

Decline in antibiotic resistance and changes in the serotype distribution of *Streptococcus pneumoniae* isolates from children with acute otitis media; a 2001–2011 survey by the French Pneumococcal Network

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Abstract

Streptococcus pneumoniae is an important cause of acute otitis media (AOM). The aim of this study was to evaluate trends in antibiotic resistance and circulating serotypes of pneumococci isolated from middle ear fluid of French children with AOM during the period 2001–2011, before and after the introduction of the PCV-7 (2003) and PCV-13 (2010) vaccines. Between 2001 and 2011 the French pneumococcal surveillance network analysed the antibiotic susceptibility of 6683 *S. pneumoniae* isolated from children with AOM, of which 1569 were serotyped. We observed a significant overall increase in antibiotic susceptibility. Respective resistance (I+R) rates in 2001 and 2011 were 76.9% and 57.3% for penicillin, 43.0% and 29.8% for amoxicillin, and 28.6% and 13.0% for cefotaxime. We also found a marked reduction in vaccine serotypes after PCV-7 implementation, from 63.0% in 2001 to 13.2% in 2011, while the incidence of the additional six serotypes included in PCV-13 increased during the same period, with a particularly high proportion of 19A isolates. The proportion of some non-PCV-13 serotypes also increased between 2001 and 2011, especially 15A and 23A. Before PCV-7 implementation, most (70.8%) penicillin non-susceptible pneumococci belonged to PCV-7 serotypes, whereas in 2011, 56.8% of penicillin non-susceptible pneumococci belonged to serotype 19A. Between 2001 and 2011, antibiotic resistance among pneumococci responsible for AOM in France fell markedly, and PCV-7 serotypes were replaced by non-PCV-7 serotypes, especially 19A. We are continuing to assess the impact of PCV-13, introduced in France in 2010, on pneumococcal serotype circulation and antibiotic resistance.

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Introduction

Acute otitis media (AOM) is the most common bacterial infection in childhood and one of the most frequent indications

for antibiotic treatment of young children [1]. Among the pathogens involved in this infection, *Streptococcus pneumoniae* is the most frequently isolated, together with *Haemophilus influenzae* [2]. In recent decades the emergence and spread of penicillin non-susceptible pneumococci (PNSP) has limited the therapeutic options in AOM, as penicillin non-susceptibility is associated with reduced activity of other oral β -lactams [3–5]. In France, a steady increase in the percentage of PNSP isolates in both invasive and non-invasive infections has been observed since the 1990s, reaching 55.4% overall in 2001 and 77% among paediatric AOM isolates [6]. In 2001 the French national health

insurance system initiated an extensive programme to avoid inappropriate antibiotic use among outpatients [6]. The heptavalent pneumococcal conjugate vaccine (PCV-7) was recommended for at-risk children under 2 years of age in 2002, and for all children in 2006 [7]. PCV-7 vaccine coverage increased slowly: at least one dose was received by 62% of children under 2 years old in 2006, rising to 69% in 2007, 85% in 2008 and 90% in 2009 [7]. PCV-13 gradually replaced PCV-7 over a 3-month period between June and early September 2010 [8]. In 2011, the full primary vaccination coverage rate was 91.7% among 9-month-old infants [Public Health Institute unpublished data, available at <http://www.invs.sante.fr/Dossiers-thematiques/Maladies-infectieuses/Maladies-a-prevention-vaccinale/Couverture-vaccinale/Donnees/Pneumocoque>].

The aim of this study was to evaluate, through the French pneumococcal surveillance network (Observatoires Régionaux du Pneumocoque, Centre National de Référence des Pneumocoques, Institut de Veille Sanitaire), changes in antibiotic resistance and circulating serotypes of *S. pneumoniae* isolated from middle ear fluid of children (0–16 years) with AOM over an 11-year period (2001–2011) following the introduction of PCV-7 and PCV-13.

Materials and Methods

Data and strain collection

Surveys were conducted every 2 years from January 2001 to December 2011 by the French pneumococcal network, which includes 396 public (75%) and private (25%) laboratories in 23 regions, as previously described [6]. All isolates from middle ear fluid of children aged 0–16 years were included in the study. Only one strain was considered per AOM episode and per child.

