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Changes in antimicrobial resistance, serotypes and genotypes in *Streptococcus pneumoniae* over a 30-year period

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Abstract

Over the past three decades, antimicrobial resistance in *Streptococcus pneumoniae* has dramatically increased worldwide. Non-susceptibility to penicillin in *S. pneumoniae* was first described in Australia in 1967, and later in New Guinea (1974), South Africa (1977), and Spain (1979). Most of these strains showed resistance to multiple antibiotics and belonged to serotypes 6A, 6B, 19A, 19F, and 23F. By the late 1980s and 1990s, the emergence and rapid dissemination of antibiotic-resistant pneumococci was observed in southern and eastern Europe, North America, South America, Africa, and Asia. Great geographical variability, both in serotype distribution and in the prevalence of resistant pneumococci, has been reported. However, the highest rates of resistance to penicillin and erythromycin worldwide were found in serotypes 6B, 6A, 9V, 14, 15A, 19F, 19A, and 23F. The introduction of the seven-valent pneumococcal conjugate vaccine (PCV7) in the 2000s and a reduction in antimicrobial use were associated with a significant decline in the incidence of invasive pneumococcal infections and in rates of antibiotic resistance in the USA. However, an increase in the incidence of infections caused by non-PCV7 serotypes, especially multiresistant serotype 19A pneumococci, has been observed in many countries over the last 5 years. The dynamic character of serotypes and antibiotic resistance in *S. pneumoniae* should be controlled by a policy of prudent antibiotic use and by implementation of the new generation of conjugate vaccines.

Keywords: Genotypes, penicillin-resistant, review, serotypes, *Streptococcus pneumoniae*

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Introduction

Streptococcus pneumoniae is the most important pathogen in otitis, sinusitis, bronchitis, and community-acquired pneumonia, as well as a predominant cause of meningitis and bacteraemia. Before 1967, this pathogen was uniformly susceptible to penicillin and most other antimicrobial agents. The first penicillin-non-susceptible pneumococcus was described in Australia in 1967; it had an MIC of penicillin of 0.6 mg/L, and it also showed resistance to tetracycline (MIC 5 mg/L) [1]. In 1974, 12% of 518 New Guinean isolates were penicillin-non-susceptible pneumococci (PNSP) [2]. Pneumococci with much higher resistance to penicillin and other antibiotics were first detected in 1977 in South Africa [3,4]. These multidrug-resistant pneumococci of serotypes 6A and 19A had penicillin MICs ranging from 0.12 to 4 mg/L, and were isolated from hospitalized paediatric carriers (29% of 543), causing bacteraemia in four patients [4]. In Spain, the first PNSP strain with a penicillin MIC of 0.5 mg/L was isolated from the blood from an adult patient in a hospital in Barcelona in 1979, and a year later one invasive isolate with a penicillin MIC of 2 mg/L was detected in the same hospital in Barcelona [5]. By the 1980s, a high prevalence of antibiotic resistance among pneumococci was being reported in other countries, leading to serious therapeutic problems, mainly in the treatment of pneumococcal meningitis [3,4,6–10]. The emergence and rapid dissemination of antibiotic-resistant pneumococcal clones in areas of southern and eastern Europe, North America, South America and Asia in the 1990s was associated with an increase in antibiotic consumption [11,12].

Although 92 pneumococcal serotypes have been described, the highest penicillin and erythromycin resistance proportions worldwide were associated with serotypes 6B, 6A, 9V, 14, 15A, 19F, 19A, and 23F, the so-called 'paediatric serotypes'. The introduction of the seven-valent pneumococcal conjugate vaccine (PCV7) for children in the 2000s, which included serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, was associated with a significant decline in penicillin resistance rates in *S. pneumoniae* in many countries [13–16]. However, an increase in the incidence of non-PCV7 serotypes, especially multiresistant serotype 19A strains, has been observed in many countries over the last 5 years [16–22].

This review analyses geographical trends in antibiotic resistance and the serotypes/genotypes in *S. pneumoniae* over the last three decades. It also includes the evolution of penicillin, erythromycin and fluoroquinolone resistance among invasive pneumococci received at the Spanish Reference Laboratory for Pneumococci over a 30-year period (1979–2008).

