

RESEARCH ARTICLE

Open Access

Macrolide susceptibility and serotype specific macrolide resistance of invasive isolates of *Streptococcus pneumoniae* in Germany from 1992 to 2008

Matthias Imöhl^{1*}, Ralf René Reinert^{1,3}, Christina Mutscher², Mark van der Linden¹

Abstract

Background: Macrolide resistant *Streptococcus pneumoniae* has been on a gradual increase in Germany for over a decade. The current study was undertaken against the background of the recent observation of declining macrolide resistance rates especially among German children. Nationwide surveillance of invasive pneumococcal disease has been conducted in Germany since 1992. A population- and laboratory-based approach was used to collect data on invasive pneumococcal disease, and isolates sent to the National Reference Center for Streptococci by diagnostic microbiological laboratories from 1992 to 2008 were included in this study.

Results: From 1992 to 2008, data on macrolide susceptibility were available for 11,807 invasive isolates. 8,834 isolates (74.8%) were from adults (≥ 16 years), and 2,973 isolates (25.2%) from children (< 16 years). The overall nonsusceptibility rate of all isolates was 16.2% (intermediate, 0.2%; resistant, 16.0%). Higher resistance rates were observed among children (intermediate, 0.2%; resistant, 23.8%) than among adults (intermediate, 0.3%; resistant 13.4%). Maximum nonsusceptibility rates during the period under study were observed in 2005 (children: intermediate, 0.3%; resistant, 32.3%; adults: intermediate, 0.0%; resistant, 18.6%), while nonsusceptibility rates in 2008 were considerably lower, especially for children (children: intermediate, 0.0%; resistant, 15.2%; adults: intermediate, 0.1%; resistant, 12.9%). The rate of resistance was higher among the vaccine serotypes (7-valent, 36.6%; 10-valent, 28.2%; 13-valent, 24.3%) than among the non vaccine serotypes (non 7-valent, 6.5%; non 10-valent, 7.4%; non 13-valent, 6.3%). Serotype 14 (69.6% nonsusceptibility) proved to be the most resistant serotype.

Conclusions: There has been a considerable and statistically significant decrease in macrolide nonsusceptibility in Germany since 2005, especially among children.

Background

Streptococcus pneumoniae is a leading pathogen in bacterial pneumonia, sepsis and meningitis in humans worldwide [1,2]. In many European countries the rate of resistance of *S. pneumoniae* to macrolides has exceeded that of penicillin [3]. Concerning penicillin, it has been described that treatment of patients with nonmeningeal invasive pneumococcal infections with nonsusceptible isolates was not associated with higher mortality rates [4-6].

In 2008 new penicillin breakpoints for *S. pneumoniae* were published by the CLSI [7], differentiating meningitis and non-meningitis cases of invasive pneumococcal disease (IPD). Their impact on susceptibility categorisation in Germany was described previously by our group [8]. However, for macrolides an increased risk of macrolide failure has been reported for pneumococcal isolates nonsusceptible in vitro [9].

The aim of this study was to evaluate macrolide susceptibility of all isolates of *S. pneumoniae* with IPD that were sent to the German National Reference Center for Streptococci (NRCS) between 1992 and 2008 and to evaluate potential trends in nonsusceptibility over time.

* Correspondence: mimoehl@ukaachen.de

¹National Reference Center for Streptococci, Department of Medical Microbiology, University Hospital RWTH Aachen, Pauwelsstrasse 30, D-52074 Aachen, Germany

Full list of author information is available at the end of the article

The description of serotype specific resistance, was a major aim of the study.

The study was undertaken against the background of the recent observation of declining macrolide resistance rates especially among German children.

Methods

Study design

The NRCS has conducted surveillance for invasive pneumococcal disease in Germany since 1992. A population- and laboratory-based approach was used to collect data on invasive pneumococcal disease among children < 16 years and adults \geq 16 years in Germany. Isolates were sent to the NRCS by diagnostic microbiological laboratories throughout Germany on a voluntary basis. Cases from January 1, 1992 to December 31, 2008 were included in this study. A case of IPD was defined by the isolation of *S. pneumoniae* from a normally sterile site.

Microbiological investigations

Isolates were identified by standard procedures including bile solubility and optochin sensitivity. Minimal inhibitory concentrations (MIC) testing was performed using the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [7]. Macrolide resistance was investigated using erythromycin or clarithromycin, in which testing with erythromycin was replaced by clarithromycin over the years. 425 isolates were tested both for erythromycin and clarithromycin. The susceptible, intermediate, and resistant breakpoints (MIC) were \leq 0.25, 0.5, and \geq 1 μ g/ml, both for erythromycin and clarithromycin [7]. *Streptococcus pneumoniae* ATCC 49619 was used as a control strain.

Statistical analysis

All categorical data were expressed as frequencies. To analyse a severe increase or decrease over time the Cochran-Armitage test was used. The overall significance level was adjusted using the Bonferroni correction to account for the problem of multiple testing. Due to 14 tests p-values \leq 0.0036 were considered as statistically significant test results. All statistical analyses were conducted using SAS Version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

In total, 12,136 isolates from invasive pneumococcal disease were collected between January 1, 1992 and December 31, 2008. The number of cases for each year vary between 297 and 2,037 (median: 505 cases). Data on macrolide susceptibility were available for 11,807 isolates, whereas 8,834 isolates (74.8%) originated from adults, 2,973 isolates (25.2%) were from children.

The overall nonsusceptibility rate of all isolates was 16.2% (intermediate, 0.2%; resistant, 16.0%). Higher resistance rates were observed among children (intermediate, 0.2%; resistant, 23.8%) than among adults (intermediate, 0.3%; resistant 13.4%) (Table 1).

The sampling source related nonsusceptibility is shown in Table 2. Highest nonsusceptibility rates were observed for pharyngeal isolates (75%, n = 4), pericardium (50%, n = 8) and mastoid (40%, n = 10). Nonsusceptibility rates for CSF and blood were 17.8% (n = 1824) and 15.9% (n = 9352), respectively. The serotype distribution broken down to the sampling source is shown in Table 3. For blood, CSF and BAL serotype 14 is most prevalent, whereas for pleural fluid serotypes 1 and 3 are most often found.

As for the childhood IPD isolates in the first year of this study (1992), 2.0% were intermediate and 10.0% resistant to macrolides. Maximum nonsusceptibility rates during the period under study were observed in 2005 (intermediate, 0.3%; resistant, 32.3%), while in 2008, 0.0% of isolates were intermediate and 15.2% resistant. IPD isolates obtained from adults were intermediate in 0.0% and resistant in 2.9% in 1992. Maximum nonsusceptibility rates were observed in 2005 as well (intermediate, 0.0%; resistant, 18.6%). Nonsusceptibility rates in 2008 were 0.1% (intermediate) and 12.9% (resistant). The increase in macrolide nonsusceptibility from 1992 to 2005 was statistically significant for children ($P < 0.0001$) and adults ($P < 0.0001$), as well as the decrease from 2005 to 2008 (children, $P < 0.0001$; adults, $P < 0.0001$). Concerning the intermediate resistant isolates no significant trends were observed (1992-2005: children ($P = 0.8942$), adults ($P = 0.4302$); 2005-2008: children ($P = 0.6282$), adults ($P = 0.5960$)). Detailed results of the macrolide susceptibility testing are shown in Figure 1. The MICs of all invasive isolates are illustrated in Figure 2.

Overall, the leading serotypes were serotypes 14 (16.4% of serotyped isolates), 3 (8.1%), 7F (7.6%), 1 (7.3%) and 23F (5.9%). A ranking of serotype specific macrolide nonsusceptibility of IPD isolates is shown in Table 1. Serotype 14 (69.5% nonsusceptibility) was by far the most resistant serotype, followed by serotypes rough, 19B, 45 (33.3% each), 6B (32.9%), 15A (31.3%), 19F (26.1%), and 19A (25.5%). However, absolute numbers for rough, 19B and 45 were very low.

