Pneumococcal bacteraemia in Belgium (1994–2004): the pre-conjugate vaccine era

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Objectives: To analyse the evolution of antibiotic resistance and serotype distribution in pneumococcal bacteraemia before the introduction of the 7-valent pneumococcal conjugate vaccine (7PCV).

Methods: Serotyping and susceptibility testing for penicillin and erythromycin were performed on 11 163 blood isolates of Streptococcus pneumoniae collected between 1994 and 2004.

Results: Penicillin resistance rose from 4.7% in 1994 to 15.2% (P < 0.001) in 2000 and decreased thereafter to 9.7% (P < 0.001) in 2004. Erythromycin resistance rose from 20.4% in 1994 to 34.4% (P < 0.001 in 2001) and stabilized thereafter. Paediatric serogroups/serotypes (SGTs) (SGTs 6, 9, 14, 19 and 23; 47.4% of bacteraemic isolates), characterized by decreasing penicillin and stable erythromycin resistance, decreased by the end of the study period. Non-paediatric SGTs (SGTs 1, 5 and 7; 20.5% of bacteraemic isolates), characterized by temporal fluctuations, the absence of penicillin resistance and rising erythromycin resistance, increased significantly by the end of the study period. The age group 5–59 years was most affected by these changes. Compared with the age group <5 years, the age group ≥60 years has a relative risk of 7.6 (CI: 4–11.6; P < 0.001) of having a pneumococcal bacteraemia with SGT 3. The overall coverage rate of bacteraemic SGTs offered by the 7PCV is 81.9% in the <5 years age group with an additional coverage of 11.6% offered by the 13-valent pneumococcal conjugate vaccine (13PCV) in this age group (P < 0.001). The coverage of bacteraemic isolates offered by the 13PCV and 23-valent pneumococcal polysaccharide vaccine (23PPV) in the ≥60 years age group is 78.7% and 95%, respectively.

Conclusions: Although the 7PCV was not used in Belgium during the study period, the overall prevalence in paediatric SGTs decreased significantly. This may be linked to secular trends in SGTs not included in the 7PCV and/or herd effects at the international level. Overall penicillin resistance decreased as well and this may be due to a shift towards susceptible serotypes and/or a decrease in antibiotic use in our country. Antibiotic resistance and trends in SGT distribution will need further surveillance in order to assess 7PCV effects on pneumococcal epidemiology, to adapt future vaccine formulations and to target the population at risk.

Keywords: Streptococcus pneumoniae, antibiotic resistance, pneumococcal vaccination, epidemiology

Introduction

Streptococcus pneumoniae is a leading cause of bacteraemia, meningitis, pneumonia and upper respiratory tract infection worldwide.

The annual incidence of pneumococcal bacteraemia is estimated at 15–30 cases/100 000 population for all persons. Invasive pneumococcal disease (IPD) affects mostly children, the elderly and immunocompromised individuals with an estimated annual incidence of 45–90/100 000 in the elderly (≥65 years of age) and >150/100 000 in children under 2 years of age.1–4

Resistance of S. pneumoniae to the major classes of antibiotics (penicillins and macrolides) used to treat invasive
disease is rising in many countries. Introduction of resistant clones as well as de novo resistance, often due to horizontal transfer of DNA between streptococcal species, results in resistance.5–7

Because of the high incidence of pneumococcal disease and the problem of rising resistance, there is a need for adequate prevention of invasive disease and of transmission in risk groups. The 23-valent pneumococcal polysaccharide vaccine (23PPV) has been shown to prevent invasive disease in the elderly and in patients with chronic underlying conditions such as heart failure, chronic obstructive pulmonary disease and splenectomy.8,9 Because infants ≤ 2 years of age are unable to mount an adequate immunological response to polysaccharides, conjugate vaccines were introduced in this population to tackle the problem of pneumococcal disease and carriage. However, pneumococcal conjugate vaccines may be beneficial in other age groups as well.4,10,11

In this article, we describe the evolution of penicillin and erythromycin resistance and serogroup/serotype (SGT) distribution of blood isolates over an 11 year period (1994–2004) in Belgium for four different age groups (0–4, 5–19, 20–59 and ≥ 60 years) before the use of the 7-valent pneumococcal conjugate vaccine (7PCV) in children. Implications for the 7PCV and 13-valent pneumococcal conjugate vaccine (13PCV) formulations and the 23PPV are discussed.

