JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1995, p. 3159–3163 Vol. 33, No. 12
0095-1137/95/$04.00+0

Antimicrobial Susceptibilities and Serotypes of Invasive *Streptococcus pneumoniae* Strains in Switzerland

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Received 15 June 1995/Returned for modification 15 August 1995/Accepted 13 September 1995

In 1993 and 1994, 10 microbiological laboratories in Switzerland collected 351 strains of *Streptococcus pneumoniae* from invasive infections. Susceptibilities to the main representatives of the chemical classes were as follows: penicillin, 93%; chloramphenicol, 92%; erythromycin, 94%; sulfamethoxazole-trimethoprim, 86%; tetracycline, 92%; vancomycin, 100%. Forty-three strains showed resistance to one agent, and 35 strains showed resistance to two or more antimicrobial agents simultaneously; i.e., 22% of the strains were resistant to at least one antimicrobial agent. Four strains (1%) were fully resistant to penicillin, whereas 21 strains (6%) showed reduced susceptibility. Of these 25 strains not fully susceptible to penicillin, 10 were resistant to one, 3 were resistant to two, and 8 were resistant to three additional antimicrobial agents. Of the quinolones, sparfloxacin was the most active substance, with an MIC at which 90% of the strains are inhibited of 0.5 mg/liter. The most common serotypes were types 6 (13.6% of isolates), 7 (10.5%), 19 (10.5%), 14 (9.1%), and 1 (8.5%) as well as 3 and 23 (8.0% each). Reduced susceptibility to penicillin was found mainly among serotypes 6, 14, 19, and 23. The currently available 23-valent pneumococcal vaccine covers 320 (91%) of the pneumococci isolated. Regional differences within Switzerland with regard to serotypes and antimicrobial resistance were not observed.

Streptococcus pneumoniae is a significant cause of morbidity and mortality (22, 23). The use of penicillin as the drug of choice is hampered by increasing resistance of strains to the drug in many parts of the world (2, 12, 19). In Europe, penicillin resistance is high in Spain, Greece, Hungary, Poland, and Romania (16, 30). In the countries neighboring Switzerland, resistance rates including strains with reduced susceptibility to penicillin are 6% in Germany, 5% in Austria, approximately 5% in Italy, and 11 to 17% in France (1, 8, 13, 21, 30, 31). Strains with resistance to other antimicrobial agents, such as erythromycin, sulfamethoxazole-trimethoprim, tetracycline, and chloramphenicol, as well as strains with resistance to multiple agents are also of concern (2, 12, 19).

In Zurich, strains with reduced susceptibility to penicillin are relatively rare. In 1978, no such strains could be found among 180 isolates (34). From 1984 to 1994, 2.5 to 5% of the pneumococci tested on an annual basis by the oxacillin disk screening test (26) showed reduced susceptibility to penicillin, with no trend towards higher rates in recent years. In the same period, 3 to 5% of strains were resistant to macrolides. The aim of this study was to determine the exact situation in Switzerland by investigation of strains isolated in 10 laboratories situated in various parts of Switzerland. Pneumococcal strains causing invasive infections, i.e., isolates from blood, cerebrospinal fluid, bronchoalveolar lavage fluid, pleural fluid, and other usually sterile body fluids, were tested against a large series of antimicrobial agents by determination of MIC values. Because many pneumococcal infections occur in nonhospitalized patients, emphasis was placed on oral antimicrobial agents, including the new oral cephalosporins, the new macrolides, and fluoroquinolones. The serotypes were determined to assess the coverage by the pneumococcal vaccine currently available.

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MATERIALS AND METHODS

Bacterial strains. Three hundred fifty-one strains of *S. pneumoniae* were collected by 10 laboratories located all over Switzerland from July 1992 to April 1994. Only isolates from blood, cerebrospinal fluid, bronchoalveolar lavage fluid, pleural fluid, and other usually sterile body fluids such as joint, bone, sinus, and ear aspirates were accepted. Pneumococci from sputum and tracheal secretions were excluded. Identification was made by standard criteria (7).