Serotypes contributing considerably to pneumococcal macrolide nonsusceptibility by combination of frequency among invasive isolates and relatively high macrolide nonsusceptibility are especially serotypes 14, 6B, 19F, 19A, 9V and 23F. The development of nonsusceptibility of these serotypes over the years is shown in Figure 3. The nonsusceptibility among serotype 14 isolates increases considerably over the years up to around 80% ($P < 0.0001$). For serotype 19F a significant increase ($P = 0.0033$) in

Table 1 Ranking of serotype specific macrolide nonsusceptibility among IPD isolates in Germany from 1992 to 2008 (n, overall = 11,807; n, adults = 8,834; n, children = 2,973)

| Sero type | children | | | | adults | | | | overall | | | | |
|-----------|----------|------|-------|-----------|--------|------|-------|-----------|---------|------|-------|------------------------|------------------------|
| | I% | R% | S% | total (n) | I% | R% | S% | total (n) | I% | R% | S% | total [†] (n) | total [‡] (%) |
| 14 | 0.0 | 67.4 | 32.6 | 663 | 0.2 | 71.0 | 28.8 | 883 | 0.1 | 69.5 | 30.4 | 1546 | 16.4 |
| 45 | - | - | - | - | 0.0 | 33.3 | 66.7 | 3 | 0.0 | 33.3 | 66.7 | 3 | 0.0 |
| 19B | 0.0 | 0.0 | 100.0 | 1 | 0.0 | 50.0 | 50.0 | 2 | 0.0 | 33.3 | 66.7 | 3 | 0.0 |
| rough | 0.0 | 25.0 | 75.0 | 8 | 0.0 | 40.0 | 60.0 | 10 | 0.0 | 33.3 | 66.7 | 18 | 0.2 |
| 6B | 0.0 | 29.3 | 70.7 | 215 | 0.4 | 36.2 | 63.4 | 232 | 0.2 | 32.9 | 66.9 | 447 | 4.8 |
| 15A | 4.8 | 28.6 | 66.7 | 21 | 0.0 | 33.3 | 66.7 | 27 | 2.1 | 31.3 | 66.7 | 48 | 0.5 |
| 19F | 0.0 | 24.5 | 75.5 | 212 | 0.4 | 27.5 | 72.0 | 236 | 0.2 | 26.1 | 73.7 | 448 | 4.8 |
| 19A | 0.0 | 24.4 | 75.6 | 90 | 0.9 | 26.0 | 73.2 | 231 | 0.6 | 25.5 | 73.8 | 321 | 3.4 |
| 10B | - | - | - | - | 0.0 | 20.0 | 80.0 | 10 | 0.0 | 20.0 | 80.0 | 10 | 0.1 |
| 19C | 0.0 | 0.0 | 100.0 | 2 | 0.0 | 33.3 | 66.7 | 3 | 0.0 | 20.0 | 80.0 | 5 | 0.1 |
| 15B | 0.0 | 23.1 | 76.9 | 26 | 0.0 | 17.5 | 82.5 | 57 | 0.0 | 19.3 | 80.7 | 83 | 0.9 |
| 23F | 0.5 | 20.4 | 79.1 | 201 | 0.6 | 18.3 | 81.2 | 356 | 0.5 | 19.0 | 80.4 | 557 | 5.9 |
| 9V | 0.9 | 13.2 | 85.8 | 106 | 1.3 | 20.1 | 78.5 | 298 | 1.2 | 18.3 | 80.4 | 404 | 4.3 |
| NT | 0.0 | 10.0 | 90.0 | 10 | 0.0 | 20.7 | 79.3 | 29 | 0.0 | 17.9 | 82.1 | 39 | 0.4 |
| 11F | - | - | - | - | 0.0 | 16.7 | 83.3 | 6 | 0.0 | 16.7 | 83.3 | 6 | 0.1 |
| 15C | 0.0 | 15.4 | 84.6 | 26 | 0.0 | 14.8 | 85.2 | 27 | 0.0 | 15.1 | 84.9 | 53 | 0.6 |
| 9A | 0.0 | 9.5 | 90.5 | 21 | 0.0 | 19.2 | 80.8 | 26 | 0.0 | 14.9 | 85.1 | 47 | 0.5 |
| 33B | 0.0 | 0.0 | 100.0 | 3 | 0.0 | 25.0 | 75.0 | 4 | 0.0 | 14.3 | 85.7 | 7 | 0.1 |
| 33A | 0.0 | 11.1 | 88.9 | 9 | 0.0 | 14.3 | 85.7 | 21 | 0.0 | 13.3 | 86.7 | 30 | 0.3 |
| 33F | 0.0 | 0.0 | 100.0 | 17 | 0.0 | 17.6 | 82.4 | 51 | 0.0 | 13.2 | 86.8 | 68 | 0.7 |
| 12B | 0.0 | 0.0 | 100.0 | 3 | 0.0 | 20.0 | 80.0 | 5 | 0.0 | 12.5 | 87.5 | 8 | 0.1 |
| 6A | 0.0 | 5.5 | 94.5 | 128 | 0.4 | 9.7 | 89.9 | 277 | 0.2 | 8.4 | 91.4 | 405 | 4.3 |
| 28A | 0.0 | 0.0 | 100.0 | 4 | 0.0 | 12.5 | 87.5 | 8 | 0.0 | 8.3 | 91.7 | 12 | 0.1 |
| 35F | 0.0 | 10.0 | 90.0 | 10 | 0.0 | 7.8 | 92.2 | 64 | 0.0 | 8.1 | 91.9 | 74 | 0.8 |
| 24F | 0.0 | 6.8 | 93.2 | 44 | 0.0 | 6.9 | 93.1 | 72 | 0.0 | 6.9 | 93.1 | 116 | 1.2 |
| 13 | 0.0 | 0.0 | 100.0 | 3 | 0.0 | 8.3 | 91.7 | 12 | 0.0 | 6.7 | 93.3 | 15 | 0.2 |
| 16F | 0.0 | 0.0 | 100.0 | 7 | 3.7 | 7.4 | 88.9 | 27 | 2.9 | 5.9 | 91.2 | 34 | 0.4 |
| 17F | 0.0 | 12.5 | 87.5 | 8 | 0.0 | 3.2 | 96.8 | 31 | 0.0 | 5.1 | 94.9 | 39 | 0.4 |
| 38 | 0.0 | 0.0 | 100.0 | 23 | 0.0 | 7.9 | 92.1 | 38 | 0.0 | 4.9 | 95.1 | 61 | 0.6 |
| 34 | 0.0 | 16.7 | 83.3 | 6 | 0.0 | 0.0 | 100.0 | 15 | 0.0 | 4.8 | 95.2 | 21 | 0.2 |
| 9N | 0.0 | 0.0 | 100.0 | 25 | 0.0 | 5.5 | 94.5 | 145 | 0.0 | 4.7 | 95.3 | 170 | 1.8 |
| 11A | 0.0 | 0.0 | 100.0 | 15 | 0.0 | 5.2 | 94.8 | 135 | 0.0 | 4.7 | 95.3 | 150 | 1.6 |
| 18A | 0.0 | 0.0 | 100.0 | 10 | 0.0 | 8.3 | 91.7 | 12 | 0.0 | 4.5 | 95.5 | 22 | 0.2 |
| 1 | 0.4 | 5.2 | 94.4 | 232 | 0.2 | 3.5 | 96.3 | 458 | 0.3 | 4.1 | 95.7 | 690 | 7.3 |
| 7F | 0.0 | 3.9 | 96.1 | 203 | 0.4 | 3.7 | 95.9 | 515 | 0.3 | 3.8 | 96.0 | 718 | 7.6 |
| 5 | 0.0 | 0.0 | 100.0 | 19 | 0.0 | 5.4 | 94.6 | 37 | 0.0 | 3.6 | 96.4 | 56 | 0.6 |
| 10A | 0.0 | 4.0 | 96.0 | 50 | 0.0 | 2.5 | 97.5 | 122 | 0.0 | 2.9 | 97.1 | 172 | 1.8 |
| 4 | 0.0 | 2.9 | 97.1 | 102 | 0.0 | 2.2 | 97.8 | 409 | 0.0 | 2.3 | 97.7 | 511 | 5.4 |
| 20 | 0.0 | 0.0 | 100.0 | 5 | 0.0 | 2.6 | 97.4 | 38 | 0.0 | 2.3 | 97.7 | 43 | 0.5 |
| 18C | 0.6 | 1.7 | 97.8 | 181 | 0.0 | 2.8 | 97.2 | 145 | 0.3 | 2.1 | 97.5 | 326 | 3.5 |
| 3 | 0.0 | 3.1 | 96.9 | 96 | 0.2 | 1.8 | 98.0 | 663 | 0.1 | 2.0 | 97.9 | 759 | 8.1 |
| 12F | 0.0 | 0.0 | 100.0 | 16 | 0.0 | 1.9 | 98.1 | 105 | 0.0 | 1.7 | 98.3 | 121 | 1.3 |
| 8 | 0.0 | 0.0 | 100.0 | 18 | 0.5 | 1.6 | 97.9 | 190 | 0.5 | 1.4 | 98.1 | 208 | 2.2 |
| 23A | 0.0 | 0.0 | 100.0 | 14 | 0.0 | 1.4 | 98.6 | 74 | 0.0 | 1.1 | 98.9 | 88 | 0.9 |
| 22F | 0.0 | 0.0 | 100.0 | 20 | 0.5 | 0.5 | 98.9 | 186 | 0.5 | 0.5 | 99.0 | 206 | 2.2 |
| 2 | 0.0 | 0.0 | 100.0 | 1 | 0.0 | 0.0 | 100.0 | 11 | 0.0 | 0.0 | 100.0 | 12 | 0.1 |
| 31 | 0.0 | 0.0 | 100.0 | 1 | 0.0 | 0.0 | 100.0 | 25 | 0.0 | 0.0 | 100.0 | 26 | 0.3 |
| 12A | 0.0 | 0.0 | 100.0 | 3 | 0.0 | 0.0 | 100.0 | 9 | 0.0 | 0.0 | 100.0 | 12 | 0.1 |
| 18F | 0.0 | 0.0 | 100.0 | 5 | 0.0 | 0.0 | 100.0 | 10 | 0.0 | 0.0 | 100.0 | 15 | 0.2 |

