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MINIREVIEW

Evolution of *Streptococcus pneumoniae* Serotypes and Antibiotic Resistance in Spain: Update (1990 to 1996)

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INTRODUCTION

The surveillance in Spain of *Streptococcus pneumoniae* isolated in hospitals from different regions of the country was initiated in our laboratory in 1979. In that year we found that 6% of the pneumococci isolated from blood, cerebrospinal fluid (CSF), or lower respiratory tract (LRT) specimens were not penicillin susceptible and that these strains were distributed only among particular serogroups or serotypes (SGTs). We thus realized that although penicillin resistance was rare in most other countries, it was clearly significant in Spain (6). Rates of penicillin resistance increased continuously in the following years, reaching values up to 44.3% among invasive strains in 1989, and both highly penicillin-resistant and multidrug-resistant strains reached high levels (15.3 and 7.9%, respectively) (11). In the present decade, resistance to penicillin has spread throughout the world and numerous countries are observing the same pattern that occurred in our country during the 1980s (1, 2, 16). The situation with antimicrobial resistance in Spain at the end of the 1980s caused concern (3, 22), and knowledge of its evolution could be of interest for those countries where increasing resistance is being observed. This survey presents an update of the previous microbiological surveillance for pneumococcal infections. Here, we have included both invasive and noninvasive pneumococci isolated from 1990 to 1996 in Spanish hospitals. For comparative purposes, some tables and figures present data obtained in the period 1979 to 1989, including analysis of not only invasive strains (6, 11) but also noninvasive strains.

PNEUMOCOCCAL STRAINS AND LABORATORY DIAGNOSIS

From January 1990 to December 1996 a total of 9,243 pneumococcal isolates were received at the Pneumococcal Reference Laboratory, Madrid, Spain, for typing purposes and antibiotic resistance surveillance. There was an increasing number of isolates every year, from 779 in 1990 to 1,633 in 1996. Pneumococci were isolated from patients and healthy carriers at 62 hospitals from 13 (of the 17) autonomous communities of Spain. Of the isolates received, 51% were from patients with systemic infections (pneumonia, sepsis, or meningitis) found mostly in the adult population. Among the pneumococci from other sources, approximately half (mostly isolated from sputum) belonged to adults and the other half were from children with local (ear, sinus, and conjunctive) infections and from the nasopharynges of healthy carriers (Table 1). Among 8,740 isolates, 5,917 were from males and 2,823 were from females (ratio, 2.09). The ratio of males to females was 2.6 for adults and 1.3 for children.

All isolates were confirmed to be *S. pneumoniae* by colony morphology on blood agar, optoquine susceptibility, and sodium deoxycholate solubility. Those strains that were negative for one or more of these tests (most of them unencapsulated) were studied by hybridization with two probes based on the autolysin and pneumolysin genes (12, 40). These two pneumococcal proteins have been demonstrated to be species specific (12, 15, 21, 32, 35, 45). Isolates resistant to optoquine and/or insoluble in deoxycholate but positive by hybridization were regarded as atypical pneumococcal strains and were included in the study. Serotyping was carried out by the Quellung reaction, with the use of 46 antisera provided by the Statens Seruminstitut (Copenhagen, Denmark). Some of the pneumococci received in 1996 were serotyped by a dot blot assay using the same antisera (10). Susceptibilities to penicillin, tetracycline, chloramphenicol, erythromycin, cefotaxime, and vancomycin were determined by the agar dilution technique as previously described (11), according to criteria from the National Committee for Clinical Laboratory Standards, guideline M-100-S6, for interpretation (28). In the present text, the term penicillin-resistant pneumococci (PRP) refers to both moderately and highly resistant strains, unless otherwise specified.

EVOLUTION OF SGTs

Thirty-eight different SGTs were found among the 9,243 pneumococci, but only 15 of them accounted for 83% of the strains (Fig. 1). The six SGTs found most frequently were SGTs 19 (12%), 6 (11.8%), 23 (10.5%), 3 (9.7%), 14 (9.6%), and 9 (7.4%), representing 61% of the pneumococci, while the remaining nine SGTs made up only 22% of isolates. The distribution of pneumococci of these SGTs isolated from invasive and noninvasive diseases in children and adults is also shown in Fig. 1. Figure 2 shows details of the SGT distribution of isolates from different sources in children and adults. SGTs 1, 4, 5, 7, and 12 were isolated with a greater frequency from blood and CSF than from other sources, in both children and adults. In contrast, serogroup 18 was associated with blood and CSF only in children. SGTs 6, 14, 19, and 23 were found more frequently in children, regardless of source, and SGTs 3, 8, and 9 had an obvious predilection for adults. In adults, serotype 3 ranked first or second most common among invasive isolates and those from most other sources. However, in children with invasive disease serotype 3 isolates were rarely recovered, although these isolates were an important cause of otitis media

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TABLE 1. Distribution of 9,243 pneumococcal strains according to source, age, and penicillin resistance in Spain from 1990 to 1996 No. (%) Pen^r

Strain group and source	Strains detected in:						
	Patients of all ages		Children (0–14 yr)		Adults (>14 yr)		Patients of unknown age (total)
	Total	No. (%) Pen^r	Total	No. (%) Pen^r	Total	No. (%) Pen^r	
Invasive							
Blood	3,319	1,143 (34.4)	424	216 (50.9)	2,160	681 (31.5)	735
CSF	384	166 (43.2)	80	46	233	89 (38.2)	71
LRT[a]	1,017	551 (54.2)	76	54	754	395 (52.4)	187
Total	4,720	1,860 (39.4)	580	316 (54.5)	3,147	1,165 (37.0)	993
Noninvasive							
Sputum	1,447	863 (59.6)	44	26	1,145	669 (58.4)	258
Ear	691	426 (61.6)	512	325 (63.5)	107	54 (50.5)	72
Nose	492	332 (67.5)	386	265 (68.7)	64	40	42
Eye	668	396 (59.3)	368	244 (66.3)	200	95 (47.5)	100
Pharynx	447	238 (53.2)	343	175 (51.0)	33	23	71
Pus	147	85 (57.8)	33	26	91	46	23
Total	3,892	2,340 (60.1)	1,686	1,061 (62.9)	1,640	927 (56.5)	
Other[b]	631	327	129	74	300	134	202
Total	9,243	4,527 (49.0)	2,395	1,451 (60.6)	5,087	2,226 (43.7)	1,761

[a] LRT includes strains isolated from pleural fluid transtracheal puncture, bronchial protected brush, and pulmonary biopsy specimens.
[b] No data (237 strains) and miscellaneous, (394 strains isolated from peritoneum, articular fluid, urine, vagina, Bartholin's gland, and catheter specimens).

and were the fourth most common serotype among isolates from the pharynges of carriers.