Susceptibility testing. MICs were determined at the department of medical microbiology of the University of Zurich by the dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (25). Ninety-four strains were tested on the same day against all antimicrobial agents. Mueller-Hinton agar (Difco, Detroit, Mich.) supplemented with 5% sheep blood was used (29). When testing susceptibility to sulfamethoxazole-trimethoprim, 0.2 IU of thymidine phosphorylase (Sigma Chemical Co., St. Louis, Mo.) per ml was added. The inoculum applied with a Steers replicator was 10^4 CFU per spot. Incubation was at 35°C in ambient air. Quality control strains *S. pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 29213 were included in all runs. The oxacillin screening test was performed with disks containing 1 μg of oxacillin as described in the NCCLS procedure (26).

Bactericidal and lytic activity. The bactericidal and lytic activity of penicillin G, amoxicillin, cefaclor, cefuroxime, cefixime, and cefpodoxime at 2 times and 20 times the MIC was determined with the time-dependent bactericidal and lytic activity procedure against four strains exhibiting various degrees of susceptibility to penicillin G. Colonies were inoculated into brain heart infusion broth, and the cultures were incubated at 37°C until a turbidity equal to 5×10^7 CFU/ml was reached. One-half milliliter of the cultures was inoculated into 7.6 ml of preheated fresh brain heart infusion broth containing the tested antibiotic or no antibiotic for control. At appropriate time intervals, the optical density of the culture was measured at 620 nm for lytic activity, and aliquots were removed to determine the number of CFU by plating dilutions onto sheep blood agar plates as described in the NCCLS tentative guideline (24). To neutralize any antibiotic present in the aliquots, 0.02 ml of an SHV extended-spectrum beta-lactamase (Centre National de Recherche Scientifique, Paris, France) was added, and incubation was carried out for 3 min before plating.

Serotyping. Serotyping was done by the capsular reaction test with the 27 serotype and 19 serogroup serum samples available from the Statens Seruminstitut, Copenhagen, Denmark, to the type or group level. Factor sera to distinguish types within groups were not available. The Danish typing scheme was used (11).

RESULTS

Figure 1 shows the age distribution of the 335 patients for whom demographic data were available. Of the age group of patients up to 10 years, 18 of the 29 patients were less than 2 years old. In these, infections of the ear, sinus, and eye as well

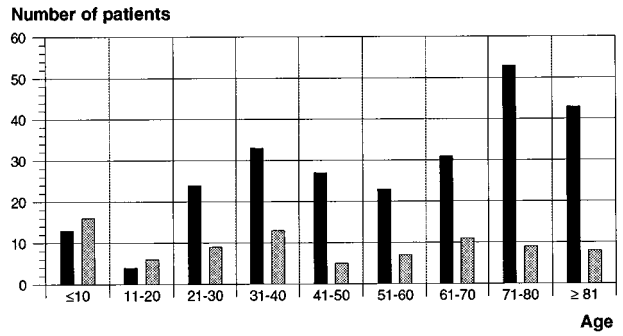

FIG. 1. Distribution of pneumococcal isolates from blood (■) and other sources (▨) by age of patients.

as meningitis were prominent. In older patients, pneumonia, meningitis, and septicemia were prevailing.

Table 1 summarizes the overall susceptibility to the antimicrobial agents examined. Percentages of strains in the fully resistant (resistant), intermediately resistant (intermediate), and susceptible categories are shown only for those drugs published in the 1994 NCCLS document (27). Six percent of pneumococci had a reduced susceptibility to penicillin, and 1% were resistant. Amoxicillin was equally active as penicillin, and oxacillin was less active than penicillin. Of the oral cephalosporins, cefpodoxime and cefuroxime had the highest activity, equalling the activity of penicillin and amoxicillin. When tested against macrolides, 94% of the strains were susceptible and 5% were resistant by the NCCLS criteria, with MIC values of >128 mg/liter. Twelve of the 21 strains resistant to erythromycin were also resistant to clindamycin. Of the quinolones, sparfloxacin was the most active substance, closely followed by ciprofloxacin.