Table 1 Ranking of serotype specific macrolide nonsusceptibility among IPD isolates in Germany from 1992 to 2008 (n, overall = 11,807; n, adults = 8,834; n, children = 2,973) (Continued)

| | | | | | | | | | | | | | |
|---------------|-----|------|-------|------|-----|------|-------|------|-----|------|-------|-------|-------|
| 23B | 0.0 | 0.0 | 100.0 | 6 | 0.0 | 0.0 | 100.0 | 11 | 0.0 | 0.0 | 100.0 | 17 | 0.2 |
| 35B | 0.0 | 0.0 | 100.0 | 3 | 0.0 | 0.0 | 100.0 | 8 | 0.0 | 0.0 | 100.0 | 11 | 0.1 |
| 9L | 0.0 | 0.0 | 100.0 | 5 | 0.0 | 0.0 | 100.0 | 12 | 0.0 | 0.0 | 100.0 | 17 | 0.2 |
| Others* | 0.0 | 0.0 | 100.0 | 31 | 0.0 | 0.0 | 100.0 | 62 | 0.0 | 0.0 | 100.0 | 93 | 1.0 |
| not serotyped | 0.0 | 4.4 | 95.6 | 45 | 0.2 | 0.0 | 99.8 | 2360 | 0.2 | 0.1 | 99.8 | 2405 | - |
| total (%) | 0.2 | 23.8 | 76.1 | - | 0.3 | 13.4 | 86.3 | - | 0.2 | 16.0 | 83.7 | - | 100.0 |
| total (n) | 5 | 707 | 2261 | 2973 | 24 | 1184 | 7626 | 8834 | 29 | 1891 | 9887 | 11807 | 9402 |

I%, intermediate isolates in percent; R%, resistant isolates in percent; S%, susceptible isolates in percent; n, number of isolates tested.

Total (n)[†] represents the number of isolates collected from both children and adults. Total (%)[‡] represents the serotype distribution in percent in relation to the number of 9402 IPD isolates serotyped.

Others* includes the serotypes (number of isolates, overall): 15F (9), 18B (9), 7C (8), 10F (6), 11B (6), 35A (6), 29 (5), 21 (4), 24A (4), 28F (4), 35C (4), 37 (4), 22A (3), 36 (3), 7A (3), 24B (2), 25F (2), 39 (2), 48 (2), 7B (2), 17A (1), 18 (1), 19 (1), 35 (1), 9 (1).

nonsusceptibility was observed as well. No significant trends were found for serotypes 6B ($P = 0.0040$), 9V ($P = 0.3554$), 19A ($P = 0.0740$) and 23F ($P = 0.0529$).

The peak in nonsusceptibility among 7-, 10- and 13-valent serotypes in adults from 1998 to 2002 (Figure 4) correlates to an increased incidence of serotype 14 during that time [10]. Generally, the rate of resistance is higher among the vaccine serotypes (7v, 36.6%; 10v, 28.2%; 13v, 24.3%) (Figure 4) than among the non vaccine serotypes (non 7v, 6.5%; non 10v, 7.4%; non 13v, 6.3%) (Figure 5). The proportion of nonsusceptible 7-valent vaccine serotypes remained largely constant from 2000 to 2007 among children (Figure 4). Among the non PCV7 serotypes the rate of nonsusceptibility is lower (Figure 5). Concerning adults, an increase of isolates sent to the NRCS can be noticed (Figures 4 and 5). The fraction of nonsusceptible isolates has declined

Table 2 Ranking of macrolide nonsusceptibility among IPD isolates in Germany from 1992 to 2008 related to the sampling source (n = 11,807)

| Sampling source | I% | R% | I+R% | total (n) |
|-----------------|------------|-------------|-------------|--------------|
| Pharynx | 0.0 | 75.0 | 75.0 | 4 |
| Pericard | 0.0 | 50.0 | 50.0 | 8 |
| Mastoid | 0.0 | 40.0 | 40.0 | 10 |
| BAL | 0.6 | 18.7 | 19.4 | 154 |
| Others/unknown | 0.0 | 18.3 | 18.3 | 131 |
| CSF | 0.2 | 17.7 | 17.8 | 1824 |
| Blood | 0.3 | 15.6 | 15.9 | 9352 |
| Pleural fluid | 0.4 | 14.7 | 15.1 | 252 |
| Eye | 0.0 | 11.1 | 11.1 | 9 |
| Ascites | 0.0 | 8.7 | 8.7 | 23 |
| Joint | 0.0 | 5.6 | 5.6 | 36 |
| Ear | 0.0 | 0.0 | 0.0 | 4 |
| Total | 0.3 | 16.0 | 16.3 | 11807 |