Materials and methods

Blood isolates of S. pneumoniae

More than 90% of blood isolates of S. pneumoniae are sent to the national reference laboratory at the University Hospital Leuven by more than 100 Belgian laboratories, covering more than 50% of the Belgian population. Isolates are mailed to the reference laboratory on blood agar. Identification of S. pneumoniae is first confirmed in the reference laboratory by appearance of colonies, α-haemolysis and optochin susceptibility on blood agar.

Typing of S. pneumoniae isolates

The isolates are typed by phase-contrast microscopy using Neufeld’s reaction with 46 SGT sera obtained from the Statens Serum Institut (Copenhagen).

Susceptibility testing

Susceptibility to penicillin and erythromycin is tested by the standardized disc diffusion test on Mueller–Hinton agar according to the CLSI recommendations.12 Oxacillin (1 μg) discs are used to screen for strains with diminished susceptibility to penicillin.

For all isolates with inhibition zones ≤ 19 mm, the MICs of penicillin G (≤ 0.06 mg/L for fully susceptible strains, 0.12–1.0 mg/L for immediately resistant strains and ≥ 2 mg/L for highly resistant strains).

Vaccine formulations

The 7PCV includes conjugates derived from polysaccharides or oligosaccharides from types 4, 6B, 9V, 14, 18C, 19F and 23F. The 13PCV comprises additional serotypes 1, 3, 5, 6A, 7F and 19A. The 23PPV has all SGTs of the 13PCV except 6A plus the additional SGTs 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F and 33F.

In the analysis of the theoretical coverage of pneumococcal vaccines, vaccine serotypes (VTs; serotypes present in the vaccine formulation) and vaccine-related serotypes (VRTs; serotypes belonging to the same serogroup but not present in the vaccine formulation) were not differentiated.

Analysed dataset

Bacteraemic isolates of S. pneumoniae obtained in the period 1994–2004 were analysed for four age groups (0–4, 5–19, 20–59 and ≥ 60 years).

Statistical analysis

Comparisons between age groups and year of isolation by χ² or Fisher’s exact test when appropriate. A P value < 0.05 was considered significant. A Bonferroni correction was used on the P value for comparison of two variables between age groups or within time series.

Results

A total of 11 163 blood isolates of S. pneumoniae were examined in the national reference laboratory between 1994 and 2004.

Antibiotic resistance in S. pneumoniae bacteraemic isolates (Figure 1)

Penicillin resistance. The percentage of pneumococci not fully susceptible to penicillin increased significantly until the year 2000 and decreased significantly thereafter [1994, 4.7%; 2000, 15.2% (P = 0.001); and 2004, 9.7% (P = 0.001)]. These significant trends were present in all age groups except in the group of 5–19 years. The average penicillin resistance was significantly higher in the 0–4 (13.3% penicillin resistance) and ≥ 60 years age group (12.2% penicillin resistance) compared with the two other age groups (5–19 years, 5.2% penicillin resistance and 20–59 years, 7.3%; P = 0.001). Before 1996, 4.7% (1994) to 6.2% (1995) of isolates showed intermediate penicillin resistance (MIC between 0.12–1.0 mg/L) and no fully resistant isolates

