ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2002, p. 2665–2667 Vol. 46, No. 8
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.8.2665–2667.2002

Activities of Six Different Quinolones against Clinical Respiratory Isolates of *Streptococcus pneumoniae* with Reduced Susceptibility to Ciprofloxacin in Spain

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Received 8 October 2001/Returned for modification 24 March 2002/Accepted 13 April 2002

Six quinolones were tested on 817 consecutive pneumococcal isolates for which ciprofloxacin MICs were high (≥2 μg/ml); the isolates had been collected during two recent Spanish surveillance studies. For strains for which the ciprofloxacin MIC was ≥4 μg/ml, the MICs at which 90% of the isolates tested against gemifloxacin, moxifloxacin, gatifloxacin, sparfloxacin, levofloxacin, and ofloxacin were inhibited were 0.25, 1, 1, 1, 4 and 16 μg/ml, respectively, and the corresponding prevalences of resistance were 0, 1, 4.5, 9.5, 8.4 and 23%. The proportion of isolates for which the ciprofloxacin MIC is high has increased over time.

Increases in ciprofloxacin MICs have a distinct influence on the activity of the other drugs in the quinolone family (4). Although the prevalence of resistance to the newer quinolones in *Streptococcus pneumoniae* is presently low, tracking the pharmacoepidemiological dynamics of *S. pneumoniae* resistance to quinolones is important, since it provides a measure of confidence when selecting among these agents for the empirical therapy of community-acquired respiratory tract infections. It also helps to design tailored antibiotic use policies to hamper the societal spread of drug resistance.

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This study aimed to characterize the intrinsic activity of different quinolones against recent respiratory *S. pneumoniae* isolates for which the ciprofloxacin MIC was high (≥2 μg/ml). The isolates were collected during two multicenter surveillance studies carried out by the Spanish Surveillance Group for Respiratory Pathogens (SAUCE Program) in Spain from 1996 to 1997 (2) and from 1998 to 1999 (6).

Confirmation of the initial identification made by each of the 20 participating laboratories and susceptibility testing were performed at a central location (Instituto Valenciano de Microbiología, Valencia, Spain). The identification, storage, and shipping procedures are described elsewhere (2, 6). The determination of the MIC for every isolate was done with a semiautomated broth microdilution method with microtiter-customized Sensititre panels (Trek Diagnostics, Westlake, Ohio). NCCLS recommendations (5) were followed, and testing was carried out at twofold-dilution increases for ciprofloxacin (concentration range, 0.5 to 128 μg/ml), ofloxacin (0.004 to 64 μg/ml), levofloxacin (0.004 to 64 μg/ml), sparfloxacin (0.004 to 64 μg/ml), gatifloxacin (0.004 to 16 μg/ml), moxifloxacin (0.004 to 16 μg/ml), and gemifloxacin (0.004 to 8 μg/ml). Panels were inoculated with isolates suspended in cation-adjusted Mueller-Hinton broth with 3% lysed horse blood to achieve an inoculum of 5×10^5 CFU/ml. Incubation was carried out at 35°C in ambient air for 24 h before an automated MIC reading was performed. *S. pneumoniae* ATCC 49619 was used as a quality control organism.

All consecutive isolates for which the ciprofloxacin MIC was ≥2 μg/ml and which were collected in the two previous SAUCE surveys (2, 6) from patients with community-acquired respiratory infections were analyzed. We used the breakpoints issued by the NCCLS (5) with the exception of breakpoints for ciprofloxacin and gemifloxacin, which have not yet been issued. Although a non-NCCLS breakpoint of 0.5 to 1 μg/ml has been proposed for gemifloxacin (7), we chose to use the more conservative breakpoints of ≤0.25 μg/ml and ≥1 μg/ml for the

TABLE 1. Activity of six quinolones against *S. pneumoniae* isolates according their susceptibility to ciprofloxacin[a]