Table 3 Serotype distribution among IPD isolates from different sampling sites in Germany from 1992 to 2008 in percent (n = 11,807)

| Sero type | Ascites | BAL | Blood | CSF | Joint | Pleural fluid | Total (%) | Total (n) |
|-----------|---------|------|-------|------|-------|---------------|-----------|-----------|
| 14 | 9,1 | 10,7 | 17,4 | 14,8 | 0,0 | 11,0 | 16,5 | 1546 |
| 3 | 0,0 | 6,0 | 8,6 | 5,7 | 3,0 | 13,8 | 8,1 | 759 |
| 7F | 4,5 | 1,2 | 8,0 | 7,2 | 0,0 | 7,2 | 7,7 | 718 |
| 1 | 4,5 | 6,0 | 8,3 | 2,6 | 12,1 | 15,5 | 7,4 | 690 |
| 23F | 4,5 | 8,3 | 5,7 | 7,1 | 3,0 | 6,6 | 5,9 | 557 |
| 4 | 4,5 | 7,1 | 6,0 | 3,8 | 6,1 | 3,9 | 5,5 | 511 |
| 19F | 9,1 | 7,1 | 4,2 | 6,8 | 3,0 | 3,9 | 4,8 | 448 |
| 6B | 9,1 | 6,0 | 4,3 | 6,6 | 12,1 | 4,4 | 4,8 | 447 |
| 6A | 4,5 | 2,4 | 4,0 | 5,7 | 12,1 | 4,4 | 4,3 | 405 |
| 9V | 0,0 | 4,8 | 4,8 | 2,3 | 6,1 | 2,8 | 4,3 | 404 |
| 18C | 4,5 | 3,6 | 2,8 | 5,8 | 9,1 | 3,3 | 3,5 | 326 |
| 19A | 4,5 | 4,8 | 3,5 | 2,7 | 3,0 | 1,7 | 3,4 | 321 |
| 8 | 9,1 | 1,2 | 2,4 | 2,0 | 0,0 | 0,6 | 2,2 | 208 |
| 22F | 0,0 | 0,0 | 2,3 | 2,0 | 6,1 | 0,6 | 2,2 | 206 |
| 10A | 4,5 | 1,2 | 1,6 | 2,7 | 3,0 | 1,7 | 1,8 | 172 |
| 9N | 0,0 | 2,4 | 1,9 | 1,7 | 0,0 | 1,1 | 1,8 | 170 |
| 11A | 0,0 | 1,2 | 1,6 | 1,7 | 0,0 | 2,8 | 1,6 | 150 |
| 12F | 4,5 | 2,4 | 1,3 | 1,2 | 0,0 | 0,6 | 1,3 | 121 |
| 24F | 0,0 | 0,0 | 1,2 | 1,6 | 0,0 | 0,6 | 1,2 | 116 |
| 23A | 0,0 | 1,2 | 0,8 | 1,2 | 0,0 | 2,8 | 0,9 | 88 |
| 15B | 0,0 | 0,0 | 0,7 | 1,5 | 3,0 | 1,7 | 0,9 | 83 |
| 35F | 4,5 | 0,0 | 0,7 | 1,0 | 0,0 | 1,7 | 0,8 | 74 |
| 33F | 0,0 | 1,2 | 0,6 | 1,2 | 3,0 | 0,6 | 0,7 | 68 |
| 38 | 0,0 | 0,0 | 0,6 | 0,8 | 0,0 | 0,0 | 0,7 | 61 |
| 5 | 0,0 | 0,0 | 0,7 | 0,3 | 0,0 | 0,6 | 0,6 | 56 |
| 15C | 4,5 | 1,2 | 0,5 | 0,7 | 3,0 | 0,0 | 0,6 | 53 |
| 15A | 0,0 | 0,0 | 0,5 | 0,7 | 0,0 | 1,1 | 0,5 | 48 |
| 9A | 0,0 | 1,2 | 0,5 | 0,4 | 0,0 | 1,1 | 0,5 | 47 |
| 20 | 0,0 | 0,0 | 0,4 | 0,5 | 0,0 | 1,1 | 0,5 | 43 |
| 17F | 4,5 | 3,6 | 0,3 | 0,6 | 0,0 | 0,0 | 0,4 | 39 |
| NT | 0,0 | 2,4 | 0,4 | 0,3 | 3,0 | 0,0 | 0,4 | 39 |
| 16F | 0,0 | 0,0 | 0,3 | 0,6 | 0,0 | 0,6 | 0,4 | 34 |
| 33A | 0,0 | 0,0 | 0,3 | 0,4 | 0,0 | 0,6 | 0,3 | 30 |

Table 3 Serotype distribution among IPD isolates from different sampling sites in Germany from 1992 to 2008 in percent (n = 11,807) (Continued)

| | | | | | | | | |
|---------------|-------|-------|-------|-------|-------|-------|-------|------|
| 31 | 0,0 | 2,4 | 0,3 | 0,2 | 0,0 | 0,0 | 0,3 | 26 |
| 18A | 0,0 | 0,0 | 0,2 | 0,4 | 3,0 | 0,0 | 0,2 | 22 |
| 34 | 0,0 | 0,0 | 0,1 | 0,6 | 0,0 | 0,6 | 0,2 | 21 |
| Others* | 9,1 | 10,7 | 2,3 | 4,5 | 6,1 | 1,7 | 2,8 | 264 |
| Total | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 9371 |
| not serotyped | 4,3 | 45,5 | 23,8 | 2,1 | 8,3 | 28,2 | 20,6 | 2436 |

Only sampling sites with ≥ 20 isolates were included in this table.
 NT: nontypeable; n: number of isolates tested.

Others* includes the serotypes (number of isolates): rough (18), 9L (17), 23B (17), 13 (15), 18F (15), 12A (12), 2 (12), 28A (12), 35B (11), 10B (10), 15F (9), 18B (9), 12B (8), 33B (7), 7C (6), 10F (6), 11B (6), 11F (6), 35A (6), 7A (5), 19C (5), 29 (5), 21 (4), 24A (4), 28F (4), 35C (4), 37 (4), 19B (3), 22A (3), 36 (3), 45 (3), 7B (2), 24B (2), 25F (2), 39 (2), 48 (2), 9(1), 17A (1), 18 (1), 19 (1), 35 (1).

during the last years among 7-valent vaccine serotypes after a notable increase from 1992 to 1999 (Figure 4).

Discussion and conclusions

This paper presents the results of 17 years of surveillance for macrolide susceptibility of invasive pneumococcal disease in Germany. The prevalence of antibiotic-resistant *S. pneumoniae* continues to increase worldwide but varies widely between countries [11-13]. In Europe, high resistance rates for macrolides have been reported from France, Spain, Italy and Belgium [12,13]. Pneumococcal macrolide resistance rates reported from Germany were low [12-17]. Nevertheless, a continuous and statistically significant increase of