Resistance to β-Lactam Antibiotics

The mechanism of penicillin resistance in *S. pneumoniae* involves structural changes in the penicillin targets, the penicillin-binding proteins 1A, 2X, and 2B [23]. These changes result in reduced affinity for penicillin as well as for other β-lactam antibiotics. However, ceftriaxone, cefotaxime and carbapenems are less affected, and are generally the most active compounds [24].

Previous studies have demonstrated that PNSP (MICs ≥0.12 mg/L) can be associated with treatment failures in patients with meningitis, because the cerebrospinal fluid levels achieved with penicillin or the standard dosage of third-generation cephalosporins are insufficient to eradicate the infecting organism. The classical NCCLS breakpoints for penicillin (susceptible, ≤0.06 mg/L; intermediate, 0.12–1 mg/L; and resistant, ≥2 mg/L) were established in the late 1970s in order to prevent failures in patients with pneumococcal meningitis caused by PNSP. In contrast, *S. pneumoniae* strains with penicillin MICs of 0.12–2 mg/L had little effect on outcome in patients with pneumonia and other non-meningeal systemic pneumococcal infections who were treated with parenteral penicillin, amoxycillin, cefotaxime, or ceftriaxone [25–31]. According to these observations, the CLSI breakpoints for ceftriaxone and cefotaxime were modified in 2002, distinguishing between meningeal infections (susceptible, MIC ≤0.5 mg/L; intermediate, MIC 1 mg/L; and resistant, MIC ≥2 mg/L) and non-meningeal infections (susceptible, MIC ≤1 mg/L; intermediate, MIC 2 mg/L; and resistant, MIC ≥4 mg/L). However, penicillin breakpoints were not modified until 2008, when the site of infection and route of administration were considered. The current penicillin parenteral breakpoints for non-meningeal infections are MIC ≤2 mg/mL (susceptible), MIC 4 mg/mL (intermediate), and MIC ≥8 mg/mL (resistant), whereas MICs ≥0.12 mg/L for strains causing meningeal infection are considered to reflect resistance [32].

In the USA, *S. pneumoniae* was uniformly susceptible to penicillin until 1987. After that, rates of PNSP with a penicillin MIC ≥0.1 mg/L progressively increased from very low rates (5%) before 1991 to 14% in 1993–1994 [33,34]. In 1997, among 3237 invasive isolates, 25% were PNSP, and among these, 13.6% had a penicillin MIC ≥2 mg/L [35].

Global surveillance studies have shown that β-lactam-non-susceptibility rates increased worldwide during the 1990s and 2000s. The Alexander Project monitored resistance in *S. pneumoniae* from 1992 to 2001, and reported increases in the levels of non-susceptibility to penicillin from 24.9% in 1992 to 30.2% in 2001 in Spain, from 7.7% to 35.8% in France, and from 5.6% to 20.4% in the USA, whereas in Italy, Germany, and the UK, resistance rates remained below 5% during this period [36].

The PROTEKT US study, with 39 495 pneumococcal isolates from patients with community-acquired respiratory tract infections in the USA, showed that intermediate non-susceptibility to penicillin (MIC 0.12–1 mg/L) increased from 12.5% in 2000–2001 to 20.0% in 2003–2004, whereas penicillin resistance (MIC ≥2 mg/L) declined from 26.3% in 2000–2001 to 16.5% in 2003–2004. However, the percentage of resistance to amoxycillin (MIC ≥8 mg/L) remained low and stable, from 4.4% in 2000–2001 to 4.1% in 2003–2004 [37]. The PROTEKT study also analysed the antibiotic susceptibility of 20 142 pneumococcal isolates collected worldwide from respiratory tract infections in 2001–2004. The highest penicillin-non-susceptiblity rates were found in South Africa (74%), the Far East (63%), and the Middle East (54%). In southern European countries, rates of PNSP were higher than those found in northern European countries. The highest rates of PNSP (intermediate and resistant isolates) were found in France (40.4% and 15.9%), Greece (42.0% and 15.9%), and Spain (29.4% and 13.1%) [38]. In seven Latin American countries, a study of 1561 pneumococcal isolates collected from 1997 to 2001 revealed a global rate of PNSP of 30.7%, ranging from 25% in Mexico to 2.8% in Venezuela. Resistance to penicillin (MIC ≥2 mg/L) and cefotaxime (MIC ≥4 mg/L) was found in 11.9% and 0.4% of isolates, respectively [39]. Southern and eastern countries of the Mediterranean region reported that 26% (335) of the 1298 invasive isolates studied were PNSP, with the highest proportions being reported in Algeria (44%) and Lebanon (40%) [40].