The predominant SGTs have remained the same over time, although the relative frequency of some of them has varied significantly. Figure 3 shows the changes in the prevalence of invasive and noninvasive isolates of the 15 most-common SGTs since 1979. The prevalence of SGTs 9, 14, and 19 increased from 2 to 7%, 6 to 14%, and 5 to 7.5%, respectively. The prevalence of SGTs 1, 5, and 7 decreased from 10 to 2%, 7 to 1%, and 6 to 1%, respectively. The prevalence of serogroup 6 rose until 1987, when it reached 18.5%. It then slowly decreased but in the last 4 years has gradually risen again. The prevalence of serogroup 23 increased rapidly at the beginning of the 1980s from 5% to

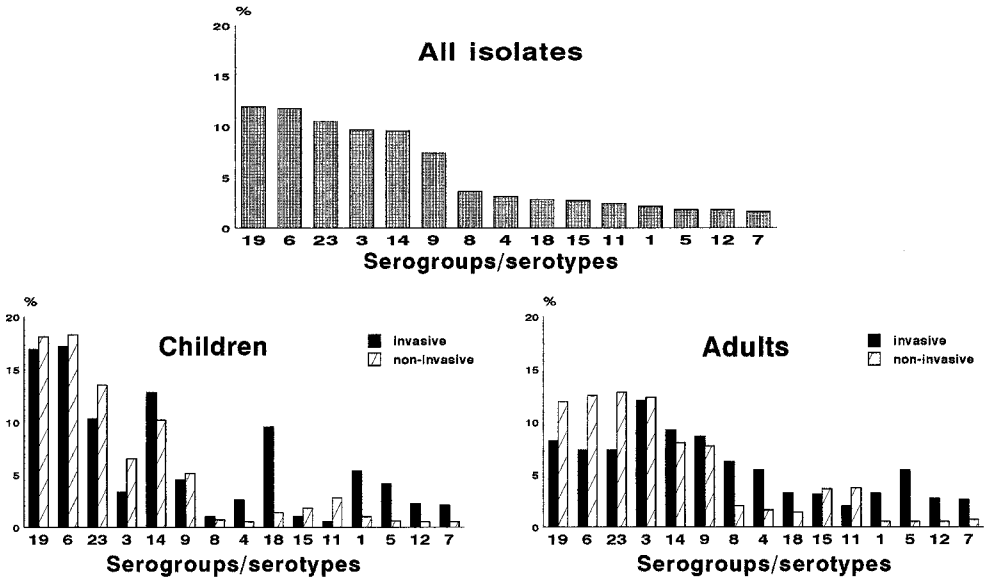

FIG. 1. Percentages of the 15 top-ranking pneumococcal SGTs isolated from all sources and ages (top) and from children and adult with invasive and noninvasive disease (bottom) in Spain from 1990 to 1996.

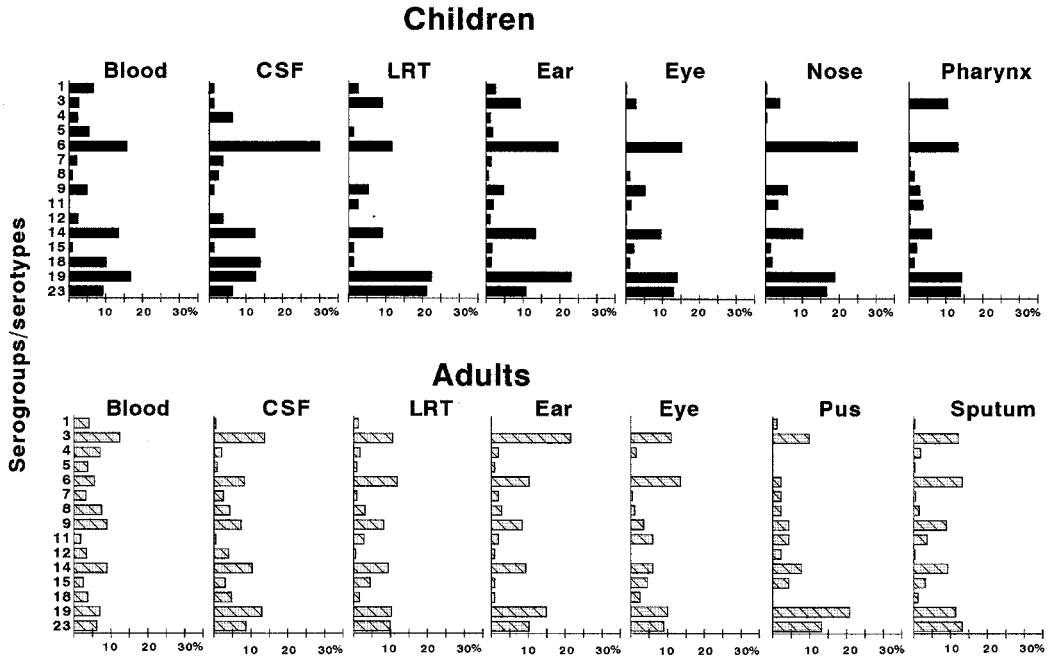

FIG. 2. Distribution of the 15 most-common pneumococcal SGTs from different specimen sources in children and adults in Spain from 1990 to 1996.

nearly 20% and then decreased, representing 10% of the total isolates in 1996.

