ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1999, p. 357–359 Vol. 43, No. 2
0066-4804/99/$04.00+0

Antimicrobial Resistance of 1,113 *Streptococcus pneumoniae* Isolates from Patients with Respiratory Tract Infections in Spain: Results of a 1-Year (1996–1997) Multicenter Surveillance Study

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Received 22 May 1998/Returned for modification 7 August 1998/Accepted 12 November 1998

A nationwide susceptibility surveillance of 1,113 *Streptococcus pneumoniae* isolates was carried out and found the following percentages of resistance: cefuroxime, 46%; penicillin, 37%; macrolides, 33%; aminopenicillins, 24%; cefotaxime, 13%; and ceftriaxone, 8%. A significant ($P < 0.05$) seasonality pattern for β-lactam antibiotics was observed. Resistance to macrolides was higher ($P < 0.05$) in middle-ear samples. Higher percentages of resistance to cefuroxime and macrolides were observed among penicillin-intermediate and -resistant strains, whereas high frequencies of resistance to aminopenicillins and expanded-spectrum cephalosporins were observed only among penicillin-resistant strains.

Resistance to penicillin among *Streptococcus pneumoniae* strains is increasing, with geographical variations (2, 6), making it important to conduct surveillance studies (1). In Spain penicillin resistance increased from 6% (1979) to 44% (1989) (5). Penicillin resistance in *S. pneumoniae* is often associated with resistance to other antibiotics, such as erythromycin (4, 5).

The aim of this study was to describe the susceptibility of *S. pneumoniae* in a nationwide antimicrobial surveillance prospective study. All consecutive clinical isolates collected (between May 1996 and April 1997) from patients with community-acquired infections at 14 hospital centers selected on the basis of geographical location were included.

At each center, isolates kept at −70°C were thawed once a month, seeded onto an enriched transport medium, incubated overnight at 35 to 37°C, and shipped to a central laboratory (Instituto Valenciano de Microbiología, Valencia, Spain), where the identities of isolates were confirmed by conventional tests and criteria. Isolates were kept frozen at −70°C in duplicate until antimicrobial susceptibility testing was performed.

Susceptibility testing was performed by using a semiautomated microdilution method in Mueller-Hinton broth supplemented with 3% lysed horse blood according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (9), with a final inoculum of 5×10^5 CFU/ml, and cultures were incubated 24 h at 35°C under a 5% carbon dioxide atmosphere, with antimicrobials commonly used in empiric therapy in Spain (Table 1). *Haemophilus influenzae* ATCC 49247, *S. pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922 were used as control strains. The mechanism of resistance to erythromycin was evaluated by using a double diffusion disk test as described elsewhere (13), with erythromycin (15 μg) and clindamycin (2 μg) disks placed 20 mm apart onto 5% defibrinated horse blood agar and incubated overnight at 35°C under a 5% carbon dioxide atmosphere (10).

Statistical analysis of data was performed by the chi-square test, with the Yates correction when necessary and correction for multiple comparisons (Bonferroni's method).

In vitro susceptibilities are shown in Table 1. Forty percent of isolates were penicillin susceptible, 24% showed intermediate resistance to penicillin, and 36% were penicillin resistant. Extended-spectrum cephalosporins, amoxicillin-clavulanate, and ciprofloxacin exhibited the highest activities (MIC at which 90% of the isolates are inhibited [MIC_{90}] ≤ 2 μg/ml), whereas macrolides, cefaclor, cefixime, and cefuroxime exhibited the lowest activities (MIC_{90} ≥ 8 μg/ml). The prevalence of resistance was around 10% for extended-spectrum cephalosporins, 25% for aminopenicillins, >30% for penicillin and macrolides, and 46% for cefuroxime. Among the oral antibiotics, amoxicillin-clavulanate exhibited the highest intrinsic activity (MIC_{90} = 2 μg/ml) and the lowest prevalence of resistant strains (25.5%).

Resistance to both macrolides and cefuroxime was markedly

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TABLE 1. In vitro activities of antimicrobial agents[a] against 1,113 *S. pneumoniae* isolates classified by penicillin susceptibility, and proportions of fully resistant strains

Antimicrobial agent	All strains (n = 1,113)			Penicillin-susceptible strains (n = 444 [39.9%])			Penicillin-intermediate strains (n = 263 [23.6%])			Penicillin-resistant strains (n = 406 [36.5%])		
	MIC (μg/ml)		No. (%) resistant[b]	MIC (μg/ml)		No. (%) resistant[b]	MIC (μg/ml)		No. (%) resistant[b]	MIC (μg/ml)		No. (%) resistant[b]
	90%	Range		90%	Range		90%	Range		90%	Range	
Penicillin	4	≤0.015–≥8	406 (36.5)	0.06	≤0.015–0.06		1	0.12–1		4	2–≥8	
Amoxicillin	4	≤0.015–≥8	264 (23.7)	0.03	≤0.015–0.06	0 (0)	1	≤0.015–4	11 (4.2)	≥8	0.5–≥8	253 (62.3)
Amox-clav[c]	2	≤0.015–8	284 (25.5)	0.03	≤0.015–0.5	0 (0)	1	≤0.015–4	12 (4.6)	4	≤0.015–8	272 (67.0)
Cefixime	≥8	≤0.25–≥8	NA[d]	0.5	≤0.25–4	NA	≥8	0.25–≥8	NA	≥8	1–≥8	NA
Cefaclor	≥64	≤1–≥64	NA	≤1	≤1–8	NA	32	≤1–≥64	NA	≥64	1–≥64	NA
Cefuroxime	8	≤0.25–16	511 (45.9)	≤0.25	≤0.25–1	0 (0)	2	≤0.25–8	106 (40.3)	8	1–16	405 (99.8)
Cefotaxime	2	≤0.25–4	141 (12.7)	≤0.25	≤0.25–0.5	0 (0)	1	≤0.25–2	4 (1.5)	2	≤0.25–4	137 (33.7)
Ceftriaxone	1	≤0.25–2	91 (8.2)	≤0.25	≤0.25–≤0.25	0 (0)	0.5	≤0.25–2	1 (0.4)	2	≤0.25–2	90 (22.2)
Erythromycin	≥16	≤0.12–≥16	375 (33.7)	≤0.12	≤0.12–≥16	26 (5.9)	≥16	≤0.12–≥16	135 (51.3)	≥16	≤0.12–≥16	214 (52.7)
Azithromycin	≥16	≤0.12–≥16	377 (33.9)	≤0.12	≤0.12–≥16	26 (5.9)	≥16	≤0.12–≥16	136 (51.7)	≥16	≤0.12–≥16	215 (53.0)
Clarithromycin	≥64	≤0.25–≥64	340 (30.5)	≤0.25	≤0.25–≥64	23 (5.2)	≥64	≤0.25–≥64	116 (44.1)	≥64	≤0.25–≥64	201 (49.5)
Ciprofloxacin	2	≤0.5–≥16	NA	2	≤0.5–≥16	NA	2	≤0.5–≥16	NA	2	≤0.5–≥16	NA