The European Antimicrobial Resistance Surveillance System (EARSS), an international network of national surveillance systems that has been collecting antimicrobial susceptibility testing data on invasive *S. pneumoniae* isolates since 1999, also reported that most northern European countries had levels of PNSP below 5%, whereas southern and eastern European countries had PNSP levels above 25% [41]. In 2008, 1152 (10%) of the 11 584 invasive *S. pneumoniae* isolates reported to the EARSS by 32 countries were PNSP [41]. Among the northern European countries, only in Finland and Ireland had the frequency of PNSP risen significantly from 2005 to 2008 (7% (37/525) vs. 11% (71/642), and 11% (43/397) vs. 23% (101/441), respectively). In contrast, four countries with the highest levels of PNSP in the early 2000s (France, Spain, Belgium, and Israel) showed significantly decreasing rates of PNSP over the subsequent years.

Data from the Spanish Reference Laboratory for Pneumococci are shown in Fig. 1. A total of 25 166 invasive pneumococci were received during the period 1979–2008. Rates of invasive PNSP isolates progressively increased from 6% in 1979 to 44.4% in 1989 ($p < 0.001$), and the proportion of resistant isolates (MIC ≥2 mg/L) increased from 0% to 15.4% during this period ($p < 0.001$). During the period 1990–1998, the proportion of PNSP plateaued, oscillating between 34.5% and 43.7%. In the last decade, rates of PNSP progressively decreased from 33.9% in 1999 to 22.3% in 2008 ($p < 0.001$). This decline was especially marked in 2005–2008, and was associated with the implementation of PCV7 for children (Table 1). Moreover, among 1397 pneumococcal isolates from cerebrospinal fluid, non-susceptibility rates for cefotaxime (MIC ≥1 mg/L) decreased from 21.7% in 2000 to 10.5% in 2008 [16].

In a study performed in 33 US medical centres, the rate of ceftriaxone non-susceptibility (MIC ≥2 mg/L) decreased from 14.4% among 1531 pneumococcal isolates collected in 1999–2000 to 5.9% among 1647 pneumococcal isolates collected in 2004–2005 in 41 medical centres [42,43].

In spite of these alarming penicillin resistance levels, pneumococci with penicillin or cefotaxime/ceftriaxone MICs ≥4 mg/L are rarely described worldwide. If the revised CLSI breakpoints for parenteral penicillin are applied to pneumococci isolated from non-meningeal infections, more than 95% of invasive pneumococcal isolates collected worldwide are currently susceptible to penicillin and third-generation cephalosporins [32]. These antibiotics should continue to be first-line therapy for these infections [25–27,31].

Macrolides

The most frequently encountered mechanism of macrolide resistance in pneumococci is target site modification mediated by the *ermB*-encoded methylase, conferring resistance to all macrolides, lincosamides, and streptogramin B (MLS_B phenotype). The expression of this phenotype can be constitutive or inducible, becoming active only in the presence of inducing macrolides. The *ermB* gene is the major cause of macrolide resistance in most European countries,