EVOLUTION OF ANTIMICROBIAL RESISTANCE

Of the 9,243 strains studied, 4,527 (49%) showed decreased susceptibility to penicillin (MIC > 0.06 μg/ml), 4,011 (43.4%) showed decreased susceptibility to tetracycline (MIC > 2 μg/ml), 2,852 (30.9%) showed decreased susceptibility to chloramphenicol (MIC > 4 μg/ml), 2,077 (22.5%) showed decreased susceptibility to erythromycin (MIC > 0.25 μg/ml), and 2,008 (21.7%) showed decreased susceptibility to cefotaxime (MIC > 0.5 μg/ml). All strains were susceptible to vancomycin (MIC ≤ 0.5 μg/ml). Among pneumococci isolated from systemic infections, 39.4% were resistant to penicillin, 36% were resistant to tetracycline, 25.7% were resistant to chloramphenicol, 16.7% were resistant to erythromycin, and 18.6% were resistant to cefotaxime; for noninvasive pneumococci, the values were 59.4, 51.6, 36.5, 28.8, and 25.1%, respectively. Figure 4 shows how the percentages of resistant isolates have varied through the years. Among pneumococci causing systemic infections, there was an increasing trend towards penicillin resistance in the 1980s, reaching a maximum of 44.3% in 1989. From that year on, the rate decreased to 34.5% in 1992 and then rose again to around 42% in the final 3 years of the study. Tetracycline and chloramphenicol resistance followed the descending pattern observed in previous years. In contrast, erythromycin resistance has been growing continuously, doubling from 10.2% (in 1990) to 23.8% (in 1996) in invasive pneumococci and from 20.7 to 40.4% in noninvasive pneumococci.

Table 1 summarizes the percentage of PRP found in different specimens from children and adults. Blood pneumococci

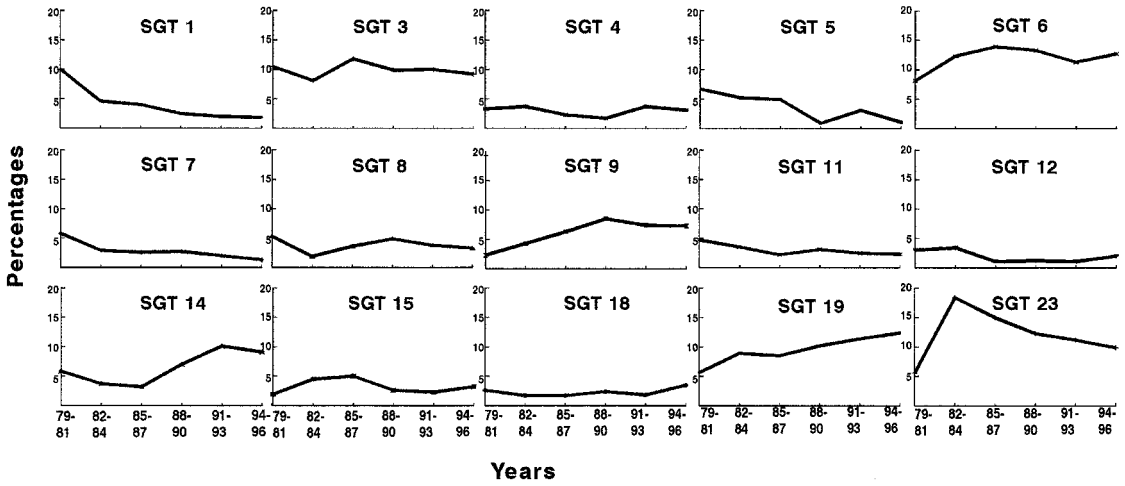

FIG. 3. Annual frequency of the 15 most-common SGTs in Spain from 1979 to 1996.

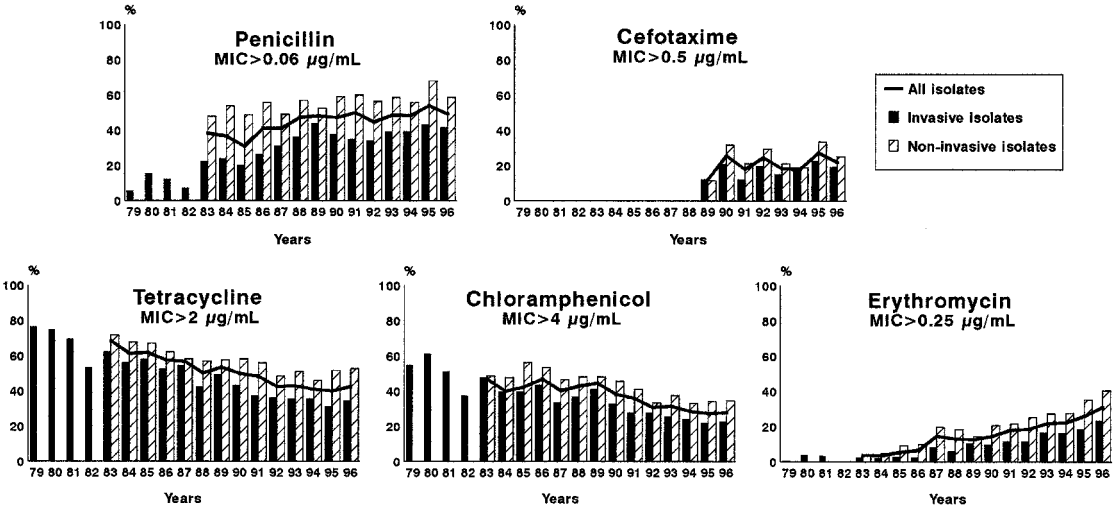

FIG. 4. Development of antibiotic resistance in pneumococcal isolates in Spain from 1979 to 1996. From 1979 to 1983 only invasive isolates were studied. Cefotaxime susceptibility testing started in 1989.

presented the lowest rates of penicillin resistance (50.9% in children and 31.5% in adults), whereas nasal isolates showed the highest (68.7 and 62.5%, respectively). Rates of PRP were lower among adults than among children, regardless of the source of the specimen.