Susceptibility testing

Minimal inhibitory concentrations (MICs) of penicillin G, amoxicillin and cefotaxime were determined with the agar dilution method according to the guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie (www.sfm.org). Three quality-control strains (R6 (WT), ATCC49619 and

CNRP32475) provided by the French National Reference Centre for Pneumococci (CNRP) were included. Erythromycin and cotrimoxazole susceptibility was determined using the disk diffusion method, the VITEK2[®] kit or the ATB-Pneumo[®] kit. The results were interpreted according to 2009 French guidelines, with the following breakpoints: 0.064–2 mg/L for penicillin, and 0.5–2 mg/L for amoxicillin and cefotaxime. PNSP were defined as strains with a penicillin MIC >0.064 mg/L.

Serotyping

A subset of isolates was selected for serotyping by random systematic sampling. Serotyping was performed by the National Pneumococcal Reference Centre, using the capsular swelling method with commercial antisera (Statens Serum Institute, Copenhagen, Denmark). The panel of antisera covered the 92 known serotypes.

Statistical analysis

Data were monitored and validated with CAPTURE SYSTEM software. SAS software (version 9.1.3., SAS Institute, Cary, NC, USA) was used for statistical analysis, using the chi-square test for trends. Values of $p < 0.05$ were considered to denote significant differences.

Results

We studied 6683 isolates recovered from middle ear fluid of 6683 children with AOM. The annual number of pneumococcal isolates declined gradually during the study period, from 1694 in 2001 to 1378 in 2003, 1159 in 2005, 998 in 2007, 922 in 2009 and 560 in 2011. The children's mean age \pm SD was 1.9 ± 2.0 years. A total of 2189 isolates (32.8%) were recovered from children less than 12 months old, and 2314 (34.6%) from children between 1 and 2 years old.

Antimicrobial resistance

Trends in resistance (I+R) to penicillin, amoxicillin, cefotaxime, erythromycin and cotrimoxazole among the 6683 isolates are shown in Table 1. A significant decrease in overall antibiotic

TABLE 1. Resistance of pneumococcal isolates from children's middle ear fluid to five antibiotics; France 2001–2011

	2001 (n = 1694)	2003 (n = 1378)	2005 (n = 1159)	2007 (n = 998)	2009 (n = 922)	2011 (n = 560)	p
PEN I+R, n (%)	1302 (76.9)	959 (69.6)	736 (63.5)	604 (60.5)	581 (63.0)	321 (57.3)	<10 ⁻⁵
AMX I+R, n (%)	729 (43.0)	572 (41.5)	405 (34.9)	298 (29.9)	338 (36.7)	167 (29.8)	<10 ⁻⁵
CTX I+R, n (%)	485 (28.6)	369 (26.8)	259 (22.3)	164 (16.4)	192 (20.8)	73 (13.0)	<10 ⁻⁵
ERY ^a I+R, n (%)	1303 (77.6)	1016 (74.1)	708 (61.9)	585 (59.3)	541 (58.7)	310 (55.4)	<10 ⁻⁵
SXT ^b I+R, n (%)	862 (53.8)	572 (47.7)	386 (39.3)	306 (31.7)	419 (45.4)	130 (23.2)	<10 ⁻⁵

Abbreviations: AMX, amoxicillin; CTX, cefotaxime; ERY, erythromycin; PEN, penicillin G; SXT, cotrimoxazole.

Note: p values are from a χ^2 -test for trends

^aFor erythromycin (ERY), the number and percentage of I+R isolates were calculated for 1680, 1370, 1143 and 986 isolates in 2001, 2003, 2005 and 2007, respectively

^bFor cotrimoxazole (SXT), the number and percentage of I+R isolates were calculated for 1602, 1200, 981 and 968 isolates in 2001, 2003, 2005 and 2007, respectively.

resistance (% of I+R) was observed ($p < 10^{-5}$); 76.9% of the strains were PNSP in 2001 compared with 57.3% in 2011. Concerning the two other β -lactams tested, 43.0% and 28.6% of the strains were non-susceptible (I+R) to amoxicillin and cefotaxime, respectively, in 2001, compared with 29.8% and 13.0% in 2011. The largest decreases concerned cotrimoxazole (53.8% in 2001 versus 23.2% in 2011) and erythromycin (77.6% in 2001 versus 55.4% in 2011).