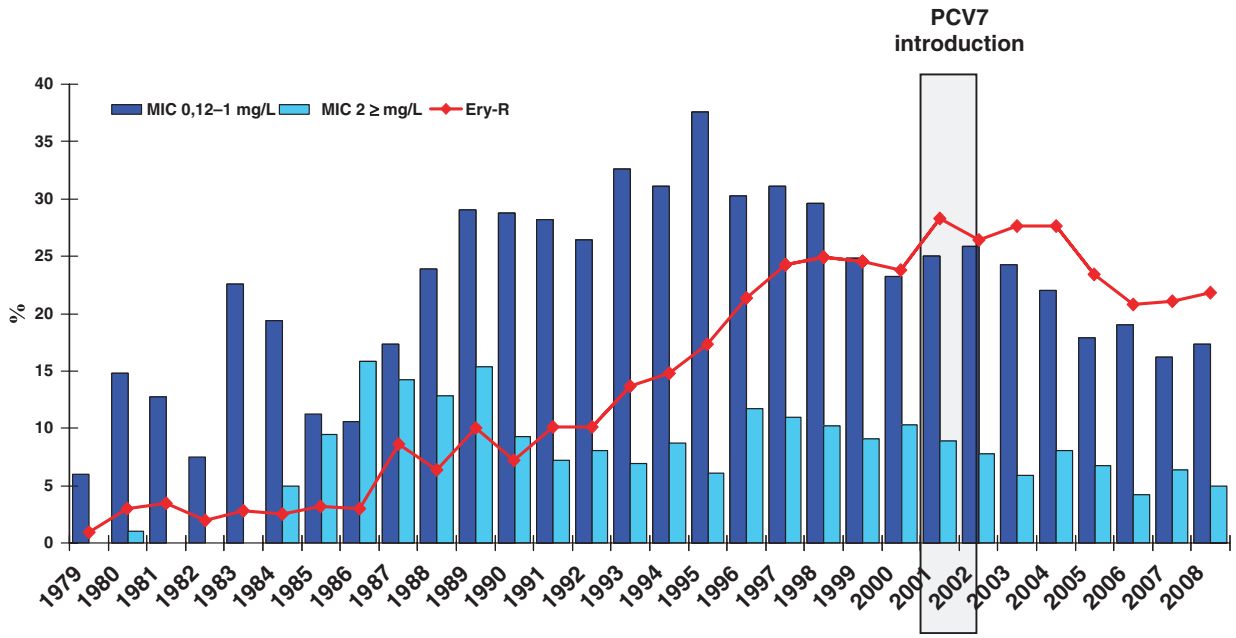

FIG. 1. Trends in penicillin and erythromycin resistance among 19 266 invasive pneumococcal isolates in patients of all ages in Spain (1979–2008). Ery-R, erythromycin resistance; PCV7, seven-valent pneumococcal conjugate vaccine.

TABLE 1. Trends in antibiotic resistance of 19 266 invasive pneumococcal isolates (all ages) tested at the Spanish Reference Laboratory for Pneumococci according to the old and the new breakpoints for penicillin of the CLSI

	Proportions of isolates resistant to antibiotics at indicated MIC breakpoints (%)													
Antibiotic breakpoints	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**Patients**	**p-value (1997 vs. 2008)**
Old breakpoints														
Penicillin-intermediate MIC of 0.12–1 mg/L	30.0	26.2	22.9	19.8	21.3	23.5	21.0	19.5	16.1	18.2	15.3	16.3	Adults	<0.001
	36.4	42.2	39.6	39.0	36.0	34.1	33.3	28.6	22.5	20.8	19.2	21.8	Children[a]	<0.001
Penicillin-resistant MIC of >2 mg/L	11.6	9.7	9.1	9.4	7.6	7.0	6.1	7.8	6.9	4.1	6.2	4.8	Adults	<0.001
	11.1	11.6	8.9	12.4	13.9	11.0	5.3	9.7	6.7	5.0	7.2	5.6	Children	0.03
New penicillin parenteral breakpoints (non-meningitis)														
Penicillin-intermediate MIC of 4 mg/L	0.6	0.1	0	0.3	0.7	0.4	0.4	1.0	0.7	0.1	0.7	0.4	Adults	0.54
	0.6	0	0	0	1.9	0.6	0.8	1.0	1.4	0.2	1.8	0.2	Children	0.42
Penicillin-resistant MIC of >8 mg/L	0	0	0	0	0	0	0	0	0	0	0	0	Adults and children	
New penicillin parenteral breakpoints (meningitis)														
Penicillin-resistant MIC of >0.12 mg/L	41.5	35.9	32.1	29.2	28.9	30.4	27.0	27.3	23.1	22.3	21.5	21.1	Adults	<0.001
	47.5	53.8	48.4	51.4	49.8	45.1	38.7	38.3	29.2	25.8	26.4	27.4	Children	<0.001
Erythromycin-resistant MIC of >1 mg/L	22.7	21.8	21.9	20.4	24.0	23.6	22.9	25.2	20.7	20.8	21.5	20.7	Adults	0.24
	32.1	39.3	39.6	39.5	42.7	35.3	42.9	34.4	30.0	20.8	22.3	26.6	Children	0.001[b]
Dual resistance	18.9	18.1	16.8	14.1	15.2	15.6	13.9	15.4	11.0	11.6	12.3	11.5	Adults	<0.001
	26.5	35.3	26.6	31.0	33.3	24.3	25.1	20.4	16.7	12.5	14.2	18.1	Children	0.02
													Total	
IPD episodes (adults)	721	729	1129	1013	914	1107	1140	1132	1513	1454	2039	2188	15 079	
IPD episodes (children)	162	173	192	210	267	317	375	392	510	457	613	519	4187	