The MIC distribution for penicillin versus cefotaxime is shown in Table 2. Of the total isolates, 39% were moderately resistant and 10% were highly resistant to penicillin (of those, 31 and 8.4%, respectively, were invasive isolates). For cefotaxime these percentages were 19.2 and 2.5%, respectively (of those, 16.6 and 2%, respectively, were invasive isolates). For most of the highly resistant pneumococci the MICs of the two β-lactams were 2 μg/ml. The MICs of cefotaxime correlated well with those of penicillin. For 6,764 isolates (73.2%) the MICs of the two antibiotics were identical, and for most other strains they differed by only 1 or 2 dilutions. For 264 (2.8%) strains, the MICs of cefotaxime were higher than those of penicillin. In contrast, the MICs of cefotaxime were lower in 2,215 (24%) strains. None of the penicillin-susceptible pneumococci were resistant to cefotaxime, but among the cefotaxime-susceptible strains, 74 were resistant to penicillin. Except for one serogroup 19 strain (MIC of penicillin and cefotaxime = 8 μg/ml) and a serotype 14 strain (MIC of penicillin = 0.25 μg/ml; MIC of cefotaxime = 8 μg/ml), all other isolates for which the MICs were 8 μg/ml were atypical pneumococci (noncapsulated, optoquine resistant, and/or bile negative). Among isolates for which the MICs were 4 μg/ml, 50% were atypical pneumococci and the rest belonged mostly to SGT 23. Seventy percent of the isolates for which the MICs were 2 μg/ml belonged to SGTs 6, 14, and 23, while isolates of SGTs 9 and 19 were moderately resistant in the majority of cases.

Table 3 summarizes the antibiotic resistance patterns found in this 7-year study, comparing them with those found in previous periods. Among PRP, 72% were also resistant to other antibiotics, while among penicillin-susceptible pneumococci, only 21.5% were resistant to other drugs. Overall, 5,540 pneumococci were resistant to one or more drugs (60%), this being less than that found in previous years (74% in 1979 to 1984 and 68% in 1985 to 1989). Although the total percentage of pneumococci resistant to one or more drugs has decreased through the years, the number of strains with certain patterns of penicillin and/or erythromycin resistance has risen. Among them, the important and continuous increase in multidrug-resistant (PTCE) pneumococci (i.e., those resistant to penicillin, tetracycline, chloramphenicol, and erythromycin) is of particular concern. The average incidence of these isolates increased from 1.1% in 1979 to 1984 to 7.7% in 1985 to 1989 and 12.5%

TABLE 2. Distribution of 9,243 pneumococcal isolates according to MICs of penicillin and cefotaxime in Spain from 1990 to 1996[a]

MIC cefotaxime (μg/ml):	No. of strains for which the MIC of penicillin (μg/ml) is:								Total no. of strains
	≤0.06	0.12	0.25	0.5	1	2	4	8	
≤0.06	4,650	114	21	2	2	0	0	0	4,789
0.12	52	175	223	28	1	0	0	0	479
0.25	8	41	282	233	21	1	0	0	586
0.5	6	4	41	451	806	73	0	0	1,381
1	0	0	4	64	1,049	652	7	0	1,776
2	0	0	1	0	37	134	22	2	196
4	0	0	0	1	1	3	16	7	28
8	0	0	1	0	0	0	0	7	8
Total no. of strains	4,716	334	573	779	1,917	863	45	16	9,243

[a] Susceptibility categorizations for penicillin and cefotaxime: highly resistant, MIC ≥ 2 μg/ml (both); moderately resistant, 0.12 μg/ml ≤ MIC ≤ 1 μg/ml and MIC = 1 μg/ml, respectively; and susceptible, MIC ≤ 0.06 μg/ml and ≤ MIC ≤ 0.5 μg/ml, respectively.

TABLE 3. Comparison of antibiotic resistance patterns of 9,243 pneumococcal isolates with those found in previous periods in Spain

Antibiotic(s)[a]	No. (%) of strains resistant during:		
	1979–1984[b] (*n* = 1,381)	1985–1989[b] (*n* = 2,577)	1990–1996 (*n* = 9,243)
P	63 (4.6)	207 (8.0)	1262 (13.7)
T	235 (17.0)	290 (11.3)	388 (4.2)
C	13 (0.9)	44 (1.7)	29 (0.3)
E	0 (—)[c]	7 (0.3)	53 (0.6)
PT	59 (4.3)	84 (3.3)	233 (2.5)
PC	9 (0.7)	27 (1.0)	55 (0.6)
PE	3 (0.2)	1 (—)	107 (1.2)
TC	342 (24.8)	264 (10.2)	337 (3.6)
TE	2 (0.1)	7 (0.3)	104 (1.1)
CE	0 (—)	0 (—)	4 (—)
PTC	252 (18.2)	570 (22.1)	1159 (12.5)
PTE	5 (0.4)	25 (1.0)	541 (5.9)
PCE	0 (—)	6 (0.2)	19 (0.2)
TCE	21 (1.5)	18 (0.7)	98 (1.1)
PTCE	15 (1.1)	199 (7.7)	1151 (12.5)
Total	1,019 (73.8)	1,749 (67.9)	5,540 (59.9)

[a] P, penicillin; T, tetracycline; C, chloramphenicol; and E, erythromycin.
[b] Data include invasive and noninvasive pneumococci.
[c] —, 0% or an insignificant percentage.

in 1990 to 1996. It should also be pointed out that within the period 1990 to 1996, the incidence of PTCE isolates increased notably (from 9.5% in 1990 to 16.6% in 1996).

RELATIONSHIP BETWEEN SGTs AND PENICILLIN RESISTANCE

Table 4 shows the distribution of the PRP patterns by SGTs. Resistant pneumococci belonged to 20 different SGTs, but 86% of the isolates were confined to the 5 SGTs classically associated with penicillin resistance: SGTs 6 (19.7%), 9 (12.3%), 14 (18.7%), 19 (17.2%), and 23 (18%). Of the serogroup 6 resistant isolates, 55% demonstrated the PTCE pattern, 87% of serogroup 9 and 53% of serotype 14 were resistant only to penicillin, and 47% of serogroup 19 and 60% of serogroup 23 belonged to the PTC pattern (resistance to penicillin, tetracycline, and chloramphenicol).

