory D ($P = 0.05$ for median MICs, Kruskal-Wallis test). Penicillin resistance rates were similar for children (age, ≤12 years) and adults (age, >12 years) (73.8 versus 66.4%, respectively; $P > 0.05$) but penicillin resistance was less common for blood isolates than for isolates from other sites (33.3 versus 73.8%, respectively; $P < 0.01$). Thirty-four (18.8%) isolates were highly resistant to penicillin with MICs of >2 μg/ml. For the most resistant isolate, the MIC was 6 μg/ml. The MICs of ampicillin and piperacillin-tazobactam for most strains were within one dilution difference of that of penicillin. However, the MIC of ticarcillin-clavulanate increased disproportionately with an increasing level of resistance to penicillin (data not shown). Ceftriaxone had the lowest MIC_{50} (MIC at which 50% of isolates were inhibited) and MIC_{90} among the cephalosporins, followed by cefepime, cefpodoxime, and cefuroxime. Multiple drug resistance was found in 12.1% (22 of 181) of the isolates.

The distribution of MICs for the fluoroquinolones are shown in Fig. 1. Ciprofloxacin was the least active fluoroquinolone with a MIC of >2 μg/ml for 12.1% (22 of 181) of the isolates. Importantly, resistance to levofloxacin and trovafloxacin was only found in the penicillin-resistant isolates. Trova-

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TABLE 1. Susceptibilities of 181 isolates of *S. pneumoniae* stratified by penicillin susceptibility

Antimicrobial agent and penicillin susceptibility (*n*)[a]	Breakpoint (μg/ml)[b]	MIC (μg/ml)[c]			No. (%) of isolates not susceptible to indicated antimicrobial agent[b]
		Range	50%	90%	
Penicillin	>0.06				
S (56)		0.023–0.064	0.032	0.064	0 (0)
I (27)		0.094–1	0.19	1	27 (100)
R (98)		1.5–6	2	4	98 (100)
Total (181)		0.023–6	1.5	3	125 (69.1)
Cefuroxime	>0.5				
S		0.023–0.19	0.023	0.064	0 (0)
I		0.064–3	0.38	4	12 (44.4)
R		2–16	4	6	98 (100)
Total		0.023–16	3	4	110 (60.8)[d]
Cefpodoxime	NA				
S		0.023–0.94	0.032	0.064	NA
I		0.05–2	0.38	2	NA
R		1–8	3	4	NA
Total		0.023–8	2	3	NA
Ceftibuten	NA				
S		2–48	4	12	NA
I		6–>256	96	>256	NA
R		>256	>256	>256	NA
Total		2–>256	>256	>256	NA
Ceftriaxone	>0.5				
S		0.012–0.19	0.023	0.032	0 (0)
I		0.032–0.5	0.19	0.5	0 (0)
R		0.25–3	0.75	1	80 (81.6)
Total		0.012–3	0.5	1	80 (44.2)[d]
Cefepime	>0.5				
S		0.023–0.94	0.047	0.094	1 (1.8)
I		0.094–1.5	0.5	1.5	12 (44.4)
R		0.75–3	1.5	2	98 (100)[d]
Total		0.023–3	1.5	2	111 (61.3)
Meropenem	>0.25				
S		0.012–0.12	0.012	0.02	0 (0)
I		0.023–0.38	0.064	0.38	5 (18.5)
R		0.094–0.75	0.5	0.75	95 (96.9)
Total		0.012–0.75	0.38	0.5	100 (55.2)[d]
Erythromycin	>0.25; ≥0.5				
S		0.032–>256	0.13	>256	22 (39.3); 22 (39.3)
I		0.13–>256	>256	>256	23 (85.2); 23 (85.2)
R		0.13–>256	4	>256	97 (98.9); 97 (98.9)
Total		0.032–>256	4	>256	142 (78.5); 142 (78.5)[d]
Azithromycin	>0.5; ≥4				
S		0.094–>256	1.5	>256	45 (80.3); 22 (39.3)
I		0.75–>256	>256	>256	27 (100); 23 (85.2)
R		1–>256	24	>256	98 (100); 91 (92.9)
Total		0.094–>256	24	>256	170 (93.9); 136 (75.1)[d]
Clindamycin	>0.25				
S		0.023–>256	0.19	>256	12 (21.4)
I		0.094–>256	>256	>256	17 (62.9)
R		0.064–>256	0.19	>256	16 (16.3)
Total		0.023–>256	0.19	>256	45 (24.7)
Ciprofloxacin	NA				
S		0.38–8	1	2	NA
I		0.5–>32	1	4	NA
R		0.38–>32	1	12	NA
Total		0.38–>32	1	6	NA