The most common resistance phenotypes are shown in Table 2. In 2001, 15.1% of the isolates were susceptible to all the antibiotics tested, compared with 34.5% in 2011. No pneumococcal strains susceptible to penicillin but exhibiting reduced susceptibility to amoxicillin or cefotaxime were found. Multidrug resistance, defined as resistance to at least three antibiotic classes [9], was found in 60.3% of isolates in 2001, compared with 31.8% in 2011. It is particularly noteworthy that the proportion of strains resistant to penicillin + amoxicillin + erythromycin + cotrimoxazole fell from 31.7% in 2001 to 7.3% in 2011. Most multidrug-resistant strains showed diminished susceptibility to β -lactams and resistance to macrolides.

The resistance was explored as a function of patients' age. The proportion of PNSP isolated from AOM in children under 12 months of age was near 30%, without major variation between the studied years. The proportion of PNSP reached 70% for those aged between 13 and 24 months. For children from the age of 24 months the PNSP rate was almost 50%. In all the cases, there were no major variations between the studied years (data not shown).

Serotype distribution

A total of 1569 *S. pneumoniae* isolates (23.5%) were serotyped: 341 in 2001, 367 in 2003, 200 in 2005, 308 in 2007, 201 in 2009, and 152 in 2011. Between 2001 and 2011, we observed a

significant decline in PCV-7 serotypes (63.0% vs 13.2%, $p < 0.05$) (Fig. 1). The prevalence of serotype 19F fell from 16.1% to 9.9%; serotype 14 from 19.1% to 1.3%; serotype 6B from 10.6% to 0.0%; serotype 23F from 10.9% to 1.3%; serotype 9V from 4.7% to 0.0% ($p < 0.01$); serotype 18C from 1.2% to 0.7%, and serotype 4 from 0.6% to 0.0%. The prevalence of the six additional serotypes covered by PCV-13 rose between 2001 (27.9%) and 2009 (70.6%, $p < 0.05$), then fell in 2011 (49.3%) ($p < 0.05$). Serotype 19A represented 19.6% of isolates in 2001 and 51.2% in 2009, falling to 38.2% in 2011. Finally, some non-PCV-13 serotypes became more prevalent between 2001 and 2011, particularly serotype 15A (2001: 0.3%; 2011: 6.6%) and serotype 23A (2001: 0.6%; 2011: 3.9%).

Trends in PNSP serotypes

In 2001, before the introduction of PCV-7 in France, most PNSP (70.8%) belonged to PCV-7 vaccine serotypes (Fig. 2). The proportion of PNSP declined after 2003, mainly as the result of a fall in PCV-7 serotypes: 66.8% of PNSP belonged to PCV-7 serotypes in 2003, compared with 52.6% in 2005, 26.3% in 2007, 8.9% in 2009, and 17.9% in 2011. Among non-PCV-7 serotypes, serotype 19A was most prevalent, rising steadily until 2009, when it represented a large majority of PNSP: 23.6% in 2001, 29.0% in 2003, 37.1% in 2005, 62.5% in 2007, 76.0% in 2009, and 56.8% in 2011. Concerning the other non-vaccine serotypes, isolates belonging to serotypes 15A and 35B were the most resistant to penicillin G.

Discussion

This is the first nationwide long-term follow-up study of trends in *S. pneumoniae* resistance and serotype distribution in French children with AOM. As expected, routine paediatric

TABLE 2. Most common resistance phenotypes of *Streptococcus pneumoniae* isolates from children's middle ear fluid; France 2001–2011