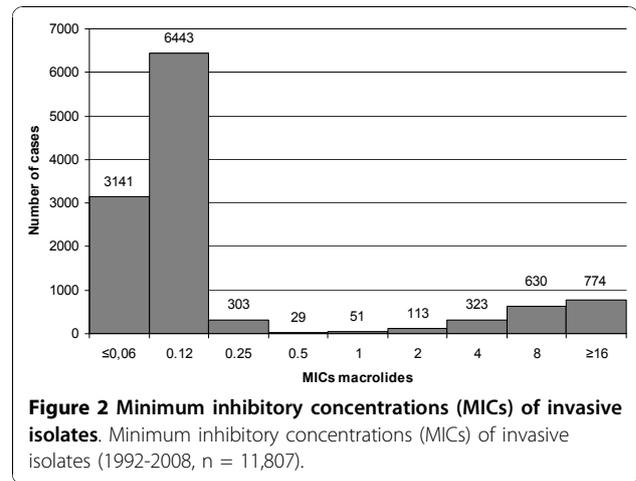
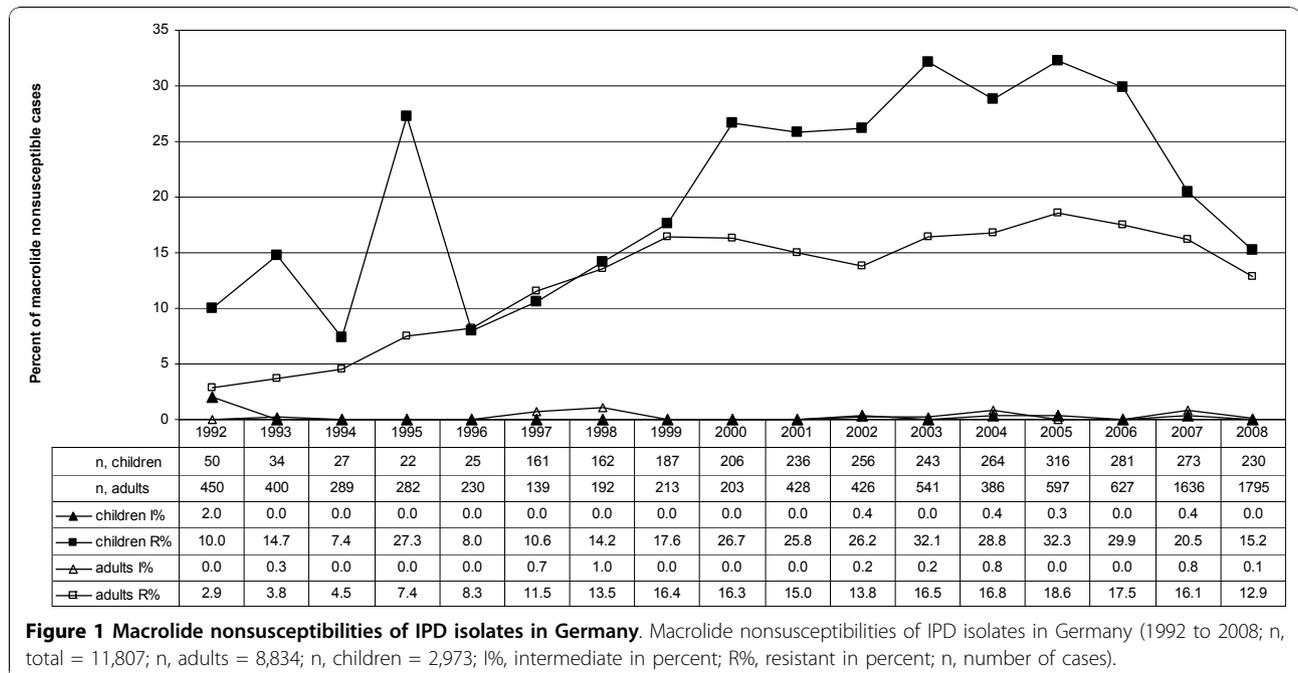


Figure 2 Minimum inhibitory concentrations (MICs) of invasive isolates. Minimum inhibitory concentrations (MICs) of invasive isolates (1992-2008, n = 11,807).

macrolide nonsusceptibility could be observed after publication of these studies, reaching maximum values in 2005 (children: intermediate, 0.3%; resistant, 32.3%; adults: intermediate, 0.0%; resistant, 18.6%). The relatively high rate of variation in resistance among childhood isolates during the first years of the study is presumably due to the low number of cases, and a suspected bias for resistant isolates among the centers sending the isolates. Since 2005, a considerable and statistically significant decrease especially for childhood nonsusceptibility has been noticed. These data are similar to those recently reported from Spain, where a reduction of erythromycin nonsusceptibility from 42.9% in 2003 to 20.0% in 2007 has been described [18].



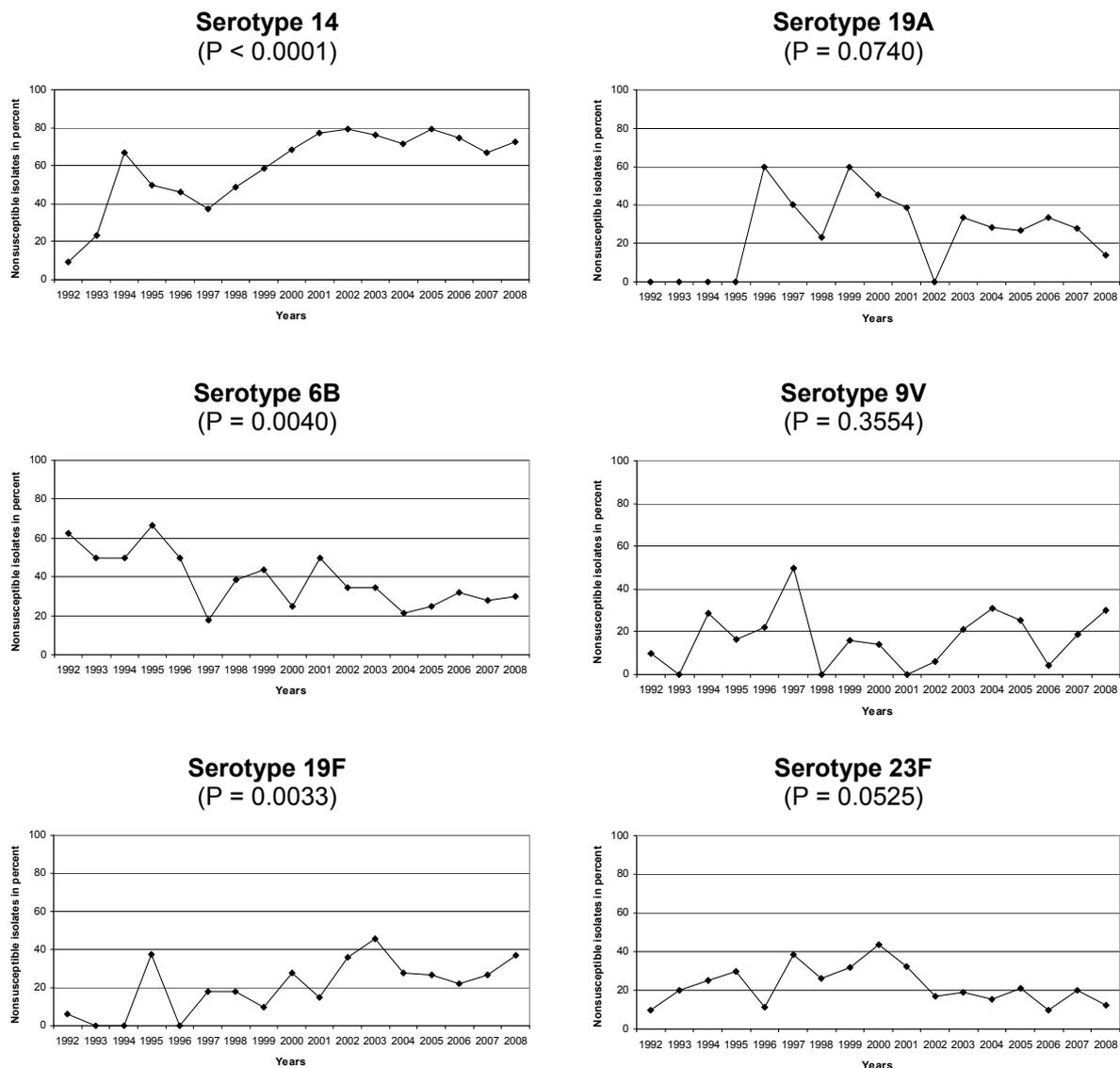


Figure 3 Serotype specific macrolide nonsusceptibility of IPD isolates in Germany. Serotype specific macrolide nonsusceptibility of IPD isolates in Germany (1992 to 2008; n, serotype 14 = 1,546; n, serotype 6B = 447; n, serotype 19F = 448; n, serotype 19A = 321; n, serotype 9V = 404; n, serotype 23F = 557).