The oxacillin screening test (26) detected all 25 strains in the intermediate and resistant categories. Of the 326 susceptible strains, 4 had zone sizes of <20 mm. This resulted in a sensitivity and specificity of the oxacillin screening test of 100 and 98.8%, respectively. This is a better specificity than the one of 3 of 56 strains reported by Kiska et al. (18), for example.

When the main antimicrobial groups (represented by penicillin, chloramphenicol, erythromycin, sulfamethoxazole-trimethoprim, and tetracycline) were considered, 43 strains were resistant to one agent, 17 were resistant to two, 6 were resistant to three, and 12 were resistant to four antimicrobial agents; i.e., 78 strains were resistant to at least one agent. Resistance to multiple agents was found especially among the pneumococci not fully susceptible to penicillin: of the 25 strains, 10 were resistant to one, 3 were resistant to two, and 8 were resistant to three additional antimicrobial agents.

Table 2 gives the results of the activity of extended-spectrum beta-lactams, other beta-lactams, and additional antimicrobial agents against the 25 strains that had a reduced susceptibility or were resistant to penicillin.

Table 3 shows the prevalent serotypes of 351 *S. pneumoniae* strains and their coverage by the pneumococcal vaccine. Overall, 91% of pneumococcal serotypes isolated in Switzerland are included in the 23-valent vaccine, the remaining 9% being either rare serotypes or nontypeable strains.

Table 4 gives the serotype distribution of the 351 *S. pneumoniae* strains by frequency, their resistance to penicillin G, and their resistance to other major antimicrobial agents. Reduced susceptibility or resistance to penicillin was found especially among serotypes 6 (9% of 44 strains), 19 (12% of 33 strains), 14 (14% of 28 strains), 1 (12% of 29 strains), and 23 (17% of 23 strains). On the other hand, the frequently encountered serotypes 7 and 3 (37 and 28 strains, respectively) were fully susceptible to penicillin.

TABLE 1. Activity of antimicrobial agents against invasive pneumococci[a]

Antimicrobial agent	MIC range (mg/liter)	MIC_{50}[b] (mg/liter)	MIC_{90}[b] (mg/liter)	Resistant strains (%)	Intermediate strains (%)	Susceptible strains (%)
Penicillin G	≤0.004–2	0.015	0.06	1.1	6.0	92.9
Oxacillin	0.008–16	0.06	0.5			
Amoxicillin	≤0.004–2	0.015	0.06			
Cefaclor	≤0.06–>128	0.5	2			
Cefetamet	0.06–>128	0.25	1			
Cefixime	0.008–>128	0.5	2			
Cefpodoxime	0.008–4	0.015	0.06			
Ceftibuten	0.06–>128	2	8			
Cefuroxime axetil (oral)	0.008–8	0.015	0.125	0	1.0	99.0
Loracarbef	0.015–128	0.5	1			
Chloramphenicol	0.125–32	1	8	7.7		92.3
Tetracycline	0.008–>64	0.125	1	7.6	0.6	91.8
Cotrimoxazole[c]	≤0.16–>160	2.5	20	6.1	7.8	86
Erythromycin	≤0.004–>128	0.03	0.06	5.4	0.9	93.7
Azithromycin	0.08–>128	0.03	0.25			
Clarithromycin	≤0.004–>128	0.03	0.06	5.3	0.6	94.1
Dirithromycin	0.008–>128	0.03	0.06			
Roxithromycin	0.008–>128	0.06	0.125			
Clindamycin	≤0.004–>128	0.015	0.06	3.7	0.3	96
Ciprofloxacin	0.06–2	0.125	1			
Fleroxacin	0.5–32	4	8			
Ofloxacin	0.25–4	1	2	0	5.2	94.8
Sparfloxacin	0.03–1	0.25	0.5			
Vancomycin	0.008–1	0.125	0.5	0	0	100
Teicoplanin	0.008–0.25	0.06	0.125			

[a] When no values are given, no NCCLS-approved breakpoints were available.
[b] MIC_{50} and MIC_{90}, MIC at which 50 or 90%, respectively, of the strains are inhibited.
[c] For cotrimoxazole, the total amounts are given (19 parts sulfamethoxazole plus 1 part trimethoprim).