Figure 1. Antibiotic resistance in S. pneumoniae bacteraemia (1994–2004). Squares, erythromycin (E) resistance; filled diamonds, penicillin (P) resistance (full and intermediate); open diamonds, fully penicillin resistant; circles, PE resistance.
(MIC > 1.0 mg/L) were detected. A significant rise followed by a significant decrease in the percentage of fully penicillin-resistant isolates was only present in the youngest and oldest age group. In children (0–4 years old), the proportion full penicillin resistance rose from 0% in 1996 to 48.3% in 1999 ($P = 0.003$) and decreased to 15.6% in 2004 ($P = 0.012$). In the elderly, the proportion of full penicillin resistance rose from 4.4% in 1996 to 43% in 2000 ($P = 0.001$) and decreased to 3.9% in 2004 ($P = 0.001$). SGTs 9, 14, 15 and 23 exhibited an average penicillin resistance of ≥10%.

**Erythromycin resistance.** The resistance to erythromycin rose from 20.4% in 1994 to 34.4% in 2001 ($P = 0.001$) and stabilized thereafter (32.8% in 2004, $P = NS$). The percentages of erythromycin resistance were significantly different between the age groups with 47.8%, 15.1%, 21.1% and 29.4% resistance in the age groups of 0–4, 5–19, 20–59 and ≥60 years, respectively ($P = 0.001$). A significant rise in erythromycin resistance was observed in the oldest age group (18.7% in 1994 to 33.2% in 1999; $P = 0.01$) before 1999 and in the age group 5–19 years after 1999 (7% in 1999 to 23.3% in 2004; $P = 0.029$). SGTs 6, 9, 11, 14, 15, 19, 21, 23, 24 and 33 exhibited an average erythromycin resistance of ≥10%.

**Combined penicillin and erythromycin (PE) resistance.** The resistance to PE increased significantly from 0.6% in 1994 to 2.3% in 2001 ($P = 0.009$) and decreased to 1.3% in 2004 ($P = NS$). The significant initial rise in PE resistance could be attributed to the oldest age group (0.3% in 1994 to 2.6% in 2001; $P = 0.017$). The percentage of PE resistance was significantly different between the age groups with 1.8%, 0.9%, 0.7% and 1.3% resistance in the age groups of 0–4, 5–19, 20–59 and ≥60 years, respectively ($P = 0.008$). SGTs 6, 10, 14, 15, 19 and 23 exhibited PE resistance with a maximum of 6.6% in SGT 23.

**SGT prevalence and distribution (Figure 2)**

In the analysis of the age distribution and prevalence of SGTs, the SGTs were grouped into paediatric SGTs (SGTs 6, 9, 14, 19 and 23) and non-paediatric SGTs (SGTs 1, 5 and 7).

The prevalence of paediatric SGTs in the youngest age group was stable over the study period [between 68% (1998) and 79% (1994); $P = NS$]. There were significant fluctuations in the prevalence of paediatric serotypes in the other age groups. These fluctuations were most prevalent in the age group 5–19 years [min, 9.3% (1999); max, 46.2% (1994); $P = 0.004$]. A significant decrease in the prevalence of these paediatric serotypes towards the end of the study period was present in the three older age groups. In the age group 5–19 years, this decrease was most pronounced (from 38.9% in 2002 to 16.7% in 2004; $P = 0.005$).

The prevalence of non-paediatric SGTs in the youngest age group was stable over the study period (3.5% in 1994 to 13.8% in 2003; $P = NS$). There were significant fluctuations in the prevalence of non-paediatric serotypes in the other age groups. These fluctuations were most prevalent in the age group 5–19 years [min, 23.1% (1994); max, 72.2% (2004); $P = 0.002$]. A significant increase in the prevalence of these non-paediatric serotypes towards the end of the study period was present in the three older age groups. In the age group 5–19 years, this

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**Figure 2.** Fifteen most prevalent SGTs causing bacteraemia in rank order per age group. Black box, SGTs included in the 7-valent pneumococcal conjugate vaccine; grey box, additional SGTs in the 13-valent pneumococcal conjugate vaccine (SGTs 1, 3, 5 and 7); white box, additional SGTs in the 23-valent pneumococcal polysaccharide vaccine (except SGT 24).
increase was most pronounced (from 38.9% in 2002 to 72.2% in 2004; \( P = 0.001 \)).