The relative frequencies of SGTs 6, 9, 14, 19, and 23 within the PRP population throughout the years is shown in Fig. 5. At the beginning of the 1980s the majority of the PRP belonged to SGTs 6 and 23 (73% in 1985). The relative importance of SGTs 6 and 23 has decreased since then, even though in the last 2 years the percentage of PRP in SGT 6 has slightly increased. The other SGTs, which prior to 1985 were very infrequent among PRP, have subsequently increased in frequency, and at present the distributions of the five SGTs among the PRP population are quite similar.

Pneumococci belonging to SGTs 6, 9, 14, 19, and 23 studied from 1990 to 1996 were almost all penicillin resistant. Penicillin resistance was found to be 95% for serotype 14 pneumococci, 84.5% for serogroup 23, 82% for serogroup 6, 80% for serogroup 9, and 70% for serogroup 19. Figure 6 compares the percentages of PRP and of pneumococci belonging to the five SGTs recovered from different Spanish communities, sources, and age groups. Both distributions are quite similar, indicating that in Spain the differences in penicillin resistance rates are mainly due to differences in the prevalence of pneumococci belonging to SGTs 6, 9, 14, 19, and 23.

COMMENTS AND CONCLUSION

The epidemiological characteristics of the different pneumococcal SGTs are extremely complex. Although the leading SGTs causing illness are the same worldwide, for reasons that are unclear significant differences exist in their relative fre-

TABLE 4. SGT distribution and resistance patterns of 4,527 penicillin-resistant pneumococcal isolates in Spain from 1990 to 1996

SGT	No. of strains resistant to[a]:								Total
	P	PT	PC	PE	PTC	PTE	PCE	PTCE	
1					1				1
3	2	1						1	4
4	1								1
6	17	20	8	13	168	165	10	495	896
7	2				1				3
8	3					1		2	6
9	488	32	1	23	4	9		2	559
11	27	2		1	3	14		2	49
13	1								1
14	451	7	5	15	70	62	1	235	846
15	7	26	1	4	23	98		7	166
18	1	1				1			3
19	96	87	11	4	371	37		175	781
21	17	2		1	2	7			29
23	42	11	26	4	496	22	5	210	816
24	1								1
29					1				1
33	1								1
34	2								2
35	8			2					10
NT[b]	95	44	3	40	19	125	3	22	351
Total	1,262	233	55	107	1,159	541	19	1,151	4,527

[a] Abbreviations: P, penicillin; T, tetracycline; C, chloramphenicol; and E, erythromycin.
[b] NT, nontypeable strains.

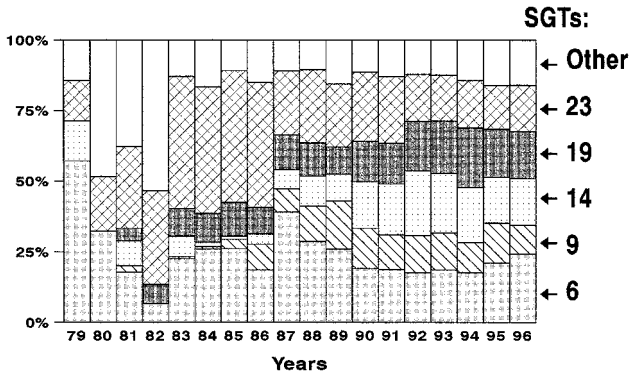

FIG. 5. Relative frequencies of SGTs 6, 9, 14, 19, and 23 within the PRP population in Spain from 1979 to 1996.

quencies as a function of time, geography, age of patient, and type of infection (14, 19, 34).

Regarding geographical variations, SGTs that are prevalent in some countries hardly cause illness in others. Serotypes 1 and 5 cause illness with much greater frequency in Latin America than elsewhere but are especially uncommon in the United States and Canada (36). In Spain these two serotypes were important at the beginning of the 1980s but presently rank among the last places in frequency. Meanwhile, in some European countries, such as Denmark and Germany, serotype 1 isolates are the most frequent cause of infections (29, 33). The incidence of serotype 3 has decreased greatly in recent years in many countries (5, 18, 43); however, in Spain this type has maintained a high frequency throughout the last 18 years. An increasing proportion of serotype 14 isolates has been observed in Spain and other countries (5, 18, 43) over the last 7 years. In general, geographical and temporal differences in the SGT distribution interfere with the development of one unique vaccine for worldwide use. In this context Sniadack et al. reviewed pneumococcal SGT data from 16 countries on six continents and estimated that the coverage for an optimal nanovalent vaccine for global use ranges from 44 to 90% of SGTs (38).

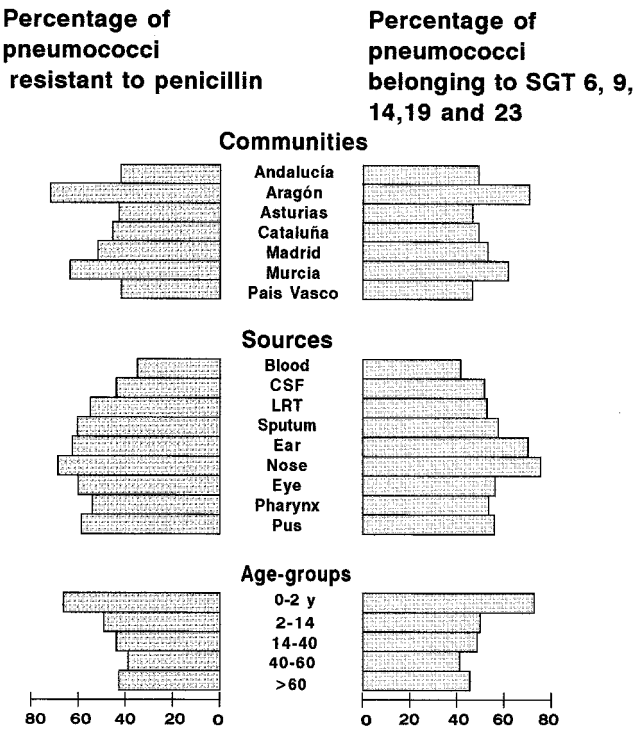

FIG. 6. Comparison of PRP and SGTs by autonomous community, source, and age groups.