IPD, invasive pneumococcal disease.
[a]Children: <14 years old.
[b]p <0.001 when comparing 1997–2004 vs. 2005–2008.

especially Belgium, France, Poland, Italy, and Spain [38]. The second most common mechanism is mediated by an efflux pump codified by the *mef* genes (*mefA*, *mefE*). This mechanism confers the M phenotype, with resistance to 14-membered and 15-membered ring macrolides, but not to lincosamides or streptogramins. The M phenotype isolates predominate in the USA, Canada, the UK, Germany, and Norway. Less common macrolide resistance could be due to mutations in the 23S rRNA and/or alterations in ribosomal proteins (L4 and L22).

The prevalence of macrolide resistance mechanisms differs considerably among countries, as shown in Table 2 [44–58]. The emergence of pneumococci that carry both *ermB* and *mefE* macrolide resistance genes is a cause for concern, especially in Asian countries, Russia, South Africa, and the USA [52,59]. Both genes (*ermB* and *mefE*), as well as the tetracycline resistance determinant (*tetM*), have been related to the composite element Tn*2010*, which is present in most multidrug-resistant isolates of serotype 19A of clonal complex 320 [18,60].

Macrolide-resistant pneumococci were first detected in 1967 in Canada, but rates of macrolide resistance among pneumococci remained low worldwide (<5%) during the 1970s [3]. By the early 1980s, the highest prevalence of erythromycin-resistant pneumococci was found among pneumococci isolated from hospital carriers in South Africa (63%), whereas, among invasive isolates, the prevalence of resistance was 8.3% in 1983. The majority of these strains showed multidrug resistance [3]. In France, macrolide resistance rates dramatically increased from 0% before 1976 to a peak of 26% in 1985 among clinical isolates at two hospitals in Paris [61]. In Spain, resistance rates increased from 0% in 1979–1980 to 9.4% in 1990 in a Barcelona hospital [9]. Thereafter, a rapid worldwide increase in the prevalence of macrolide resistance associated with an increase in macrolide consumption, especially of long-acting macrolides such as clarithromycin and azithromycin, was observed [38,62–64].

Global surveillance studies have shown that macrolide resistance rates increased during the 1990s. The Alexander Project gave a global rate of macrolide resistance of 16.5–21.9% in 1996–1997, increasing up to 24.6% in 1998–2000 [36]. The PROTEKT study showed an overall rate of 31.0% in 1999–2000, increasing to 37.2% in 2003–2004, but there was marked geographical variability [37]. The highest rates (80%) were recorded among isolates collected in the Far East, followed by South Africa (54%) and southern Europe (37%), whereas resistance was lowest in Latin America (15%), Australia (18%), and northern Europe (18%). Macrolide resistance in Europe was notably high in isolates collected from Belgium (31.5%), Spain (33.5%), Hungary (39.4%), Italy (40.8%), Greece (51.4%), and France (55.6%).

The EARSS report for 2008 shows high variability in the proportion of macrolide resistance in Europe. Whereas northern European countries, the Czech Republic and Bulgaria reported resistance rates below 5%, Italy, Turkey, France, Hungary and Cyprus reported resistance rates above 25% [41].

In Spain, overall macrolide resistance rates among invasive pneumococci received at the Spanish Reference Laboratory for Pneumococci remained below 5% until 1986, but thereafter

TABLE 2. Distribution of *ermB* and *mefA* genes among macrolide-resistant pneumococci from different countries