[a] Breakpoints used were ≥2 μg/ml for amoxicillin, amoxicillin-clavulanate, cefotaxime, ceftriaxone, cefuroxime, and azithromycin, and ≥1 μg/ml for clarithromycin and erythromycin. Penicillin breakpoints were ≤0.06 μg/ml (susceptible), 0.12 to 1 μg/ml (intermediate), and ≥2 μg/ml (resistant) (9).
[b] Refers to fully resistant isolates.
[c] Amox-clav, amoxicillin-clavulanate (2:1). The concentrations listed refer to amoxicillin.
[d] NA, (not applicable), no NCCLS breakpoint criteria (9).

increased in strains showing intermediate resistance to penicillin compared with that in penicillin-susceptible strains (51 versus 6% for macrolides and 40 versus 0% for cefuroxime); this phenomenon was not observed with amoxicillin (with or without clavulanate), cefotaxime, or ceftriaxone. Increases in resistance to aminopenicillins and extended-spectrum cephalosporins were observed only when penicillin-intermediate and -resistant strains were compared. Cefuroxime resistance increased again in the penicillin-resistant strains compared with that in the penicillin-intermediate strains (100 versus 40%, respectively). Penicillin-intermediate and -resistant strains showed the same level of resistance to macrolides (51 to 53%).

Middle-ear isolates exhibited the highest ($P < 0.05$) percentage of resistance to macrolides (49%) compared with isolates of other origins. Sixty-nine percent of pediatric samples were middle-ear isolates. As described previously (3), pediatric isolates exhibit higher resistance to penicillin compared with isolates from internal medicine patients (50 versus 37%); however, there were no significant differences between medical wards.

Seasonal variations in pneumococcal infections have been documented (7). In this 1-year surveillance study, the overall distribution of isolates by season was 10.3% in the spring of 1996, 13.5% in the summer of 1996, 25.7% in the autumn of 1996, 32.1% in the winter of 1996 to 1997, and 18.6% in the spring of 1997. Statistically significant ($P < 0.05$) differences were found with respect to seasonal rates, with higher rates of resistance to all β-lactams in summer and winter. Penicillin resistance rates of 41, 31, 43, and 30% were seen in summer, autumn, winter, and spring, respectively. No seasonality was observed for macrolide resistance rates.

Penicillin resistance among pneumococci has increased in Spain since 1989, from 44% in a previous national survey (5) to 60% in this study when penicillin-intermediate and -resistant strains are included. This increase is attributable to an increase in isolates in the penicillin-resistant category: 36.5% in the present study versus 15.3% in 1989 (5). Furthermore, 58% resistance was found in a study carried out in Madrid on isolates from children with invasive pneumococcal infection between 1989 and 1993; only 14% of the isolates exhibited intermediate resistance (12).

With respect to erythromycin resistance, an increase was identified between 1989 (5) and 1996 to 1997 (10 versus 33.7%, respectively). This increase was due to an increase in the more highly resistant strains (MIC ≥ 8 μg/ml) from 1990 (8) to 1996 to 1997 (9.4 versus 25.8%, respectively). In this study, the most common erythromycin resistance phenotype found was the constitutive phenotype (98.4%; 369 of 375 isolates); the M phenotype was found in 1.3% (5 strains), and the inducible phenotype was shown only in 1 strain. Interestingly, all five M phenotype isolates came from a single hospital (Insular Hospital, Las Palmas, Spain). Significant differences ($P < 0.001$) in M phenotype prevalence were found between this hospital (31%; 5 M phenotype isolates among 16 erythromycin-resistant isolates) and others (0%).

It has been suggested that the selective pressure of the local pattern of antibiotic consumption, together with the spread of resistant clones (11), may account for the spread of antibiotic resistance. The consumption of antibiotics has increased in areas where increases in *S. pneumoniae* resistance have been described (8). In Spain, community antibiotic consumption accounts for 90% of total antibiotic consumption; 50% of this consists of penicillins, 17% consists of macrolides, and 13% consists of oral cephalosporins (2). Antibiotic consumption may be one of the reasons for the seasonal variation in penicillin resistance observed in this study.

Increases in resistance, together with significant local differences in resistance patterns, make local susceptibility surveillance a must in order to establish guidelines for the empiric treatment of respiratory-tract infections.

This study has been supported by a grant from SmithKline Beecham Pharmaceuticals, Madrid, Spain.

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