Isolates for which the ciprofloxacin MIC (μg/ml) is:	Ofloxacin				Levofloxacin				Sparfloxacin				Gatifloxacin				Moxifloxacin				Gemifloxacin[b]			
	MIC		% S	% R	MIC		% S	% R	MIC		% S	% R	MIC		% S	% R	MIC		% S	% R	MIC		% S	% R
	50%	90%			50%	90%			50%	90%			50%	90%			50%	90%			50%	90%		
≥2 (*n* = 817)	2	4	66.8	5.3	1	2	96.5	1.8	0.25	0.5	94.4	2.1	0.5	0.5	97.8	1.0	0.25	0.5	98.9	0.2	0.03	0.12	99.6	0
≥4 (*n* = 179)	4	16	20	23	2	4	84.4	8.4	0.5	1	83.2	9.5	0.5	1	89.9	4.5	0.25	1	95	1.1	0.06	0.25	98.3	0
≥8 (*n* = 43)	16	32	2.3	55.8	4	16	48.9	32.6	0.5	8	55.8	37.2	1	4	60.5	18.6	0.5	2	79	4.7	0.12	0.25	93	0

[a] % S, percentage of susceptible strains; % R, percentage of resistant strains. Breakpoints used were ≤2 μg/ml (susceptible) and ≥8 μg/ml (resistant) for ofloxacin and levofloxacin; ≤1 μg/ml (susceptible) and ≥4 μg/ml (resistant) for gatifloxacin and moxifloxacin; and ≤0.5 μg/ml (susceptible) and ≥2 μg/ml (resistant) for sparfloxacin.

[b] No NCCLS breakpoints have been established for gemifloxacin; proposed breakpoints are ≤0.25 μg/ml (susceptible) and ≥1 μg/ml (resistant).

susceptibility and resistance categories, respectively. The resistance of an isolate to ciprofloxacin was arbitrarily defined by an MIC of ≥4 μg/ml. Statistical analyses were performed using the Epi-Info version 6.04b software package; *P* values, odds ratios (OR), and their 95% confidence intervals (95% CI) were determined by the chi-square test, with the Yates correction if necessary.

All of the 817 consecutive *S. pneumoniae* isolates for which the ciprofloxacin MIC was ≥2 μg/ml were tested. Table 1 shows the MIC at which 50% of the isolates tested were inhibited (MIC_{50}) and the MIC_{90} of each drug tested, along with the prevalence of susceptibility and resistance to each antibiotic, for pneumococcal strains for which the corresponding ciprofloxacin MICs were ≥2, ≥4, and ≥8 μg/ml. Gatifloxacin, moxifloxacin, and gemifloxacin were the most potent agents, and their potencies were the least affected by increasing ciprofloxacin MICs. The MIC_{90} of gemifloxaxin increased only twofold and those of gatifloxacin and moxifloxacin increased four- to eightfold when the ciprofloxacin MICs increased. The MIC distribution of the other quinolones for ciprofloxacin-resistant *S. pneumoniae* isolates is shown in Table 2. Figure 1 shows the frequencies of pneumococcal isolates with respect to their ciprofloxacin MICs in the first and second surveys. A small but statistically significant increase in the prevalence of strains for which the MICs were higher can be seen.

By comparing MIC_{90}s, we determined that gemifloxacin was 4-fold more active than moxifloxacin, gatifloxacin, and sparfloxacin and 16-fold more potent than levofloxacin against isolates for which the ciprofloxacin MICs were ≥2 and ≥4 μg/ml. Gemifloxacin was 8-, 16-, 32-, and 64-fold more active, respectively, than the same drugs against isolates for which the ciprofloxacin MIC was ≥8 μg/ml. As for ofloxacin, gemifloxacin was 32-, 64-, and 128-fold more potent than this drug against isolates for which the ciprofloxacin MICs were ≥2, ≥4, and ≥8 μg/ml, respectively.

Regarding cross-resistance to other antibiotics, up to 49.7% of the ciprofloxacin-resistant strains (for which MICs were ≥4 μg/ml) were also resistant to erythromycin (MIC, ≥1 μg/ml), compared to 32.9% of strains for which the ciprofloxacin MIC was ≤2 μg/ml (OR = 2.01; 95% CI, 1.47 to 2.76). As for penicillin, 42% of the ciprofloxacin-resistant isolates displayed high resistance to penicillin (MIC, ≥2 μg/ml), compared to 27.6% of strains for which the ciprofloxacin MIC was low (OR = 1.89; 95% CI, 1.37 to 2.61). This clustering of resistances would make it possible to select for unrelated resistance traits by using ciprofloxacin.

The shift in susceptibility of pneumococcal subpopulations to higher ciprofloxacin MICs also causes concern (Fig. 1). A trend toward a loss of susceptibility to levofloxacin from 1996–1997 (0.7%) to 1998–1999 (1.2%) was also found, but it did not reach statistical significance, perhaps due to the low number of pneumococcal isolates.