I+R phenotypes	2001 (n = 1598)	2003 (n = 1195)	2005 (n = 980)	2007 (n = 985)	2009 (n = 922)	2011 (n = 560)	p
No resistance marker	241 (15.1)	248 (20.8)	267 (27.2)	316 (32.1)	293 (31.8)	193 (34.5)	<10 ⁻⁵
PEN	36 (2.3)	21 (1.8)	33 (3.4)	26 (2.6)	23 (2.5)	22 (3.9)	<0.05
AMX	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NS
ERY	89 (5.6)	89 (7.4)	53 (5.4)	42 (4.3)	19 (2.1)	29 (5.2)	<0.001
SXT	12 (0.8)	17 (1.4)	20 (2.0)	25 (2.5)	23 (2.5)	12 (2.1)	<0.001
PEN+AMX	8 (0.5)	6 (0.5)	17 (1.7)	15 (1.5)	20 (2.2)	14 (2.5)	<10 ⁻⁵
PEN+ERY	211 (13.2)	135 (11.3)	116 (11.8)	138 (14.0)	90 (9.8)	102 (18.2)	NS
PEN+SXT	15 (0.9)	6 (0.5)	13 (1.3)	12 (1.2)	15 (1.6)	5 (0.9)	NS
AMX+ERY	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NS
AMX+SXT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NS
ERY+SXT	21 (1.3)	19 (1.6)	20 (2.0)	9 (0.9)	6 (0.7)	5 (0.9)	NS
PEN+AMX+ERY	145 (9.1)	124 (10.4)	109 (11.1)	137 (13.9)	106 (11.5)	108 (19.3)	<10 ⁻⁵
PEN+AMX+SXT	34 (2.1)	19 (1.6)	23 (2.3)	6 (0.6)	6 (0.7)	4 (0.7)	<0.001
PEN+ERY+SXT	278 (17.4)	171 (14.3)	116 (11.8)	126 (12.8)	114 (12.4)	25 (4.5)	<10 ⁻⁵
AMX+ERY+SXT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NS
PEN+AMX+ERY+SXT	507 (31.7)	337 (28.2)	193 (19.7)	133 (13.5)	206 (22.3)	41 (7.3)	<10 ⁻⁵

Abbreviations: AMX, amoxicillin; ERY, erythromycin; PEN, penicillin G; SXT, cotrimoxazole.
Note: Data are given as n (%); p values are from a χ^2 -test for trends

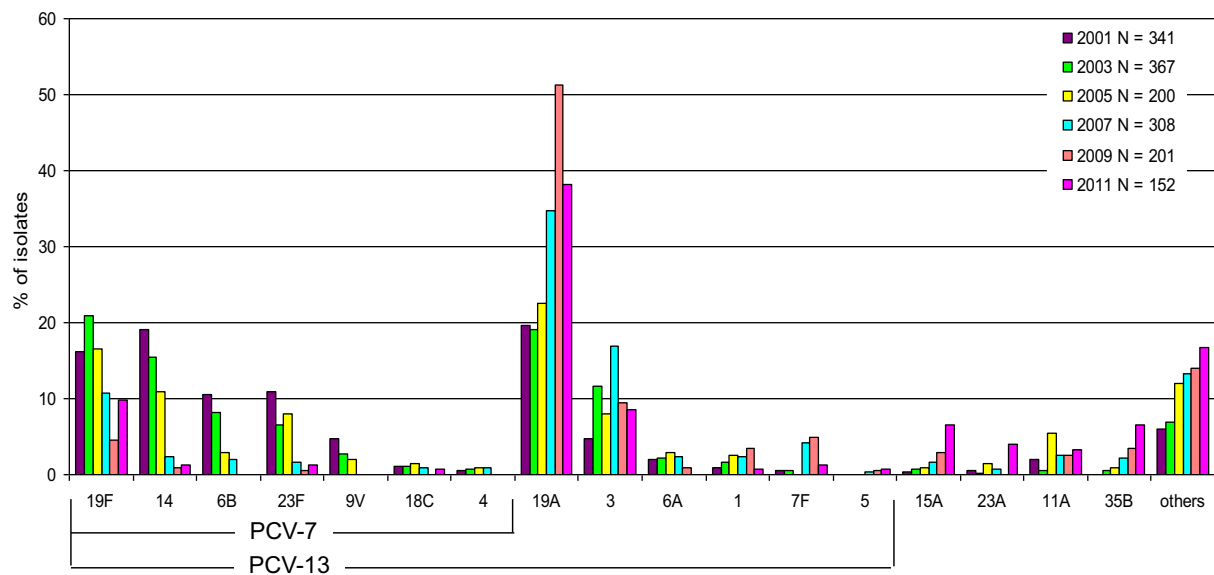


FIG. 1. Trends in *Streptococcus pneumoniae* serotypes isolated from middle ear fluid of French children with acute otitis media between 2001 and 2011.

immunization programmes with the PCV-7 vaccine, beginning in 2003, led to a decline in the proportion of cases due to PCV-7 serotypes. Similar results have been obtained in other countries such as the USA, where PCV-7 was licensed in 2000 and where several studies of AOM have shown a decline in PCV-7 serotypes in favour of non-vaccine serotypes [10–12]. In a recent Spanish study, Alonso *et al.* also observed that the prevalence of PCV-7 serotypes causing AOM declined sharply after the introduction of PCV-7 in 2001, from 62.4% in the 1999–2001 pre-vaccination period to 2.2% in the 2008–2010 late vaccination period [13]. Likewise, a decline of *S. pneumoniae* isolation has been observed in children with chronic sinusitis, as described by Olarte *et al.*, with a substantial reduction of PCV-13 serotypes, predominantly serotype 19A [14].