**Antibiotic resistance in paediatric SGTs (Figure 3).** Penicillin resistance in paediatric serotypes increased from 9.9% in 1994 to 27.3% \( (P = 0.001) \) in 2000 and decreased thereafter to 19.9% in 2004 \( (P = 0.004) \). This rise in penicillin resistance was significant in all age groups except in the age group 5–19 years. The decline of penicillin resistance was not significant when age groups were analysed apart.

Erythromycin resistance in paediatric serotypes increased significantly from 40.3% in 1994 to 58% in 2001 \( (P = 0.001) \) and stabilized thereafter. The increase was only significant in the youngest \( (44.8\% \text{ in } 1994 \text{ to } 66.7\% \text{ in } 2001; P = 0.004) \) and oldest \( (38.5\% \text{ in } 1994 \text{ to } 64.7\% \text{ in } 2001; P = 0.001) \) age groups.

**Antibiotic resistance in non-paediatric SGTs (Figure 3).** Non-paediatric serotypes showed no penicillin resistance over the study period.

Erythromycin resistance in non-paediatric serotypes increased from 1.6% in 2001 to 11.4% in 2004 \( (P = 0.001) \). This rise in erythromycin resistance was significant in all age groups, except in the youngest age group. The increase was obvious in SGT 1 isolates \( (0.8\% \text{ in } 2001 \text{ to } 19\% \text{ in } 2004; P = 0.001) \).

SGT 3. There were no significant fluctuations in the overall prevalence of SGT 3. SGT 3 remained highly susceptible to penicillin \( (99.9\%) \) and erythromycin \( (98.4\%) \). In the older population serotype 3 caused 8.9% of pneumococcal bacteraemia. Compared with the age group <5 years, the age group ≥60 years has a relative risk of 7.6 \( (CI: 4–11.6; P = 0.001) \) of having a pneumococcal bacteraemia with SGT 3.

**Vaccine coverage (Figure 4)**

Significantly more bacteraemic isolates are covered by the 7PCV \( (81.9\%) \) and the 13PCV \( (93\%) \) in the youngest age group than in the other age groups \( (P = 0.001) \). The coverage of bacteraemic isolates offered by the 23PPV is 97.2% in the 5–19 years age group, 95.8% in the 20–59 years age group and 95% in the ≥60 years age group \( (P = 0.032) \).

The coverage of bacteraemic SGTs over the study period is stable in the youngest age group for all vaccine formulations and for the 23PPV for all age groups. Over the study period, the fluctuations in vaccine coverage between the 7PCV and 13PCV in the three older age groups correlate with the fluctuations in the non-paediatric SGTs described above.

The coverage of SGTs exhibiting penicillin resistance is the same for the 7PCV and 13PCV. The 23PPV does not cover significantly more SGTs exhibiting penicillin resistance than the two PCVs in all age groups \( (P = NS) \). There are significant differences in coverage of erythromycin-resistant bacteraemic SGTs between the three vaccine formulations with a significant increasing trend when more SGTs are included in the vaccine formulations \( (P = 0.001) \).

**Discussion**

We described the evolution of antibiotic resistance and SGT distribution of bacteraemic *S. pneumoniae* in Belgium during the period 1994–2004.

We focused on pneumococcal bacteraemia as a marker of IPD. In fact pneumococcal bacteraemia represents 91% of the IPD isolates \( (86\% \text{ and } 94\% \text{ of IPD in children <5 years and adults ≥60 years, respectively}) \). Since the majority of non-bacteraemic IPD isolates come from sites that are infected secondary to bacteraemia (arthritis, peritonitis and most cases of meningitis), the bacteraemic isolates analysed in this dataset are representative for IPD in our country. In contrast to the US, where obtaining blood cultures from outpatients is common practice, in our country blood cultures are mostly drawn in hospital settings. The difference in blood culture practices can overestimate the prevalence of SGTs associated with serious IPD necessitating hospitalization compared with SGTs that cause mild or occult bacteraemia.\(^{13}\)

The stability in the distribution of SGTs over the study period was highest in young children, followed by the ≥60 years age group, the 20–59 years age group and then the 5–19 years age group.