TABLE 2. Susceptibility of 25 penicillin-resistant pneumococci to parenteral extended-spectrum beta-lactams, oral beta-lactams, and additional antimicrobial agents[a]

Antimicrobial agent	MIC range (mg/liter)	MIC_{50}[b] (mg/liter)	MIC_{90}[b] (mg/liter)	No. of strains		
				Resistant	Intermediately resistant	Susceptible
Penicillin	0.125–2	0.5	2	4	21	0
Cefepime	0.03–2	0.5	2	3	9	13
Cefotaxime	0.03–2	0.25	1	1	4	20
Ceftriaxone	0.03–1	0.25	1	0	8	17
Imipenem	0.03–0.25	0.06	0.25	0	8	17
Amoxicillin	0.125–2	0.5	2			
Cefaclor	0.5–>128	8	>128	10	1	14
Cefetamet	0.25–>128	4	>128			
Cefixime	0.25–>128	8	>128			
Cefpodoxime	0.6–4	0.5	4			
Ceftibuten	1–>128	8	>128			
Cefuroxime axetil (oral)	0.03–8	1	8	0	3	22
Loracarbef	0.125–128	4	128	11	0	14
Chloramphenicol	0.5–32	4	32	6	3	16
Tetracycline	0.25–64	0.5	32	8	0	17
Cotrimoxazole	1.25–160	40	160	10	9	6
Erythromycin	0.03–128	0.03	128	5	1	19
Clarithromycin	0.03–>128	0.06	16			
Roxithromycin	0.03–64	0.125	16			
Azithromycin	0.03–32	0.06	32			
Dirithromycin	0.008–32	0.06	4			
Clindamycin	0.015–128	0.03	32			
Ciprofloxacin	0.25–2	1	1			
Ofloxacin	1–4	2	4	0	3	22
Sparfloxacin	0.125–1	0.25	1			
Vancomycin	0.125–0.5	0.25	0.5	0	0	25

[a] When no values are given, no NCCLS-approved breakpoints were available.
[b] For abbreviations, see Table 1, footnote *b*.

Time-dependent lytic and bactericidal activity of beta-lactam agents at a fixed concentration of 20 times the MIC is shown in Fig. 2. The strain examined was susceptible to penicillin (MIC, 0.008 mg/liter). The penicillins had a better lytic and bactericidal effect than the cephalosporins did. Other susceptible and resistant strains at 20 times as well as at 2 times the MIC of the antibiotics examined showed the same response (data not shown).

DISCUSSION

S. pneumoniae continues to be a significant cause of morbidity in humans and is the leading cause of community-acquired bacterial pneumonia as well as an important cause of otitis media and meningitis in children. Until recently, *S. pneumoniae* has displayed susceptibility to most of the antimicrobial agents used in treating pneumococcal disease. However, strains resistant to the antibiotic of choice, penicillin, and to additional antibiotics were first described in 1978 in South Africa (19). Antibiotic-resistant strains now have a global distribution, with a high percentage of penicillin-resistant strains found in Africa, South and Central America, Southeast Asia, and the Middle East as well as southern and eastern Europe (2, 19). Because of this situation, it is recommended that each country establish its own surveillance system to determine the local epidemiology of this emerging problem.

In Switzerland, the penicillin resistance rate for the pneumococcus has reached a level comparable to the one in the United States (5), although locally higher rates have been reported there (6, 9, 12). In our multicenter study, 1% of the strains were inhibited by 2 mg of penicillin per liter and, thus, were resistant according to the NCCLS definition (25). Six

TABLE 3. Prevalent serotypes of 351 *S. pneumoniae* strains

Clinical diagnosis	No. of strains	No. of strains of serogroup or serotype:										Other strains[b]	No. of nontypeable strains	Coverage by vaccine (%)
		6[a]	7[a]	19[a]	14	1	3	23[a]	9[a]	18[a]	4			
Pneumonia, pleural empyema	160	19	18	17	12	22	11	11	10	6	4	15/3	12	91
Septicemia	92	18	13	9	9	4	5	5	9	2	5	5/4	4	91
Meningitis	33	1		5	4	3	3	3	1	6		3/2	2	88
Otitis, sinusitis	11	3		1			2		2	2			1	91
Eye infections	10	2	3		1			1			1		2	80
Other[c]	45	5	3	5	6	1	7	7	2		2	6/1		98
Total	351	48	37	37	32	30	28	27	24	16	12	29/10	21	91

[a] Group sera.
[b] Number of strains included in vaccine/number of strains not included in vaccine.
[c] Isolates from abscesses, wound, bone, and joint infections and strains with insufficient clinical data regarding the primary infection site.