A remarkable increase in the prevalence of pneumococcal strains with reduced susceptibility to ciprofloxacin was reported in Canada (3) and was deemed to be related to the selective pressure derived from increased use of fluoroquinolones. Likewise, there existed an association between penicillin resistance and reduced susceptibility to fluoroquinolones. Another Spanish report (C. García-Rey, L. Aguilar, F. Baquero, and the Spanish Surveillance Group for Respiratory Pathogens, Letter, Antimicrob. Agents Chemother. **44:**3481–3482, 2000) described a prevalence of as high as 5.3% of pneumococcal strains for which the ciprofloxacin MICs were ≥4 μg/ml, with a strong association with macrolide resistance. In addition, a larger proportion of strains for which ciprofloxacin MICs were high was isolated from adults than from children (6

TABLE 2. Distribution of the MICs of six fluoroquinolones for *S. pneumoniae* isolates for which the ciprofloxacin MIC is ≥4 μg/ml (*n* = 179)

Fluoroquinolone	No. of isolates inhibited (cumulative %) at a concn (μg/ml) of[a]:															
	0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128
Ofloxacin									1 (0.6)	35 (20)	102 (77)	19 (87)	**15 (96)**	6 (99)	1 (100)	
Levofloxacin								2 (1)	76 (43)	73 (84)	**13 (91)**	9 (96)	6 (100)			
Sparfloxacin						2 (1)	44 (25)	103 (83)	**13 (90)**	4 (92)	1 (93)	8 (97)	13 (99)	1 (100)		
Gatifloxacin						1 (0.6)	8 (5)	129 (77)	**23 (90)**	10 (95)	7 (99)	1 (100)				
Moxifloxacin						23 (12)	121 (80)	15 (88)	**11 (95)**	7 (98)	2 (100)					
Gemifloxacin			3 (1)	57 (33)	71 (73)	28 (88)	**17 (98)**	3 (100)								

[a] MIC_{90}s are shown in bold.

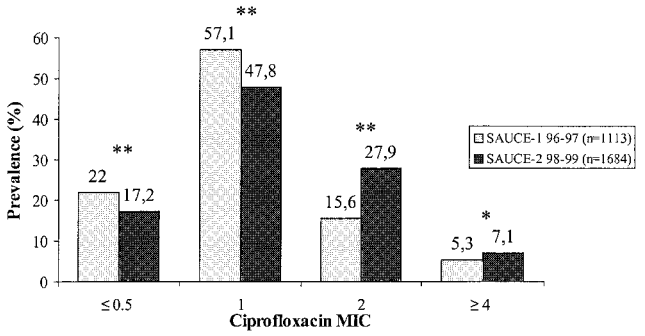

FIG. 1. Frequencies of pneumococcal isolates with respect to different ciprofloxacin MICs in two different surveillance studies in Spain. **, $P < 0.01$; *, $P < 0.05$.

versus 0% for a MIC of ≥4 μg/ml and 22.5 versus 7% for a MIC of ≥2 μg/ml). A more recent Spanish surveillance study (6) found a 7% prevalence of ciprofloxacin resistance among *S. pneumoniae* isolates, and resistance was higher among non-macrolide-susceptible (9.7 versus 5.8%; OR = 1.8) and non-penicillin-susceptible (8.4 versus 5.8%; OR = 1.5) isolates.

Whether resistant clones arise from clonal spread (1) or antibiotic use has not yet been evaluated, but since these two selective forces may differ geographically, it is not surprising that regional surveillance studies are more likely to detect the emerging resistance sooner than international surveillance studies do.

The activity of the older quinolones is more affected by increases of ciprofloxacin MICs than the activity of newer drugs, and furthermore, consumption of older drugs may result in a faster development of ciprofloxacin resistance than that which would be expected to result from the use of newer drugs with enhanced antipneumococcal activity.

With the apparently steady loss of susceptibility to ciprofloxacin in Spain that is occurring over time, neither ciprofloxacin nor ofloxacin currently provides adequate pneumococcal coverage. If the trend continues, levofloxacin and sparfloxacin would presumably be unlikely to retain their activity against ciprofloxacin-resistant isolates. Whether this potential threat will somehow affect the remaining quinolones can only be determined over time. The low prevalence of resistance to the newer quinolone members that was observed in this study cannot be attributable to the consumption of these new drugs because they were not authorized for use during the period 1996 to 1999.

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