The paediatric SGTs (SGTs 6, 9, 14, 19 and 23), representing 47% of bacteraemic SGTs in our dataset, are most prevalent in the youngest (73% of SGTs) and the oldest age group (48% of SGTs). Compared with the age group 5–19 years, the relative risk of being infected with a paediatric SGT is 2.3 \( (CI: 1.9–2.7; P = 0.001) \) in the oldest age group. The paediatric SGTs have a
Pneumococcal bacteraemia in Belgium (1994–2004)

Figure 4. Theoretical coverage of (antibiotic-resistant) bacteraemic pneumococcal SGTs. Left-hand panel: coverage of bacteraemic pneumococcal SGTs by different vaccine formulations. Black bars, 7PCV; grey bars, 13PCV; white bars, 23PPV. Right-hand panel: coverage of antibiotic-resistant bacteraemic pneumococcal SGTs by different vaccine formulations. Black bars, coverage of penicillin-resistant strains; white bars, coverage of erythromycin-resistant strains.

high carriage:invasiveness ratio and frequently cause invasive disease in children with underlying conditions.\textsuperscript{14} Probably, the SGTs that are frequently carried by young children are transmitted to parents and grandparents where they act as opportunistic pathogens and cause disease in susceptible (i.e. having underlying conditions) individuals.\textsuperscript{15} This phenomenon is temporally and geographically stable.\textsuperscript{16}

Over the study period, there was a significant increase in the overall prevalence of non-paediatric SGTs (SGTs 1, 5 and 7). The lowest prevalence of the non-paediatric SGTs, representing 20.5\% of bacteraemic SGTs in our dataset, was found in the youngest (10.4\% of SGTs) and the oldest (14.1\% of SGTs) age group. The highest prevalence (56\% of SGTs) and increase (2002–04: \(+\)33\%) of non-paediatric SGTs was found in the age group 5–19 years. SGTs 1, 5 and 7 are considered true pathogens affecting older children and adults without underlying conditions.\textsuperscript{16}

The oldest population had the highest prevalence of SGT 3 in our study. Although being frequently carried without invasive disease in children, SGT 3 reappears as a cause of bacteraemia in the older population with a subsequent high case fatality rate (up to 50\%).\textsuperscript{17}

Ninety-five per cent of bacteraemic SGTs in the population over 60 years of age are included in the 23PPV in Belgium.\textsuperscript{18} The 23PPV was introduced in Belgium by the end of 1995 and recommended for use in high-risk groups and all persons \(\geq\) 65 years of age by the Belgian High Council of Public Health and a consensus conference of scientific societies.\textsuperscript{19} The vaccine uptake in the target population was \(\sim\)20\% in 1997 and 15\% in 2004.\textsuperscript{20,21} The theoretical coverage for bacteraemic isolates of the 7PCV in young children in Belgium is 82\%. The 7PCV became available in Belgium in the autumn of 2004 and the Belgian High Council of Public Health recommended vaccination of all children under the age of 2 in 2006.\textsuperscript{22} Fifty per cent of the children were vaccinated (with three doses) by the end of 2006. The 7PCV was introduced free of charge in the vaccination schedule of all children under the age of 2 in January 2007.