TABLE 4. Serotype distribution of 351 *S. pneumoniae* strains by frequency, resistance to penicillin G, and resistance to other major groups of antimicrobial agents

Serotype or serogroup	No. of strains with resistance to:						
	Penicillin			Other antimicrobial agents			
	Susceptible	Intermediately resistant	Resistant	Chloramphenicol	Cotrimoxazole	Erythromycin	Tetracycline
6	44	3	1	11	15	11	10
7	37			0	2	0	2
19	33	4		5	2	1	3
14	28	3	1	1	5	2	2
1	29	1		0	2	1	2
3	28			0	0	0	0
23	23	3	1	2	10	1	2
9	21	2	1	0	4	1	2
18	16			0	2	0	0
4	12			0	0	0	0
11	5	2		2	4	0	1
24	1	1		0	0	0	0
Nontypeable	19	2		2	1	2	1
Other serotypes (<10 strains each)	30			4	1	3	4

percent of the isolates showed an intermediate resistance phenotype. These rates are much lower than those in countries reporting a serious problem (19). In the Zurich area, the resistance rate has even remained stable since 1984 (35). An explanation of this situation could be seen in the reserved use of antibiotics in our country and could also be explained by the compliance of patients with respect to antibiotic dosage and duration.

Penicillin resistance by pneumococci has been shown to be due to altered penicillin-binding proteins that have a decreased affinity for beta-lactam antimicrobial agents (10, 32). Therefore, strains of intermediate or high resistance to penicillin would also exhibit reduced susceptibility to other beta-lactam agents. This was also observed in the present study. Geometric mean MICs of oral cephalosporins for the 25 strains not susceptible to penicillin were 14.3 mg/liter for cefaclor, 12.1 mg/liter for cefixime, and 9.2 mg/liter for cefetamet, respectively. These drugs would be a questionable choice for the empirical treatment of suspected pneumococcal infections in an area of high incidence of resistant strains. On the other hand, cefpodoxime (mean MIC, 0.4 mg/liter) and cefuroxime (mean MIC, 0.6 mg/liter) had activities against strains with intermediate and full resistance similar to that of penicillin G (mean MIC, 0.44 mg/liter) and amoxicillin (mean MIC, 0.30 mg/liter). In time-dependent bactericidal experiments, the penicillins showed better killing properties than the oral cephalosporins. Whether this in vitro observation has any clinical relevance is unknown at the moment.

The laboratory definition of intermediate and full resistance to penicillin by pneumococci was established before enough data for the clinical relevance of the breakpoints were available. It appears now that only infections caused by resistant strains involving sites into which beta-lactams penetrate poorly such as the meninges and maybe also the middle ear respond insufficiently to therapy with penicillin or equally active beta-lactam agents in the usual dosages (for a review, see Klugman and Friedland [20]). Therefore, because of the very low incidence of highly resistant strains in Switzerland, empirical therapy with an appropriate beta-lactam can still be considered appropriate in most pneumococcal infections.