An increased awareness of IPD among adults has been observed since 2007. This correlates to the general recommendation of pneumococcal conjugate vaccination for children < 2 years in Germany at the end of July 2006 and an increased interest in serotype information of IPD. Furthermore, in January 2007 an internet based laboratory sentinel system ('PneumoWeb') was established in Germany, which enables participating laboratories to transfer anonymised basic patient information on a voluntary basis. Compared to children, only a minor reduction of nonsusceptibility has been observed among adults from 2005 (18.6%) to 2008 (13.0%), although this reduction was also statistically significant.

Possible reasons for the decrease in macrolide nonsusceptibility include a reduced macrolide consumption due to the rising resistance rates, as well as the general recommendation of pneumococcal conjugate vaccination for children < 2 years in Germany at the end of July 2006. Since the introduction of the vaccine a considerable decrease of serotypes included in the 7-valent pneumococcal conjugate vaccine has been observed among German children, but also (to a lesser extent) among adults [10], which is partly due to the association of serotypes with age [19,20].

The antibiotic prescribing practices, which are thought to be among the most significant drivers for the spread

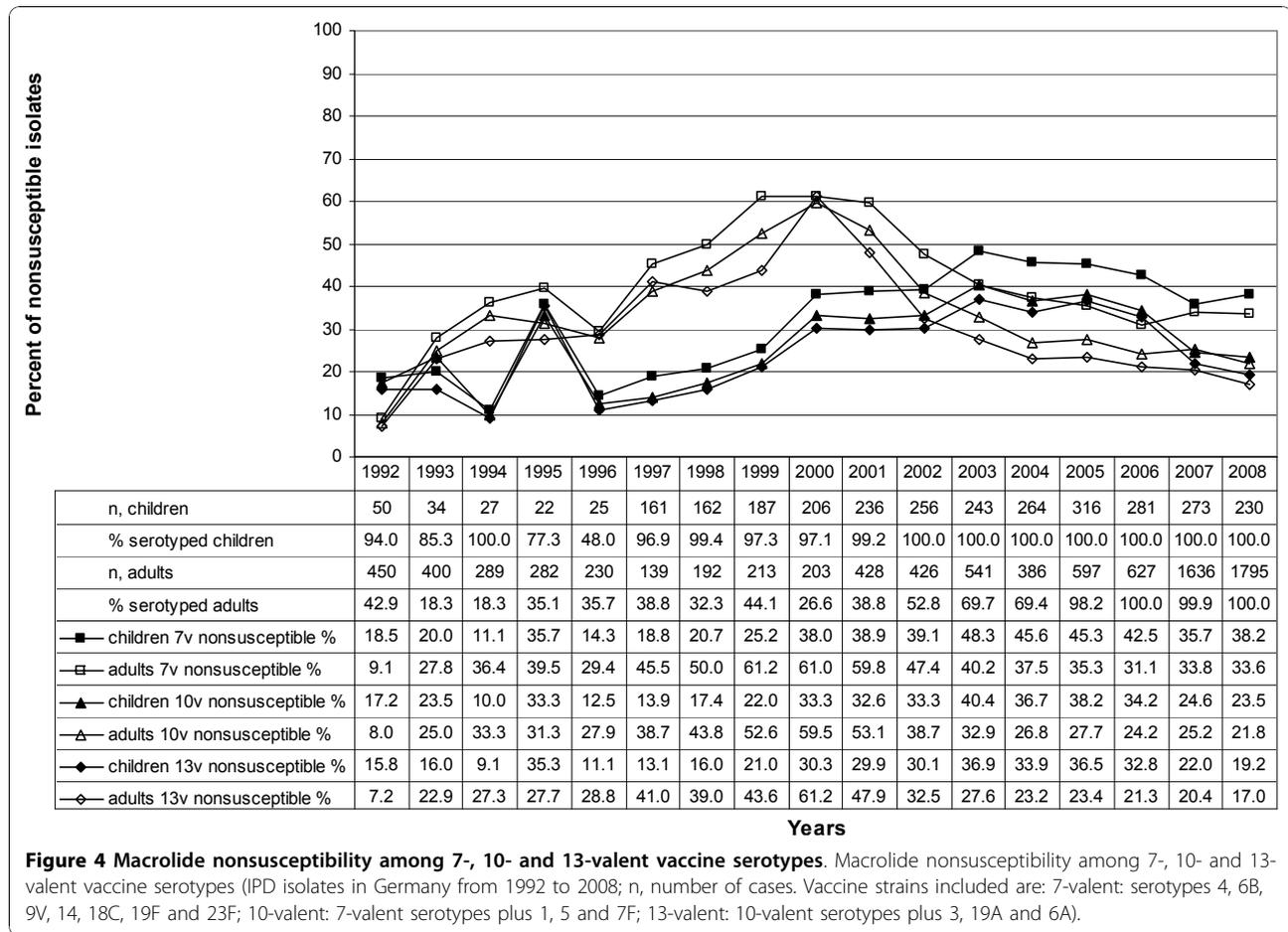


Figure 4 Macrolide nonsusceptibility among 7-, 10- and 13-valent vaccine serotypes. Macrolide nonsusceptibility among 7-, 10- and 13-valent vaccine serotypes (IPD isolates in Germany from 1992 to 2008; n, number of cases. Vaccine strains included are: 7-valent: serotypes 4, 6B, 9V, 14, 18C, 19F and 23F; 10-valent: 7-valent serotypes plus 1, 5 and 7F; 13-valent: 10-valent serotypes plus 3, 19A and 6A).

of pneumococcal resistance, differ vastly between European countries [15,21-23]. A decrease in the use of macrolides has been reported for instance in Spain [18], Portugal [24,25], Belgium [26], Slovenia [27] and Taiwan [28,29]. The influence of a decreased macrolide consumption on macrolide susceptibility is discussed controversially. In Spain a relation between the decrease in macrolide consumption and the decrease in erythromycin non-susceptibility among children could be shown, while this effect was absent among the adult population, probably due to the increase in non-vaccine serotypes such as 19A (from 3.6% of all invasive serotypes in 2000 to 10.1% in 2007) [18]. Reports from other countries showed no decrease in macrolide nonsusceptibility following a reduced macrolide consumption [25-29]. Besides the total macrolide consumption, the influence of long lasting macrolides, which may increase even in times of decreasing total macrolide consumption [25], is discussed to be a cause of the macrolide nonsusceptibility [25,30-32]. Besides antibiotics, pneumococcal conjugate vaccination is another important factor associated with changes in macrolide susceptibility [25,26,33-36].

In our study, high rates of serotype specific resistance among the more frequent serotypes were observed among the serotypes 14, 6B, 19F and 23F, in particular. These results are in line with results from Germany published previously by the NRCS for invasive [14] and respiratory tract isolates [37] and comparable to results reported from several European countries [3,13,38,39]. Notably, a statistically significant trend in increasing macrolide resistance was seen for serotypes 14 and 19F. However, since both serotypes are included in the pneumococcal conjugate vaccines, a future reduction of these serotypes can be expected. The low rate of macrolide nonsusceptibility among isolates not serotyped corresponds to the fact, that high resistance levels were a main trigger for initiation of serotyping during the early years of this study, when consistent serotyping of all isolates was not conducted due to excessive costs.