According to Rodgers *et al.* [2], the most common serotypes causing AOM worldwide in the 1970–2008 period were 6A, 6B, 14, 19A, 19F, and 23F. In our study, serotype 6A represented only 0–3% of all isolates. However, we found that serotype 3, which was not mentioned by Rodgers *et al.*, became more prevalent between 2001 and 2011 (4.7% and 8.6%, respectively). Serotype 19A became the leading non-PCV-7 serotype, accounting for more than 50% of isolates in 2009.

Our results are consistent with those of Cohen *et al.* [7], who studied nasopharyngeal samples from French children with AOM before and after PCV-7 implementation. These authors found a marked reduction in vaccine serotype carriage after PCV-7 implementation. They also observed an increase in non-PCV-7 serotypes (notably 19A, the prevalence of which rose from 6% to 22%), and also in other non-vaccine serotypes such as 15A, 23A, 35B and 23B. Recently, Cohen *et al.* [8] showed

that in French children with AOM, the carriage of serotypes 19A and 7F was significantly lower in PCV-13-vaccinated patients than in those vaccinated only with PCV-7. These observations were further confirmed by Hau *et al.* [15], who reported that PCV-13 introduction in France led to a further reduction in nasopharyngeal carriage of the six additional serotypes included in this vaccine. In our study, serotype 19A increased gradually from 2001 to 2009 but declined markedly in 2011 (51.2% in 2009 versus 38.2% in 2011), probably due to the introduction of PCV-13 in 2010. Further studies are needed to confirm this relationship, and also the decline in the other additional serotypes included in PCV-13.

The relationship between antibiotic resistance and the pneumococcal serotype is well known [16]. Before PCV-7 introduction, the most common serotypes carried by children with AOM, and also the most commonly resistant serotypes, were the vaccine serotypes 6B, 9V, 14, 19F and 23F and the non-vaccine serotypes 6A and 19A [16]. The introduction of PCV-7 was associated with a decline in vaccine serotypes, and hence in antibiotic resistance. We found that the decline in PNSP over the study period coincided with an increase in the number of isolates susceptible to all the antibiotics tested (15.1% in 2011 versus 34.5% in 2001). From 2001 to 2005, most PNSP isolates belonged to PCV-7 serotypes, and the decline in these strains accounted for the decrease in antibiotic resistance. Most isolates belonging to serotype 19A, a non-PCV-7 serotype, were resistant to antibiotics, with a PNSP rate close to 90%. This decline in antibiotic resistance is expected to continue, as a result of the decline in serotype 19A, which is covered by PCV-13. Besides, a decrease in the level of