The parenteral cephalosporins cefotaxime, ceftriaxone, and

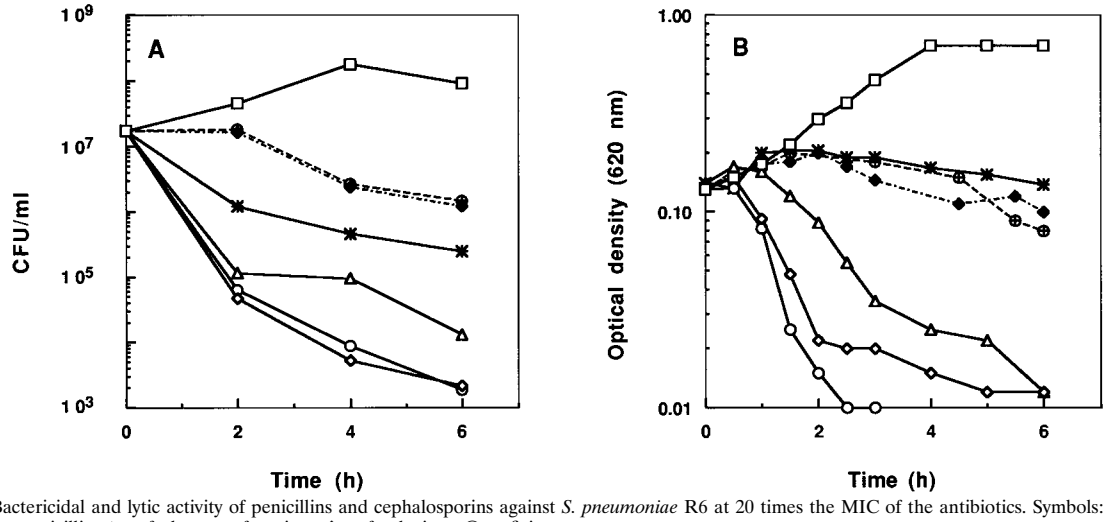

FIG. 2. Bactericidal and lytic activity of penicillins and cephalosporins against *S. pneumoniae* R6 at 20 times the MIC of the antibiotics. Symbols: □, control; ◇, penicillin; ○, amoxicillin; △, cefaclor; ∗, cefuroxime; ◆, cefpodoxime; ⊕, cefixime.

cefepime have been recommended for treatment of severe pneumococcal disease, especially meningitis (3, 9, 20), although treatment failures have been reported (14). It is important for the laboratory to test isolates not only with penicillin but also with these beta-lactams in case their use in the treatment of penicillin-resistant pneumococcal disease is considered.

Susceptibility to alternative agents such as the tetracyclines, the macrolides, or cotrimoxazole ranged between 86 and 94%, making these drugs inappropriate for empirical therapy. Currently available quinolones yielded MICs which either cluster around the susceptibility breakpoint used currently for other bacteria (ciprofloxacin and ofloxacin) or are in the intermediate or even resistant range (fleroxacin). Of the new quinolones under development, good results were obtained with sparfloxacin, a new quinolone with a better activity against gram-positive organisms than that of other quinolones (3, 15, 17, 33). Our data were in accordance with these other reports.

The 10 most prevalent serotypes or serogroups in the present collection comprised 83% of all *S. pneumoniae* isolates examined. Ninety-one percent of all serotypes or serogroups are included in the present 23-valent pneumococcal vaccine. Antimicrobial resistance in pneumococci worldwide is restricted to a surprisingly limited range of serotypes. This is especially true of penicillin resistance and multiple resistance, which is restricted mostly to the serotypes or serogroups 6, 14, 19, and 23 (19). Our data are in agreement with this observation. Resistance to penicillin among often-encountered strains of serotypes 3 and 7 was not observed, which is also in accordance with other epidemiological studies (4, 5, 28). Regional differences within Switzerland with regard to serotype distribution and susceptibility patterns were not observed.

ACKNOWLEDGMENTS

We thank the following colleagues (in Switzerland) for providing strains and relevant information: R. Auckenthaler, University Hospital, Geneva; M. Barcherini, Hôpital de zone, Nyon; D. Buhl, Institute of Medical Microbiology, St. Gallen; M. Dolina, Istituto cantonale batteriosierologico, Lugano; R. Frei, University Hospital, Basel; A. Gerber, Regional Hospital, Burgdorf; I. Heinzer, Cantonal Hospital, Aarau; L. Matter, Institute of Medical Microbiology of the University, Berne; J. Munzinger, Cantonal Hospital, Lucerne; and G. Praz, Laboratoire Central des Hôpitaux Valaisans, Sion.

This study was supported by a grant from Rhône-Poulenc, Thalwil, Switzerland.

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