In spite of all these observations, because the impact of preventive and therapeutic strategies on pneumococcal evolution not only depends on, but also influences the serotype distribution, when normal temporal [11,40] and regional [15,41,42] variations of serotype distribution are

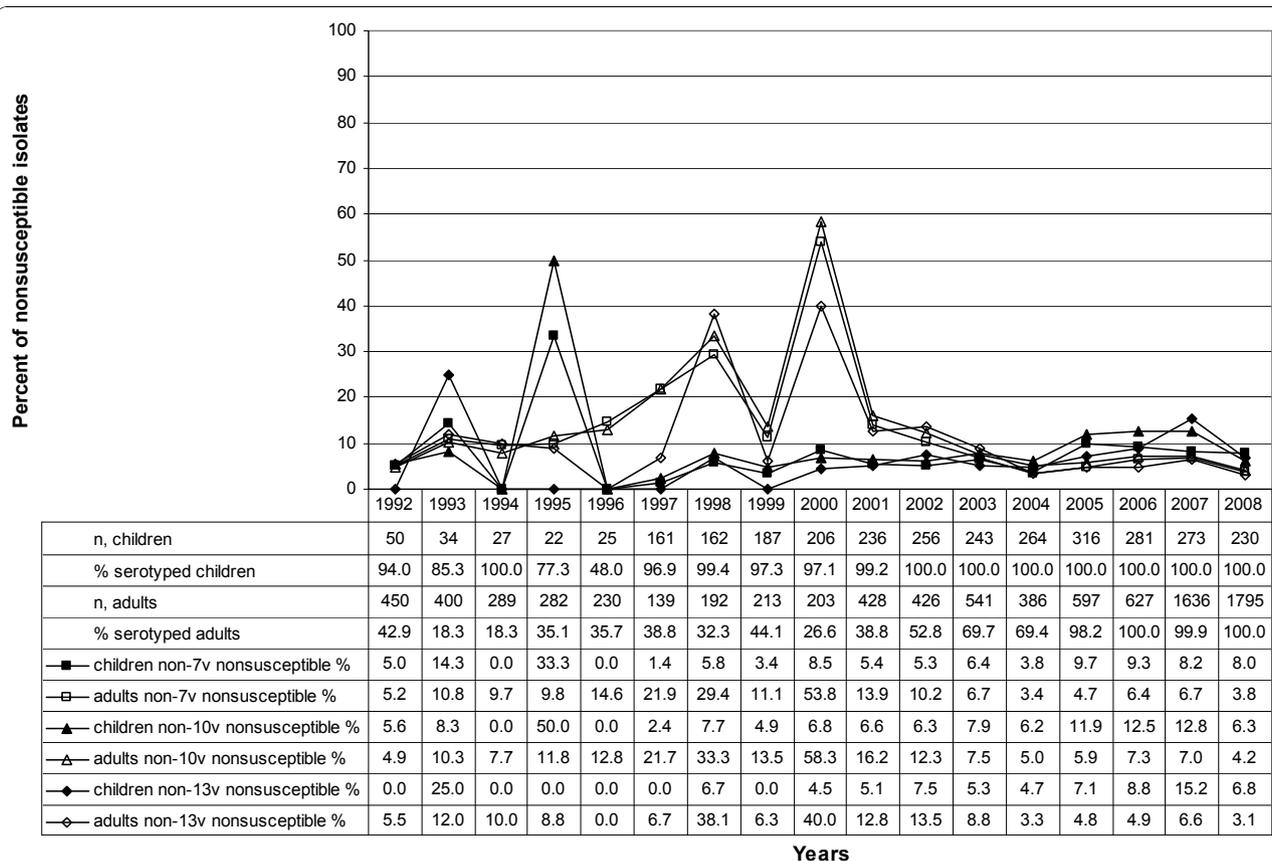


Figure 5 Macrolide nonsusceptibility among non 7-, non 10- and non 13-valent vaccine serotypes. Macrolide nonsusceptibility among non 7-, non 10- and non 13-valent vaccine serotypes (IPD isolates in Germany from 1992 to 2008; n, number of cases).

taken into consideration, future developments remain difficult to predict [32]. Ongoing nationwide surveillance is necessary to observe further developments of pneumococcal macrolide resistance in Germany.

Acknowledgements

We thank the microbiological laboratories in Germany for their cooperation and for providing the isolates. This study was supported, in part, by Wyeth Pharma GmbH, Germany.

Author details

¹National Reference Center for Streptococci, Department of Medical Microbiology, University Hospital RWTH Aachen, Pauwelsstrasse 30, D-52074 Aachen, Germany. ²Department of Medical Statistics, University Hospital RWTH Aachen, Pauwelsstrasse 30, D-52074 Aachen, Germany. ³Wyeth Vaccines Research, Paris La Défense, Paris, France.

Authors' contributions

MI performed the analysis and drafted the manuscript. CM performed the statistical analysis. MI, RRR and ML participated in the laboratory analyses. MI, RRR and ML conceived the study. All authors read and approved the final manuscript.

Received: 14 May 2010 Accepted: 25 November 2010
 Published: 25 November 2010

References

1. Austrian R: *Pneumococcus: the first one hundred years.* *Rev Infect Dis* 1981, **3**(2):183-189.
2. Musher DM: *Infections caused by Streptococcus pneumoniae: clinical spectrum, pathogenesis, immunity, and treatment.* *Clin Infect Dis* 1992, **14**(4):801-807.
3. Reinert RR, Ringelstein A, van der Linden M, Cil MY, Al-Lahham A, Schmitz FJ: *Molecular epidemiology of macrolide-resistant Streptococcus pneumoniae isolates in Europe.* *J Clin Microbiol* 2005, **43**(3):1294-1300.
4. Pallares R, Linares J, Vadillo M, Cabellos C, Manresa F, Viladrich PF, Martin R, Gudiol F: *Resistance to penicillin and cephalosporin and mortality from severe pneumococcal pneumonia in Barcelona, Spain.* *N Engl J Med* 1995, **333**(8):474-480.
5. Deeks SL, Palacio R, Ruvinsky R, Kertesz DA, Hortal M, Rossi A, Spika JS, Di Fabio JL: *Risk factors and course of illness among children with invasive penicillin-resistant Streptococcus pneumoniae. The Streptococcus pneumoniae Working Group.* *Pediatrics* 1999, **103**(2):409-413.
6. Yu VL, Chiou CC, Feldman C, Orqvist A, Rello J, Morris AJ, Baddour LM, Luna CM, Snyderman DR, Ip M, et al: *An international prospective study of pneumococcal bacteremia: correlation with in vitro resistance, antibiotics administered, and clinical outcome.* *Clin Infect Dis* 2003, **37**(2):230-237.
7. Clinical Laboratory Standards Institute: *Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement.* Wayne, PA; 2008.
8. Imöhl M, Reinert RR, van der Linden M: *New penicillin susceptibility breakpoints for Streptococcus pneumoniae and their effects on susceptibility categorisation in Germany (1992-2008).* *Int J Antimicrob Agents* 2009, **34**(3):271-273.