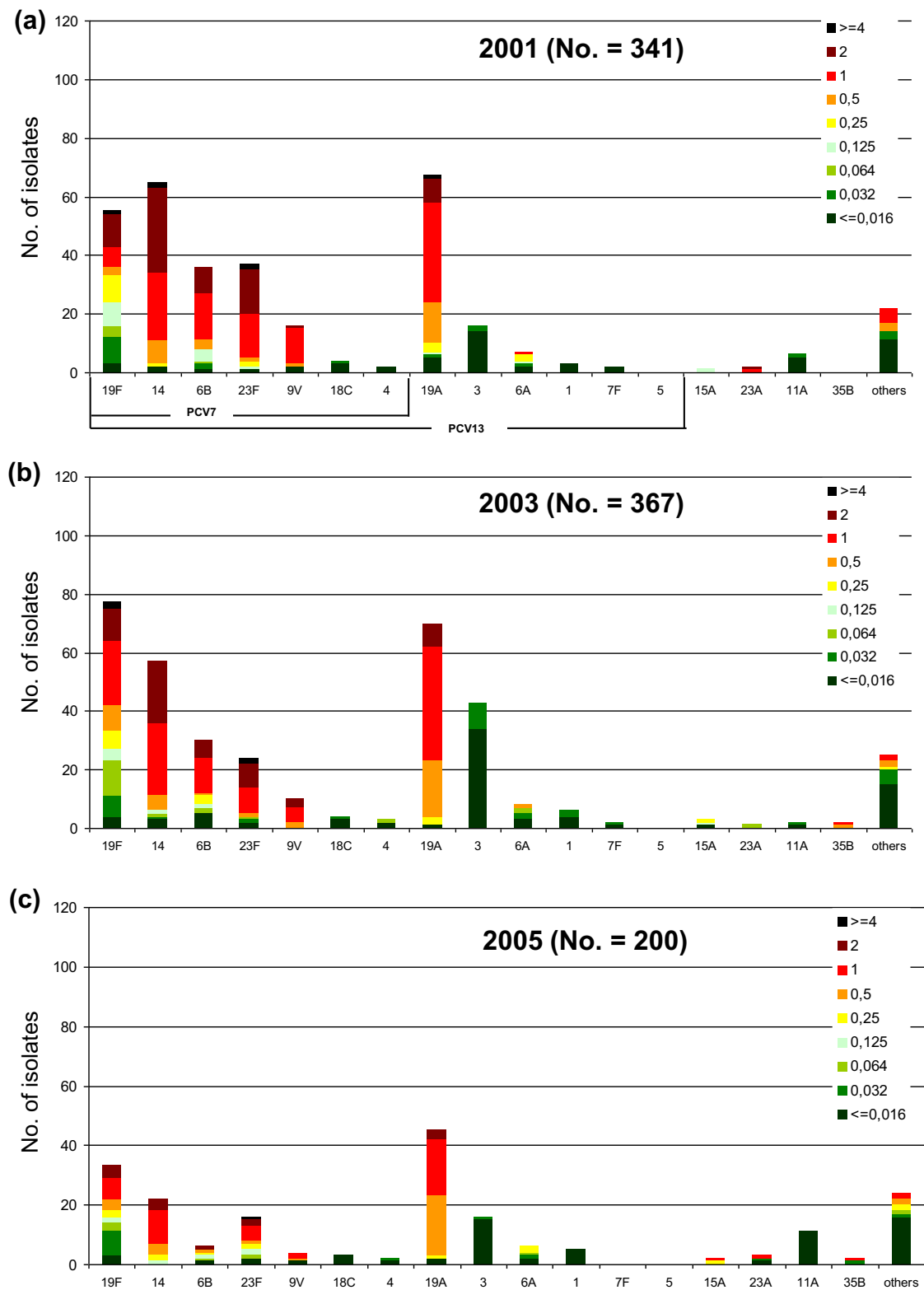


FIG. 2. Trends in the serotype distribution of *Streptococcus pneumoniae* isolated from middle ear fluid of French children with acute otitis media, according to their susceptibility to penicillin G. The colour scale highlights the penicillin minimum inhibitory concentration. Serotypes are sorted, from left to right, according to their inclusion in PCV-7 and PCV-13, and their non-inclusion in either vaccine. Each graph represents one year (a, 2001; b, 2003; c, 2005; d, 2007; e, 2009; f, 2011).