Country	Period	Samples	No. of. isolates (% erythromycin-resistant)	No. of erythromycin-resistant isolates studied	Macrolide resistance genes (%)				Major serotypes	Major genotypes	Ref.
					Positive for *ermB*	Positive for *mefA*	Positive for *ermB* and *mefA*	Negative for *ermB* and *mefA*			
Germany	1992–2004	Invasive	3845 (11.2)	430	35.6	63.5	0	0	14, 6B	CC9, CC273, CC124	[44]
UK	1998–2000	Invasive and non-invasive	1595 (5.0)	62	21.0	75.8	3.2	0	14	CC9	[45]
Norway	2001–2002	Invasive and non-invasive	2200 (2.7)	60	38.3	60.0	0	1.7	14, 6B, 19F	CC9	[46]
Belgium	1999–2000	Invasive and non-invasive	637 (36.6)	233	89.7	6	3.5	1[a]	–	–	[47]
Italy	2001–2003	Invasive	444 (34.9)	155	61.3	36.1	1.9	0.7	14, 19F 6B	CC9	[48]
France	2001–2003	Invasive and non-invasive	443 (46.1)	202	98.5	1.5	0	0	–	–	[49]
Finland	2002–2006	Invasive	3571 (16–28)	223	30.0	56.0	1.0	13.0	14, 9V, 6B, 19A	CC156 CC271	[50]
Spain	2004	Invasive and non-invasive	360 (34.7)	125	82.4	12.8	4.8	0	19F, 19A, 6B, 14, 23F	CC63,CC88 CC81, CC156	[51]
Russia	2003–2005	Invasive and non-invasive	833 (11.1)	76	50.0	19.7	30.3	0	23F, 6B, 19F, 6A	–	[52]
Korea	2000–2004	Invasive and non-invasive	–	251	54.6	14.7	30.7	0	19F, 23F, 19A, 14	–	[53]
Japan	2003–2005	Invasive and non-invasive	156 (81.4)	127	61.4	29.1	8.7	0.8	3, 19F, 23F	–	[54]
Hong Kong	1998–2001	Invasive and non-invasive	102 (76.5)	78	24.4	66.7	8.9	0	19F, 23F, 14	CC236	[55]
Vietnam	1998–2001	Invasive and non-invasive	60 (91.6)	55	45.3	5.7	49.1	0	19F, 23F	CC236	[55]
China	1998–2001	Invasive and non-invasive	86 (75.6)	65	76.9	3.1	20.0	0	19F, 23F	CC236	[55]
USA	2005–2006	Invasive and non-invasive	6747 (35.3)	2381	18.8	53.8	24.1	1.7	–	–	[56]
Canada	1998–2004	Invasive and non-invasive	–	865	42.9	46.7	5.8	4.6	–	–	[57]
South Africa	2000–2005	Invasive	15 982 (12)	260	57.0	27.0	15.0	1.0	14, 23F, 19F	CC236, CC9	[58]

[a]One isolate contained an erm (A) subclass erm (TR) gene, and the other contained an A2058G mutation in domain V or the 23S rRNA gene.

increased to 28% in 2001 and decreased to 21.8% in 2008, as shown in Fig. 1 [16]. After the introduction of PCV7 for children in June 2001 in Spain, a significant decline in macrolide resistance among invasive paediatric isolates (42.9% in 2003 vs. 20.8% in 2006, $p < 0.001$) was observed (Table 1), in agreement with previous reports [13,64]. In contrast, erythromycin resistance rates among invasive pneumococci isolated from adult patients remained stable from 1997 to 2008, fluctuating from 21% to 25% (Table 1) [16].

Several studies have associated macrolide resistance with therapy failure [65]. This argues against the empirical use of macrolides for treatment of pneumococcal pneumonia in countries with high rates of macrolide-resistant pneumococci.

Dual Non-susceptibility to Penicillin and Erythromycin

Over the 1990s, an increase in the proportion of pneumococcal isolates with combined non-susceptibility to penicillin and erythromycin was observed. This increase was related to the spread of classic penicillin-resistant clones that have acquired determinants of macrolide resistance, mostly carried by transposons of the Tn*916* family [51,66]. Data from the Alexander Project revealed a dramatic increase in the prevalence of combined resistance from 1.8% in 1992 to 32.7% in 2001 in France, from 3.7% to 17.0% in Spain, and from 3.2% to 15.3% in the USA [36].

The last EARSS report noted that the overall rate of dual non-susceptibility remained below 5% in 2008 in Europe. Although the northern European countries reported the lowest dual non-susceptibility levels, a decrease in the level of dual non-susceptibility from 2005 to 2008 was observed in Belgium (9% vs. 6%) and France (32% vs. 25%). Of concern is the increase observed over the last 4 years in the level of dual non-susceptibility in Ireland (from 3% to 12%), Hungary (from 13% to 21%), and Turkey (from 10% to 23%) [41].