9. Daneman N, McGeer A, Green K, Low DE: **Macrolide resistance in bacteremic pneumococcal disease: implications for patient management.** *Clin Infect Dis* 2006, **43**(4):432-438.
10. Imöhl M, Reinert RR, van der Linden M: **Temporal Variations among Invasive Pneumococcal Disease Serotypes in Children and Adults in Germany (1992-2008).** *Int J Microbiol* 2010, **2010**, 874189.
11. Jacobs MR, Good CE, Beall B, Bajaksouzian S, Windau AR, Whitney CG: **Changes in serotypes and antimicrobial susceptibility of invasive *Streptococcus pneumoniae* strains in Cleveland: a quarter century of experience.** *J Clin Microbiol* 2008, **46**(3):982-990.
12. Adam D: **Global antibiotic resistance in *Streptococcus pneumoniae*.** *J Antimicrob Chemother* 2002, **50**(Suppl):1-5.
13. Reinert RR, Reinert S, van der Linden M, Cil MY, Al-Lahham A, Appelbaum P: **Antimicrobial susceptibility of *Streptococcus pneumoniae* in eight European countries from 2001 to 2003.** *Antimicrob Agents Chemother* 2005, **49**(7):2903-2913.
14. Reinert RR, Al-Lahham A, Lemperle M, Tenholte C, Briefs C, Haupts S, Gerards HH, Lutticken R: **Emergence of macrolide and penicillin resistance among invasive pneumococcal isolates in Germany.** *J Antimicrob Chemother* 2002, **49**(1):61-68.
15. Reinert RR: **Pneumococcal conjugate vaccines—a European perspective.** *Int J Med Microbiol* 2004, **294**(5):277-294.
16. Kaufhold A: **Antibiotikaresistenz von *Streptococcus pneumoniae* (Pneumokokken).** *Med Klin* 1988, **83**:723-726.
17. Reinert RR, Lütticken R, Kaufhold A: **Aktuelle Daten zur Antibiotikaempfindlichkeit von *Streptococcus pneumoniae* (Pneumokokken). Die Bedeutung von penicillinresistenten Isolat.** *Med Klin* 1993, **88**(6):357-361.
18. Fenoll A, Aguilar L, Granizo JJ, Gimenez MJ, Aragonese-Fenoll L, Mendez C, Tarrago D: **Has the licensing of respiratory quinolones for adults and the 7-valent pneumococcal conjugate vaccine (PCV-7) for children had herd effects with respect to antimicrobial non-susceptibility in invasive *Streptococcus pneumoniae*?** *J Antimicrob Chemother* 2008, **62**(6):1430-1433.
19. Imöhl M, Reinert RR, Ocklenburg C, van der Linden M: **Association of serotypes of *Streptococcus pneumoniae* with age in invasive pneumococcal disease.** *J Clin Microbiol* 2010, **48**(4):1291-1296.
20. Imöhl M, van der Linden M, Mutscher C, Reinert RR: **Serotype distribution of invasive pneumococcal disease during the first 60 days of life.** *Vaccine* 2010, **28**(30):4758-4762.
21. Coenen S, Muller A, Adriaenssens N, Vankerckhoven V, Hendrickx E, Goossens H: **European Surveillance of Antimicrobial Consumption (ESAC): outpatient parenteral antibiotic treatment in Europe.** *J Antimicrob Chemother* 2009, **64**(1):200-205.
22. van de Sande-Bruinsma N, Grundmann H, Verloo D, Tiemersma E, Monen J, Goossens H, Ferech M: **Antimicrobial drug use and resistance in Europe.** *Emerg Infect Dis* 2008, **14**(11):1722-1730.
23. Goossens H, Ferech M, Coenen S, Stephens P: **Comparison of outpatient systemic antibacterial use in 2004 in the United States and 27 European countries.** *Clin Infect Dis* 2007, **44**(8):1091-1095.
24. Dias R, Canica M: **Invasive pneumococcal disease in Portugal prior to and after the introduction of pneumococcal heptavalent conjugate vaccine.** *FEMS Immunol Med Microbiol* 2007, **51**(1):35-42.
25. Dias R, Canica M: **Trends in resistance to penicillin and erythromycin of invasive pneumococci in Portugal.** *Epidemiol Infect* 2008, **136**(7):928-939.
26. Van Eldere J, Mera RM, Miller LA, Poupard JA, Amrine-Madsen H: **Risk factors for development of multiple-class resistance to *Streptococcus pneumoniae* Strains in Belgium over a 10-year period: antimicrobial consumption, population density, and geographic location.** *Antimicrob Agents Chemother* 2007, **51**(10):3491-3497.
27. Cizman M, Beovic B, Seme K, Paragi M, Strumbelj I, Muller-Premru M, Cad-Pecar S, Pokorn M: **Macrolide resistance rates in respiratory pathogens in Slovenia following reduced macrolide use.** *Int J Antimicrob Agents* 2006, **28**(6):537-542.
28. Hsueh PR: **Decreasing rates of resistance to penicillin, but not erythromycin, in *Streptococcus pneumoniae* after introduction of a policy to restrict antibiotic usage in Taiwan.** *Clin Microbiol Infect* 2005, **11**(11):925-927.
29. Hsueh PR, Shyr JM, Wu JJ: **Changes in macrolide resistance among respiratory pathogens after decreased erythromycin consumption in Taiwan.** *Clin Microbiol Infect* 2006, **12**(3):296-298.
30. Bergman M, Huikko S, Huovinen P, Paakkari P, Seppala H: **Macrolide and azithromycin use are linked to increased macrolide resistance in *Streptococcus pneumoniae*.** *Antimicrob Agents Chemother* 2006, **50**(11):3646-3650.
31. Arason VA, Sigurdsson JA, Erlendsdottir H, Gudmundsson S, Kristinsson KG: **The role of antimicrobial use in the epidemiology of resistant pneumococci: A 10-year follow up.** *Microb Drug Resist* 2006, **12**(3):169-176.
32. Fenoll A, Granizo JJ, Aguilar L, Gimenez MJ, Aragonese-Fenoll L, Hanquet G, Casal J, Tarrago D: **Temporal trends of invasive *Streptococcus pneumoniae* serotypes and antimicrobial resistance patterns in Spain from 1979 to 2007.** *J Clin Microbiol* 2009, **47**(4):1012-1020.
33. Kyaw MH, Lynfield R, Schaffner W, Craig AS, Hadler J, Reingold A, Thomas AR, Harrison LH, Bennett NM, Farley MM, et al: **Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*.** *N Engl J Med* 2006, **354**(14):1455-1463.
34. Calbo E, Diaz A, Canadell E, Fabrega J, Uriz S, Xercavins M, Morera MA, Cuchi E, Rodriguez-Carballeira M, Garau J: **Invasive pneumococcal disease among children in a health district of Barcelona: early impact of pneumococcal conjugate vaccine.** *Clin Microbiol Infect* 2006, **12**(9):867-872.
35. Reinert RR: **The antimicrobial resistance profile of *Streptococcus pneumoniae*.** *Clin Microbiol Infect* 2009, **15**(Suppl 3):7-11.
36. Hanage WP, Huang SS, Lipsitch M, Bishop CJ, Godoy D, Pelton SI, Goldstein R, Huot H, Finkelstein JA: **Diversity and antibiotic resistance among nonvaccine serotypes of *Streptococcus pneumoniae* carriage isolates in the post-heptavalent conjugate vaccine era.** *J Infect Dis* 2007, **195**(3):347-352.
37. Reinert RR, Lutticken R, Reinert S, Al-Lahham A, Lemmen S: **Antimicrobial resistance of *Streptococcus pneumoniae* isolates of outpatients in Germany, 1999-2000.** *Chemotherapy* 2004, **50**(4):184-189.
38. Garcia-Suarez Mdel M, Villaverde R, Caldevilla AF, Mendez FJ, Vazquez F: **Serotype distribution and antimicrobial resistance of invasive and non-invasive pneumococcal isolates in Asturias, Spain.** *Jpn J Infect Dis* 2006, **59**(5):299-205.
39. Clarke SC, Scott KJ, McChlery SM: **Erythromycin resistance in invasive serotype 14 pneumococci is highly related to clonal type.** *J Med Microbiol* 2004, **53**(Pt 11):1101-1103.
40. Feikin DR, Klugman KP: **Historical changes in pneumococcal serogroup distribution: implications for the era of pneumococcal conjugate vaccines.** *Clin Infect Dis* 2002, **35**(5):547-555.
41. Feikin DR, Klugman KP, Facklam RR, Zell ER, Schuchat A, Whitney CG: **Increased prevalence of pediatric pneumococcal serotypes in elderly adults.** *Clin Infect Dis* 2005, **41**(4):481-487.
42. Imöhl M, Reinert RR, van der Linden M: **Regional differences in serotype distribution, pneumococcal vaccine coverage, and antimicrobial resistance of invasive pneumococcal disease among German federal states.** *Int J Med Microbiol* 2010, **300**(4):237-47.

doi:10.1186/1471-2180-10-299

Cite this article as: Imöhl et al.: Macrolide susceptibility and serotype specific macrolide resistance of invasive isolates of *Streptococcus pneumoniae* in Germany from 1992 to 2008. *BMC Microbiology* 2010 **10**:299.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