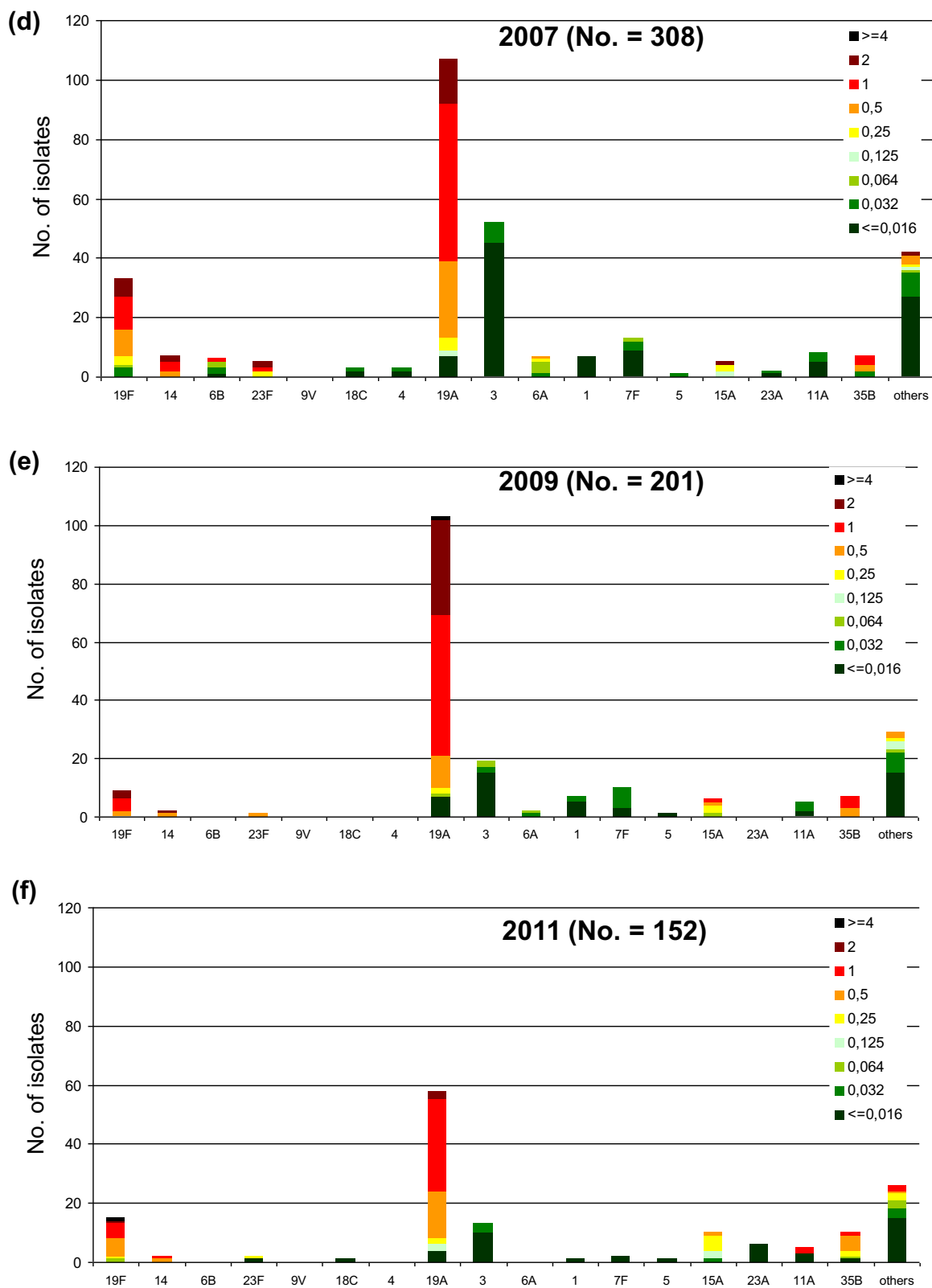


FIG. 2. (continued).

multidrug-resistant isolates has been observed (31.7% in 2001 to 7.3% in 2011), probably related to an association of multiple mechanisms of resistance in certain serotypes.

Levels of antibiotic resistance are directly proportional to antibiotic consumption in the community [16,17]. Multivariate analysis of data from 21 European countries in 2000–2005 demonstrated significant positive associations ($p \leq 0.01$) between PNSP rates and levels of penicillin consumption [18]. In France, overall community antimicrobial consumption fell from 33.0 defined daily doses per 1000 inhabitants in 2001 to 27.1 in 2004; broad-spectrum penicillin consumption fell by 20% and macrolide consumption by 39% [19]. Since then, a slight increase has been observed, reaching 28.7 defined daily doses per 1000 inhabitants in 2011 (data from the ANSM 2013 report <http://ansm.sante.fr>).

The number of *S. pneumoniae* strains recovered from middle ear fluid of children diagnosed with AOM fell from 1694 in 2001 to only 560 in 2011. This was probably due to PCV-7 vaccination, although a change in medical practices, and especially in the indications of tympanocentesis, cannot be ruled out. In France, tympanocentesis and bacteriological analysis are not usually performed at the first medical examination for AOM but are reserved for treatment failure, relapse or recurrence. However, even if practices did change, the decline in resistant pneumococci recovered from children with AOM remains remarkable.

In conclusion, the frequency of non-susceptible pneumococci isolated from middle ear fluid of children with AOM fell significantly in France between 2001 and 2011. This was mainly due to a decline in isolates belonging to PCV-7 serotypes, most of which were resistant to antibiotics. Implementation of PCV-7, followed in 2010 by PCV-13, was associated with a dramatic change in the epidemiology of pneumococcal AOM in France.

Transparency Declaration

The authors declare that they have no conflicts of interest.

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