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TABLE 1—*Continued*

Antimicrobial agent and penicillin susceptibility (*n*)[a]	Breakpoint (μg/ml)[b]	MIC (μg/ml)[c]			No. (%) of isolates not susceptible to indicated antimicrobial agent[b]
		Range	50%	90%	
Levofloxacin	>2				
S		0.5–1.5	0.75	1	0 (0)
I		0.5–24	0.75	1.5	1 (3.7)
R		0.38–>32	1	3	9 (9.2)
Total		0.38–>32	1	1.5	10 (5.5)[d]
Trovafloxacin	>1				
S		0.094–0.38	0.19	0.25	0 (0)
I		0.094–0.38	0.19	0.25	0 (0)
R		0.064–>32	0.19	0.5	4 (4)
Total		0.064–>32	0.19	0.38	4 (2.2)
Vancomycin	>1				
S		0.38–1	0.5	0.75	0 (0)
I		0.38–0.75	0.75	0.75	0 (0)
R		0.25–1	0.5	0.75	0 (0)
Total		0.25–1	0.5	0.75	0 (0)

[a] S, susceptible; I, intermediate; R, resistant.
[b] Breakpoints are given in accordance with NCCLS standard M100-S (10) or guidelines from the manufacturer of the E-test. The breakpoints and interpretations according to the recommendations of the manufacturer of the E-test are underlined. Both intermediate and resistant strains were included as nonsusceptible.
[c] 50% and 90%, MICs required to inhibit 50 and 90% of the isolates, respectively.
[d] Penicillin-susceptible versus penicillin-nonsusceptible strains. $P < 0.05$ by chi-square or Fisher exact test.

floxacin was highly active, with similar MIC_{90}s for both penicillin-susceptible and -resistant isolates. However, all four trovafloxacin-resistant isolates were also penicillin and macrolide resistant (Table 2).

A marked increase in the overall prevalence of resistance to penicillin (69.1%) was found compared to rates from previous studies in 1993 (18%) and 1995 (28.9%) in Hong Kong (5, 7). While resistance to penicillin among *S. pneumoniae* isolates is an emerging problem worldwide, this high rate of resistance (>60%) has only been reported in several Asian countries, including Korea (73.4%), Taiwan (71%), Japan (67.7%), and Thailand (63.1%) (1, 12). High rates of cross-resistance to the