The differences found in SGT distribution by age coincide with previously published data (19). SGTs 6, 14, 18, 19, and 23 are most frequently associated with infection in children, while SGTs 3, 8, and 9 are more prevalent in adults. Analyzing the SGTs of 7,000 pneumococci isolated in 13 countries, Scott et al. observed as much variation in SGT distribution by age as one might expect between different species of respiratory bacterial pathogens (36).

Although, in general, PRP have increased worldwide during recent years, the prevalence of resistance and the rates of emergence of resistance vary considerably from one country to another (1, 2, 16). Penicillin resistance increased sharply in Spain until 1989 (11), but it has since remained stable, despite annual variations, with a resistance rate among invasive pneumococci of about 42%. In many countries with low resistance rates during the previous decade, there has been a remarkable increase in the 1990s, similar to that which occurred in Spain in the 1980s. In Portugal penicillin resistance increased from 4.6% in 1989 to 17.9% in 1993 (31), in France it increased from 3.2% in 1987 to 20% in 1992 (4), and in the United States it increased from around 5% in the 1980s to 33.5% in 1996 (41). In contrast the incidence of PRP has remained stable at very low levels in other areas of Europe: Denmark (<1%), Germany (1.8%), Belgium (2 to 4%), Sweden (1.7%), Finland (1.7%), Great Britain (1.5 to 3.9%), and Italy (5.5%) (18, 20, 23, 29, 30, 33, 43). In the present situation, it is clear that in order to stop the increase and, if possible, reduce the frequency of PRP, an exhaustive surveillance of pneumococcal resistance is required, along with a strict control of antibiotic policy.

Association of penicillin resistance with only certain specific SGTs is a general finding. However, no clear explanation has been found for this association. Spanish penicillin-resistant isolates have been genetically characterized on numerous occasions, showing the existence of a number of distinct clones, the majority of which have spread globally (25, 27, 42). It has also been demonstrated that some of the resistant clones circulating in different countries (such as the multidrug-resistant serotype 19F) are serotype variants of the Spanish clone 23F, which originated by the horizontal transfer of capsular genes. In other cases new clones have emerged by the horizontal spread of altered *pbp* genes (e.g., the penicillin-resistant serotype 9V clone) (7, 8). Although clonal spread, horizontal transfer, and antibiotic policy seem to have a definitive influence on PRP prevalence, there are many findings that are not easily explained by taking only these factors into account. Thus, it is difficult to explain how the Spanish clones 6B and 23F have spread to and become established in regions as remote as Iceland or South Korea (24, 39, 44) but have not become prevalent in England or Germany, in spite of millions of tourists coming through our country each year. In the 1980s, the Spanish clone 6B was detected in Iceland and Finland simultaneously, and, surprisingly, the epidemic spread of clone 6B took place in Iceland but not in Finland (39).

Molecular analyses of PRP isolates in different countries have demonstrated that just one or two clones are responsible for the majority of the resistant population. For instance, in France 50% of the PRP are the Spanish serotype 23F clone, in

Slovakia nearly all PRP are the serotype 14 clone, and in South Africa 71% of the PRP are serotype 19A and 6B clones (9, 13, 37). During the early 1980s in Spain, two major clones, 6B and 23F, were clearly predominant, but recently they have made way for other clones. On the other hand, and in spite of selective pressure exerted by the overuse of antibiotics in our country, certain penicillin-susceptible SGTs have not been displaced by these resistant clones. Currently, serotype 3 pneumococci are uniformly penicillin susceptible, although this has been one of the most prevalent serotypes in Spain for at least the past 18 years. This serotype not only has been isolated from adults but also is one of the main causes of otitis media in children. Curiously, serotype 3 pneumococci have been recovered with high frequency from infant carriers, and in spite of the extensive use of antibiotics in infants, for reasons that are not understood this serotype has neither acquired penicillin resistance nor decreased in prevalence over time.

Although highly resistant PRP (MICs, 8 to 32 μg/ml) have been reported in some countries (17), in Spain the level of resistance remains relatively low and stable, with the penicillin MICs for the majority of the most-resistant isolates being 2 μg/ml. The finding in the United States of isolates with extremely high resistance to cefotaxime but low resistance to penicillin and the proof that such resistance could be acquired by only one transformation step caused alarm among microbiologists due to the likely spread of this resistance pattern among pneumococcal populations (26). Fortunately this has not occurred, and currently this type of resistant isolate is very rare outside the United States. In our study, among the 9,243 strains analyzed, for only 3 was the MIC of cefotaxime significantly greater than that of penicillin.

The results of the present study show that the alarming tendency toward increasing penicillin resistance in pneumococci from Spain may have ended over the last few years. In contrast, erythromycin resistance has been growing continuously.