What factors are responsible for the decline in penicillin resistance and stagnation of erythromycin resistance we observed in Belgium? Firstly, secular trends in the prevalence of SGTs can account for changes in antibiotic resistance.\textsuperscript{23} We documented a significant fluctuation in the prevalence of non-paediatric SGTs (SGT 1, 5 and 7) and a decline in the paediatric SGTs (SGTs 6, 9, 14, 19 and 23) in three of the four age groups. The prevalence was stable in the youngest age group. The absence of penicillin resistance and the rise of erythromycin resistance in the non-paediatric SGTs together with the decline of penicillin resistance and stabilization of erythromycin resistance in paediatric SGTs may have resulted in an overall decline of penicillin resistance and a stagnation of erythromycin resistance. Secondly, antibiotic (over- and mis-) use is a risk factor for the emergence of antibiotic resistance while reduction of antibiotic use can reduce resistance rates.\textsuperscript{24,25} Others also observed changes in the prevalence and resistance of VRT (SGT 19A) driven by antibiotic use rather than the use of 7PCV.\textsuperscript{26} Belgium had an average total outpatient antibiotic use of 24.73 DDD per 1000 inhabitants per day (DID) in the period 1997–2004 and ranks with the higher antibiotic consumers in Europe.\textsuperscript{27} Two public campaigns for more rational use of antibiotics in 2000–01 and 2001–02 resulted in a decrease of antibiotic sales during the 3 month campaigns of, respectively, 6.5\% and 3.4\% (in DDD) and an annual antibiotic sales decrease of 5.3\% (in DDD) between 2000 and 2002.\textsuperscript{28} The outpatient use of macrolides in Belgium is decreasing (from 3.56 DID in 1998 to 2.14 DID in 2004).\textsuperscript{29} The outpatient use of penicillins has not decreased (9.96 DID in 1999; 10.6 DDD in 2004).\textsuperscript{30} Whether these moderate changes in antibiotic use influenced antibiotic resistance in S. pneumoniae needs further study. Thirdly, the spread of successful antibiotic-resistant clones, originating de novo or from neighbouring countries, can influence antibiotic resistance and SGTs prevalence. A high population density and proximity to high resistance regions (e.g. France) in addition to antibiotic use may favour resistance.\textsuperscript{31} Finally, the introduction of the 7PCV in children \(\leq\) 2 years can decrease the incidence of vaccine-type IPD and the incidence of IPD caused by antibiotic-resistant S. pneumoniae in the target population.\textsuperscript{4} The same effects were observed in the adult and elderly population by herd immunity.\textsuperscript{11,32} France reported a decrease in IPD and antibiotic resistance after the introduction of the 7PCV in 2001.\textsuperscript{33,34} The 7PCV was not yet available during the study period in Belgium. Whether vaccine-related changes in SGT
prevalence and resistance can cross borders also needs further study.

The 7PCV coverage of 82% is probably an overestimation because serotyping within serogroups, differentiating VT from VRT, was not performed in our dataset until 2004. Based on a recent active surveillance of IPD in children <5 years in Belgium, the 7 VTs of the 7PCV covered 68.4% of pneumococcal bacteraemia. VRTs represented 20.2% and non-VTs 11.4% of pneumococcal bacteraemia in this age group. Serotype 6A represented 27.5% of serogroup 6, and 19A 62.5% of serogroup 19.35 After the introduction of the 7PCV, replacement by VRT (SGT 19A) and non-VT (SGTs 1, 3 and 15) in non-IPD and IPD has been documented in the US in children and older adults.36 Selection of transformants (by capsular switching) has also been documented recently.37 In Belgium, we also observed a shift in the SGT 19A/19F ratio (1996–2004, 1.6; 2006, 4.75) after the 7PCV introduction in 2004. The potential of replacement disease by non-VTs (e.g. the high prevalence of SGTs 1, 5 and 7 in the 5–19 years age group) is also present in Belgium. The latter SGTs are included in the future 13PCV.

Secular trends in SGT distribution, antibiotic use and vaccine use at the national and international level are likely to influence the Belgian pneumococcal epidemiology. Further surveillance, taking all these factors into account, is warranted.

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**References**