As Table 1 shows, in Spain the rate of combined penicillin and erythromycin resistance among paediatric isolates fluctuated between 24% and 35% in 1997–2003, and thereafter showed a significant and progressive decline until it reached 11.5% in 2008 ($p < 0.05$). Among adult isolates, the rate of combined resistance also declined progressively, from 18.9% in 1997 to 11.5% in 2008 ($p < 0.05$) [16].

Quinolones

The main mechanism of resistance to fluoroquinolones in *S. pneumoniae* is point mutation producing amino acid

changes in the quinolone resistance-determining regions of the subunits of DNA topoisomerase IV ($ParC_2ParE_2$) and DNA gyrase ($GyrA_2GyrB_2$). However, resistance can also be acquired by intraspecific recombination or by interspecific recombination with streptococci of the *mitis* group [67–69].

The new, or respiratory, fluoroquinolones such as levofloxacin and moxifloxacin have enhanced activity against pneumococci when compared with the older ones (ciprofloxacin), and have become therapeutic alternatives for the treatment of community-acquired pneumonia in adults, because their spectrum of activity includes *S. pneumoniae*, *Legionella pneumophila*, and other atypical pathogens. Increased use of these antimicrobials has been associated with the emergence of resistance in *S. pneumoniae* in both Canada and Spain [70,71].

A multicentre study performed in Europe in 2004–2005 involving community-acquired respiratory tract infections [72] showed a low level of quinolone resistance in the majority of European countries, with the exception of Poland (4.4%), Finland (6.6%), and Italy (7.2%). Higher rates were also detected in some Asian countries [73], as well as recently in Canada (7.3% in 2006) [74]. In this country, the increase in ciprofloxacin resistance observed between 1998 (0.6%) and 2006 (7.3%) was strongly associated with an increase in fluoroquinolone consumption [74]. In Spain, two nationwide surveillance studies performed in 2002 and 2006 showed a stable rate of ciprofloxacin resistance (2.6% and 2.3%, respectively) [68,69].

There are several reports of treatment failure with the use of quinolones in the treatment of pneumococcal infection caused by fluoroquinolone-non-susceptible isolates. The risk factors identified in these reports were previous fluoroquinolone use, chronic obstructive pulmonary disease, hospitalization, and living in nursing homes [73]. However, once patients with these risk factors were excluded, the efficacy of levofloxacin in the treatment of pneumococcal pneumonia was proven [75]. To avoid therapeutic failures, it is very important to detect strains with first-step mutations, which usually have low-level ciprofloxacin resistance, with MICs of 4–8 mg/L, and frequently appear to be levofloxacin-susceptible, with MICs of 1–2 mg/L [68,69]. These strains could become highly resistant under selective fluoroquinolone pressure. In our experience, the ciprofloxacin breakpoint of 4 μg/mL, suggested by Chen *et al.*, is the best marker for detecting strains with first-step mutations [68–70]. These strains, wrongly identified as susceptible, accounted for 2.1% of 665 pneumococcal isolates collected in 2003 during the Canadian Respiratory Organism Susceptibility Study [76].

Antibiotic Resistance and Serotypes/Genotypes

The worldwide increase in antibiotic resistance in *S. pneumoniae* has been related to the spread of several pneumococcal serotypes (6A, 6B, 9V, 14, 15A, 19F, 19A, and 23F), the so-called 'paediatric serotypes'. In the USA, the CDC's Active Bacterial Core surveillance reported that 24% of 3475 invasive pneumococcal isolates collected in 1998 were PNSP. Seven serotypes (6A, 6B, 9V, 14, 19A, 19F, and 23F) accounted for 91% of all PNSP [64]. After the introduction of PCV7 in 2000 in the USA, a dramatic fall in the incidence of invasive pneumococcal disease (IPD) was observed in children ≤5 years of age and adults ≥65 years of age, associated with a decrease in IPD caused by PCV7 serotypes [13,14]. However, an increase in IPD caused by non-PCV7 serotype 19A was observed in all age groups: from 2.6% in 1998–1999 to 47.2% in 2006–2007 in children ≤5 years of age; from 2.9% to 16.6% in adults 18–64 years of age; and from 3.7% to 14.9% in adults ≥65 years of age [14].