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REFERENCES

1. **Appelbaum, P. C.** 1992. Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. Clin. Infect. Dis. **15:**77–83.
2. **Baquero, F.** 1996. Epidemiology and management of penicillin-resistant pneumococci. Curr. Opin. Infect. Dis. **9:**372–379.
3. **Barnett, E. D., J. O. Klein, and D. W. Teele.** 1992. Pneumococcal vaccine for Olympic athletes and visitors to Spain. N. Engl. J. Med. **326:**1572.
4. **Bedos, J. P., S. Chevret, C. Chastang, P. Geslin, B. Regnier, and French Cooperative Pneumococcus Study Group.** 1996. Epidemiological features of and risk factors for infection by *Streptococcus pneumoniae* strains with diminished susceptibility to penicillin: findings of a French survey. Clin. Infect. Dis. **22:**63–72.
5. **Butler, J. C., R. F. Breiman, H. B. Lipman, J. Hofmann, and R. R. Facklam.** 1995. Serotype distribution of *Streptococcus pneumoniae* infections among preschool children in the United States, 1978–1994: implications for development of a conjugate vaccine. J. Infect. Dis. **171:**885–889.
6. **Casal, J.** 1982. Antimicrobial susceptibility of *Streptococcus pneumoniae*: serotype distribution of penicillin-resistant strains in Spain. Antimicrob. Agents Chemother. **22:**222–225.
7. **Coffey, T. J., S. Berrón, M. Daniels, M. E. García-Leoni, E. Cercenado, E. Bouza, A. Fenoll, and B. G. Spratt.** 1996. Multiple antibiotic-resistant *Streptococcus pneumoniae* recovered from Spanish hospitals (1988–1994): novel major clones of serotypes 14, 19F and 15F. Microbiology **142:**2747–2757.
8. **Coffey, T. J., C. G. Dowson, M. Daniels, and B. G. Spratt.** 1995. Genetics and molecular biology of β-lactam-resistant pneumococci. Microb. Drug Resist. **1:**29–33.
9. **Doit, C., E. Denamur, B. Picard, P. Gaslin, J. Elion, and E. Bingen.** 1996. Mechanisms of the spread of penicillin resistance in *Streptococcus pneumoniae* strains causing meningitis in children in France. J. Infect. Dis. **174:**520–528.
10. **Fenoll, A., I. Jado, D. Vicioso, and J. Casal.** 1997. Dot blot assay for the serotyping of pneumococci. J. Clin. Microbiol. **35:**764–766.
11. **Fenoll, A., C. Martín-Bourgon, R. Muñoz, D. Vicioso, and J. Casal.** 1991. Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolates causing systemic infections in Spain, 1979–1989. Rev. Infect. Dis. **13:**56–60.
12. **Fenoll, A., J. V. Martinez-Suarez, R. Muñoz, J. Casal, and J. L. García.** 1990. Identification of atypical strains of *Streptococcus pneumoniae* by a specific DNA probe. Eur. J. Clin. Microbiol. Infect. Dis. **9:**396–401.
13. **Figueiredo, A. M., R. Austrian, P. Urbaskova, L. A. Teixeira, and A. Tomasz.** 1995. Novel penicillin-resistant clones of *Streptococcus pneumoniae* in the Czech Republic and in Slovakia Microb. Drug Resist. **1:**71–77.
14. **Finland, M., and M. W. Barnes.** 1977. Changes in occurrence of capsular serotypes of *Streptococcus pneumoniae* at Boston City Hospital during selected years between 1935 and 1974. J. Clin. Microbiol. **5:**154–166.
15. **Gillespie, S., C. Ullman, M. D. Smith, and V. Emery.** 1994. Detection of *Streptococcus pneumoniae* in sputum samples by PCR. J. Clin. Microbiol. **32:**1308–1311.
16. **Goldstein, F. W., J. F. Acar, and The Alexander Project Collaborative Group.** 1996. Antimicrobial resistance among lower respiratory tract isolates of *Streptococcus pneumoniae*: results of a 1992-1993 Western Europe and USA Collaborative Surveillance Study. J. Antimicrob. Chemother. **38**(Suppl. A)**:**71–84.
17. **Goldstein, F. W., J. Liñares, J. M. Cristino, E. Malafiiej, M. Marcova, A. Marton, J. Murphy, G. Schito, Y. Trupl, P. Urbaskova, R. Vanhoof, and the European Study Group, Hospital Saint Joseph, Paris.** 1996. Comparison of the intrinsic activity of 9 betalactams against 1131 strains of Streptococcus pneumoniae isolated in the 11 european countries between 1993 and 1995, abstr. T148. First European Congress of Antimicrobial Chemotherapy, Glasgow, Scotland.
18. **Hedlund, J., S. B. Svenson, M. Kalin, J. Henrichsen, B. Alsson-Liljequist, G. Möllerberg, and G. Kállenius.** 1995. Incidence, capsular types and antibiotic susceptibility of invasive *Streptococcus pneumoniae* in Sweden. Clin. Infect. Dis. **21:**948–953.
19. **Henrichsen, J.** 1979. The pneumococcal typing system and pneumococcal surveillance. J. Infect. **1**(Suppl. 2)**:**31–37.
20. **Johnson, A. P., D. C. E. Speller, R. C. George, M. Warner, G. Domingue, and A. Efstratiou.** 1996. Prevalence of antibiotic resistance and serotypes in pneumococci in England and Wales: results of observational surveys in 1990 and 1995. Br. Med. J. **312:**1454–1456.
21. **Kalin, M., K. Klanclerski, M. Granstrom, and R. Möllby.** 1987. Diagnosis of pneumococcal pneumonia by enzyme-linked immunosorbent assay of antibodies to pneumococcal hemolysin (pneumolysin). J. Clin. Microbiol. **25:**226–229.
22. **Lancet.** 1992. Pneumococcal vaccination for travel to Spain? Lancet **340:**84–85.
23. **Marchese, A., E. A. Debbia, A. Arvigo, A. Pesce, and G. C. Schito.** 1995. Susceptibility of *Streptococcus pneumoniae* strains isolated in Italy to penicillin and ten other antibiotics. J. Antimicrob. Chemother. **36:**833–837.
24. **McGee, L., K. P. Klugman, D. Friedland, and H. J. Lee.** 1997. Spread of the Spanish multi-resistant serotype 23F clone of *Streptococcus pneumoniae* to Seoul, Korea. Microb. Drug. Resist. **3:**253–257.
25. **Muñoz, R., T. Coffey, M. Daniels, C. G. Dowson, G. Laible, J. Casal, R. Hakenbeck, M. Jacobs, J. M. Musser, B. G. Spratt, and A. Tomasz.** 1991. Intercontinental spread of a multiresistant clone of serotype 23F *Streptococcus pneumoniae*. J. Infect. Dis. **164:**302–306.
26. **Muñoz, R., C. G. Dowson, M. Daniels, T. J. Coffey, C. Martin, R. Hakenbeck, and B. G. Spratt.** 1992. Genetics of resistant to third-generation cephalosporins in clinical isolates of *Streptococcus pneumoniae*. Mol. Microbiol. **6:**2461–2465.
27. **Muñoz, R., J. M. Musser, M. Crain, D. E. Briles, A. Marton, A. J. Parkinson, U. Sorensen, and A. Tomasz.** 1992. Geographic distribution of penicillin-resistant clones of 23F *Streptococcus pneumoniae*: characterization by penicillin-binding protein profiles, surface protein A typing, and multilocus enzyme electrophoresis. Clin. Infect. Dis. **15:**112–118.
28. **National Committee for Clinical Laboratory Standards.** 1995. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
29. **Nielsen, S. V., and J. Henrichsen.** 1996. Incidence of invasive pneumococcal disease and distribution of capsular types of pneumococci in Denmark, 1989–94. Epidemiol. Infect. **117:**411–416.
30. **Nissinen, A., M. Leinonen, P. Huovinen, E. Herva, M. L. Katila, S. Kontiainen, O. Liimatainen, S. Oinonen, A. K. Takala, and P. H. Mäkelä.** 1995. Antimicrobial resistance of *Streptococcus pneumoniae* in Finland, 1987–1990. Clin. Infect. Dis. **20:**1275–1280.
31. **Pato, M. V. V., C. B. Carvalho, A. Tomasz, and The Multicenter Study Group.** 1995. Antibiotic susceptibility of *Streptococcus pneumoniae* isolates in Portugal. A multicenter study between 1989 and 1993. Microb. Drug. Resist. **1:**59–69.
32. **Pozzi, G., M. R. Oggioni, and A. Tomasz.** 1989. DNA probe for identification of *Streptococcus pneumoniae*. J. Clin. Microbiol. **27:**370–372.