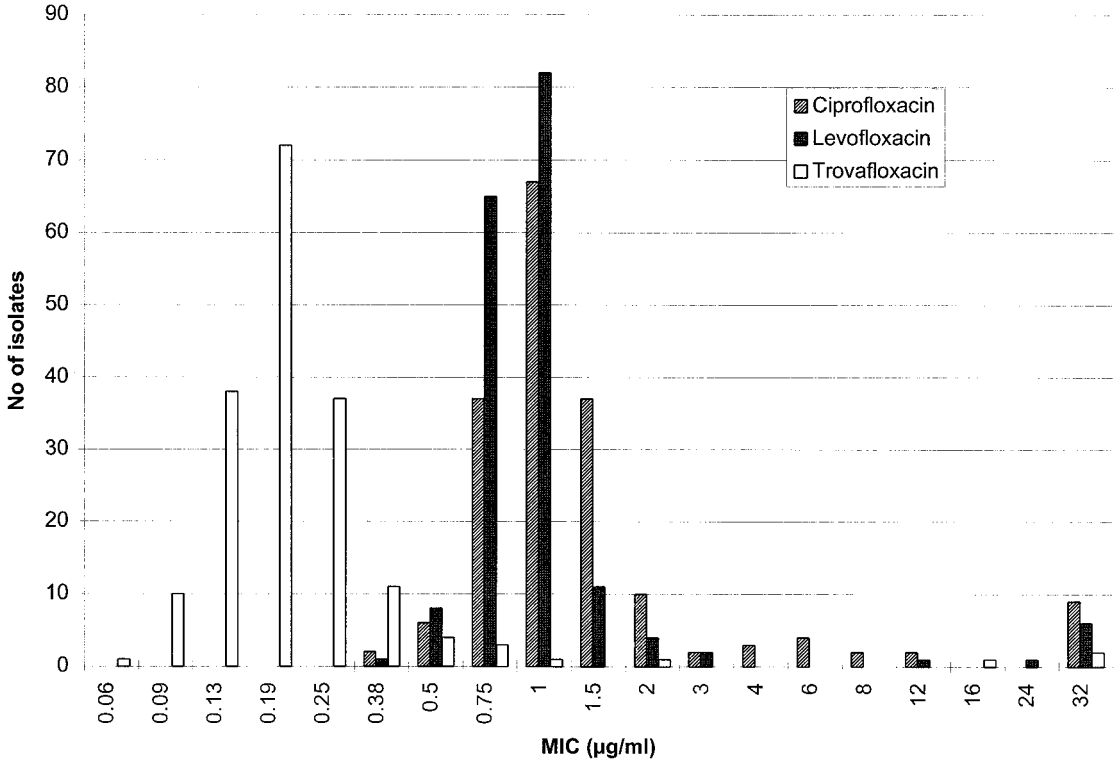

FIG. 1. Distribution of MICs of three fluoroquinolones for 181 strains of *S. pneumoniae*.

TABLE 2. Distribution and MICs of four β-lactam–macrolide–fluoroquinolone-resistant pneumococci

Strain	Laboratory	Date of isolation (mo/day/yr)	Sample	MIC[a] (μg/ml) of:				
				Trovafloxacin	Ciprofloxacin	Levofloxacin	Penicillin	Erythromycin
S10B8	A	2/11/98	Sputum	2	>32	>32	2	3
S7D7	D	3/9/98	Sputum	16	>32	>32	2	4
S7C5	C	27/8/98	Sputum	>32	>32	>32	3	6
S7E1	D	4/9/98	Sputum	>32	>32	>32	1.5	2

[a] See Table 1 for breakpoints.

cephalosporins (64 to 88%) and meropenem (80.2%) among the penicillin-intermediate or -resistant isolates were also found.

The overall rate of resistance to erythromycin also rose dramatically from 10% in 1993 and 39.2% in 1995 to 78.5% in the present study (3). The overall consumption of antimicrobial agents is frequently identified as a risk factor for the rapid emergence of resistance. Notably, the consumption of macrolides in Hong Kong increased by more than twofold from 1994 to 1997 (13). In addition, overcrowdedness may be another factor that contributes to the rapid dissemination of drug-resistant *S. pneumoniae* in Hong Kong. This is supported by findings from Ip and coworkers (6) that 70% of the penicillin-resistant strains belong to a variant of the Spanish type 23F clone.

The emergence of fluoroquinolone-resistant *S. pneumoniae* is alarming because this group of antimicrobial agents is at present the only possible option for oral therapy of infection due to β-lactam–macrolide-resistant strains. Within 3 years, the rate of resistance to fluoroquinolones has increased from <0.5% for ofloxacin (5) to 5.5% for levofloxacin (MIC > 2 μg/ml). This is in contrast to the lack of development of resistance reported in other areas (2, 11, 14). Most alarming, 4% of the penicillin-resistant isolates were already highly resistant to trovafloxacin, an agent only registered for use in Hong Kong in October 1998.

In conclusion, we have reported extremely high rates of resistance to β-lactams and macrolides among *S. pneumoniae* isolates in Hong Kong. Trovafloxacin is highly active in vitro against β-lactam–macrolide-resistant strains. However, ominous resistance to fluoroquinolones is emerging. The global mobility of human populations and the convergence of tourist and business traffic in Hong Kong will likely facilitate the worldwide spread of these very resistant clones (7).

This study was supported by grants from the Committee on Research and Conference Grant, The University of Hong Kong, and the Pfizer Corporation, Hong Kong.

We thank Allan Ronald for a critical reading of the manuscript.

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