In Spain, an increase in the prevalence of combined penicillin and erythromycin resistance among invasive pneumococci was associated with antibiotic consumption in the 1980s and 1990s [16]. Data from the Spanish Reference Laboratory for Pneumococci demonstrated that this increase was related to the rise in prevalence of PCV7 serotypes 6B, 9V, 14, 19F, and 23F, accounting for 76.6% of PNSP in 1979–1985 and 88% of PNSP in 1998–2000 [16]. The high prevalence of penicillin resistance (36.1%) observed among 5697 invasive pneumococcal isolates collected in Spain in the pre-PCV7 period (1997–2001) was reversed to 22.4% in 2007–2008 (n = 5465) ($p < 0.001$), when the PCV7 dose distribution increased. On comparison of these two periods, the proportions of four serotypes significantly decreased ($p < 0.001$) among penicillin-resistant strains—serotypes 6B (16.7% vs. 0%), 9V (15.4% vs. 9.1%), 19F (13.7% vs. 7.3%), and 23F (12.6% vs. 3.8%)—whereas the proportion of serotype 14 was similar in both periods (29.6–26.4%). The proportion of non-PCV7 serotypes among PNSP increased from 12.0% in 1997–2001 to 49.5% in 2007–2008, owing to, in particular, significant increases in the proportions of serotypes 19A (3.3–24.5%) and 24F (0.1–7.6%) [16].

The drug-resistant serotypes belong to a small number of pneumococcal clones whose nomenclature is standardized by the Pneumococcal Molecular Epidemiology Network (http://www.sph.emory.edu/PMEN/) [77]. The most important pneumococcal clones involved in the global spread of antibiotic resistance in the 1980s and 1990s were Spain^{23F}-ST81,

Spain^{6B}-ST90, Spain^{9V}-ST156, England^{14}-ST9, Taiwan^{19F}-ST236, Taiwan^{23F}-ST242, Poland^{6B}-ST315, Sweden^{15A}-ST63, and Colombia^{23F}-ST338. Although Spain^{23F}-ST81 and Spain^{6B}-ST90 were well-established clones in the 1980s and 1990s, their prevalence decreased after the introduction of PCV7 [78,79]. Spain^{9V}-ST156 (serotypes 9V and 14) has been an important cause of IPD in many countries, before and after PCV7 introduction, and usually shows resistance to penicillin and co-trimoxazole [15,78,79]. The prevalence of England^{14}-ST9, associated with the spread of macrolide resistance (*mefA*) in the 1990s in the USA, Canada, the UK, Germany, Norway, Greece, and Italy, decreased significantly after the introduction of PCV7 [44,48,80,81]. The high rate of penicillin and macrolide resistance detected in Asia and the USA was partially related to the spread of Taiwan^{19F}-ST236 [55,82]. The increasing prevalence of the multidrug-resistant serotype 19A observed in the USA is due to the spread of CC320, a double-locus variant of Taiwan^{19F}-ST236 [17]. This serotype 19A clone has also been reported in Asia and Europe [18,19]. However, in Europe, the major clone of serotype 19A is ST276, related to Denmark^{14}-ST230 [18,20–22,78].

Antimicrobial use and Antimicrobial Resistance

The occurrence of drug-resistant pneumococci has been associated with a variety of factors, antibiotic consumption being one of the most important [25]. Resistance selection has mainly occurred in pneumococci colonizing or infecting children. The frequency of children as carriers, and their exposure to antibiotics, favours the selection of drug-resistant strains [3,4].

Several studies have shown geographical differences in the prevalence of antimicrobial resistance in Europe, with lower rates in the northern countries than in the southern countries [41]. Outpatient antibiotic consumption in the USA was recently compared with data from the European Surveillance Antimicrobial Consumption. Northern European countries reported the lowest antibiotic use, and southern European countries (Greece, France and Italy) the highest, similar to that of the USA. The Netherlands was the country with the lowest reported consumption [12].

Conclusions

S. pneumoniae continues to be an important cause of invasive diseases, especially in children and the elderly. The high prevalence of multiresistant pneumococci in the 1980s and 1990s declined after the introduction of the PCV7 in the 2000s. However, the emergence of drug-resistant non-PCV7 serotypes/genotypes in the late 2000s emphasizes the importance of prudent use of antibiotics with the aim of preventing their spread. Continued surveillance of antimicrobial resistance, serotypes and genotypes is crucial in providing information on the emergence of multiresistant clones. These data are also essential for the development of appropriate guidelines for empirical therapy of pneumococcal infections and for the inclusion of emergent serotypes in the new generation of conjugate vaccines.

Transparency Declaration

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