33. **Reinert, R. R., A. Queck, A. Kaufhold, M. Kresken, and R. Lütticken.** 1995. Antimicrobial resistance and type distribution of *Streptococcus pneumoniae* isolates causing systemic infections in Germany, 1992–1994. Clin. Infect. Dis. **21:**1398–1401.
34. **Robbins, J. B., R. Austrian, C. J. Lee, S. C. Rastogi, G. Schiffman, J. Henrichsen, P. H. Mäkelä, C. V. Broome, R. R. Facklam, R. H. Tiesjema, and J. C. Parke.** 1983. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on cross-reactive types within groups. J. Infect. Dis. **148:**1136–1159.
35. **Rudolph, K. M., A. J. Parkinson, C. M. Black, and L. W. Mayer.** 1993. Evaluation of polymerase chain reaction for diagnosis of pneumococcal pneumonia. J. Clin. Microbiol. **31:**2661–2666.
36. **Scott, J. A. G., A. J. Hall, R. Dagan, J. M. S. Dixon, S. J. Eykyn, A. Fenoll, M. Hortal, L. P. Jetté, J. H. Jorgensen, F. Lamothe, C. Latorre, J. T. Macfarlane, D. M. Shlaes, L. E. Smart, and A. Taunay.** 1996. Serogroup-specific epidemiology of *Streptococcus pneumoniae*: associations with age, sex, and geography in 7,000 episodes of invasive disease. Clin. Infect. Dis. **22:**973–981.
37. **Smith, A. M., and K. P. Klugman.** 1997. Three predominant clones identified within penicillin-resistant South African isolates of *Streptococcus pneumoniae*. Microb. Drug Resist. **3:**385–389.
38. **Sniadack, D. H., B. Schwartz, H. Lipman, J. Bogaerts, J. C. Butler, R. Dagan, G. Echaniz-Aviles, N. Lloyd-Evans, A. Fenoll, N. I. Girgis, J. Henrichsen, K. Klugman, D. Lehmann, A. K. Takala, J. Vandepitte, S. Cove, and R. F. Breiman.** 1995. Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children—implications for vaccine strategies. Pediatr. Infect. Dis. J. **14:**503–510.
39. **Soares, S., K. K. G. Kristinsson, J. M. Musser, and A. Tomasz.** 1993. Evidence for the introduction of a multiresistant clone of serotype 6B *Streptococcus pneumoniae* from Spain to Iceland in the late 1980s. J. Infect. Dis. **168:**158–163.
40. **Taira, S., E. Jalonen, J. C. Paton, M. Sarvas, and K. Runeberg-Nyman.** 1989. Production of pneumolysin, an pneumococcal toxin, in *Bacillus subtilis*. Gene **77:**211–218.
41. **Thornsberry, C., P. Ogilvie, J. Kahn, Y. Mauriz, and the Laboratory Investigator Group.** 1997. Surveillance of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenza*, and *Moraxella catarrhalis* in the United States in 1996–1997 respiratory season. Diagn. Microbiol. Infect. Dis. **29:** 249–257.
42. **Tomasz, A.** 1997. Antibiotic resistance in *Streptococcus pneumoniae*. Clin. Infect. Dis. **24**(Suppl. 1)**:**S85–S88.
43. **Verhaegen, J., Y. Glupczynski, L. Verbist, M. Blogie, N. Verbiest, J. Vandeven, and E. Yourassowsky.** 1995. Capsular types and antibiotic susceptibility of pneumococci isolated from patients in Belgium with serious infections, 1980–1993. Clin. Infect. Dis. **20:**1339–1345.
44. **Versalovic, J., V. Kapur, E. O. Mason, U. Shah, T. Koeuth, J. R. Lupski, and J. M. Musser.** 1993. Penicillin-resistant *Streptococcus pneumoniae* strains recovered in Houston: identification and molecular characterization of multiple clones. J. Infect. Dis. **167:**850–856.
45. **Virolainen, A., P. Salo, J. Jero, P. Karma, J. Eskola, and M. Leinonen.** 1994. Comparison of PCR assay with bacterial culture for detecting *Streptococcus pneumoniae* in middle ear fluid of children with acute otitis media. J. Clin. Microbiol. **32:**2667–2670.