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# Antimicrobial Resistance of *Streptococcus pneumoniae* Recovered from Outpatients with Respiratory Tract Infections in Germany from 1998 to 1999: Results of a National Surveillance Study

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**Clinically significant pneumococcal isolates were prospectively collected from outpatients with respiratory tract infections by 19 different clinical microbiology laboratories in Germany. Resistance rates in a total of 961 isolates were as follows: penicillin, 6.6%; clarithromycin, 10.6%; tetracycline, 13.9%; and levofloxacin, 0.1%. Among 324 isolates from children, pneumococcal serotypes 19F (17.0%), 23F (13.0%), and 6B (11.7%) were the predominant types.**

Prior to the early 1990s, penicillin resistance remained uncommon among clinical isolates of *Streptococcus pneumoniae* in Germany despite the emergence of this problem in many parts of Europe (4, 5, 7). For Germany the resistance profile of *S. pneumoniae* isolated from patients with invasive disease is well documented (11, 15), but data on pneumococcal respiratory tract infections are scant to date. The aim of this multicenter study was to determine the prevalence of antimicrobial resistance among clinical isolates of *S. pneumoniae* during the winter of 1998 to 1999.

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A total of 961 isolates of *S. pneumoniae* were collected from 19 different medical microbiology laboratories. All strains were isolated from presumed respiratory tract infections of nonhospitalized patients and represented either colonizers or true pathogens of respiratory tract infections. MICs were determined by microbroth dilution as recommended by the National Committee for Clinical Laboratory Standards (8). For amplification of *tetM*, the primers originally published by Provvedi et al. (16) with the sequences 5′ TGG AAT TGA TTT ATC AAC GG 3′ (positions 2496 to 2515) and 5′ TTC CAA CCA TAC AAT CCT TG 3′ (positions 3575 to 3556) were used. For amplification of *tet*O, the primers 5′ GGC ACA GAC CCG TAT ACT GTT 3′ (positions 442 to 462) and 5′ TTA AAA GAG GGT CGC CAT CTG 3′ (positions 1230 to 1250) were used (16, 17). For detection of *erm* and *mef*, the primers described by Trieu-Cuot et al. (14) and Tait-Kamradt et al. (12) with the sequences (*erm*) 5′ CGA GTG AAA AAG TAC TCA ACC 3′ (positions 362 to 382), 5′ GGC GTG TTT CAT TGC

TABLE 1. MIC range, $MIC_{90}$, and resistance rate of 961 pneumococcal respiratory tract isolates in Germany, 1998-1999

| Antimicrobial agent | MIC range (mg/liter) | $MIC_{90}$ (mg/liter) | I[a] (%) | R[a] (%) |
|---|---|---|---|---|
| Penicillin G | ≤008–2 | 06 | 6.3 | 0.3 |
| Amoxicillin/ clavulanic acid | ≤008–1 | 06 | 0.6 | 0 |
| Cefuroxime | ≤008–4 | 0.125 | 0.4 | 0.6 |
| Clarithromycin | 03–≥32 | 0.5 | 3.0 | 7.6 |
| Tetracycline | 03–≥32 | 16 | 0.8 | 13.1 |
| Levofloxacin | 06–8 | 1.0 | 0 | 0.1 |
| Ciprofloxacin[b] | 0.25–8 | 1.0 | N.D. | N.D. |

[a] Breakpoints (I, intermediate; R, resistant) according to the National Committee for Clinical Laboratory Standards (NCCLS) (8). Penicillin G: 0.12–1 mg/liter, ≥2 mg/liter; amoxicillin/clavulanic acid: 4/2 mg/liter, ≥8/4 mg/liter; cefuroxime: 2 mg/liter, ≥4 mg/liter; clarithromycin: 0.5 mg/liter, ≥1 mg/liter; tetracycline: 4 mg/liter, ≥8 mg/liter; levofloxacin: 4 mg/liter, ≥8 mg/liter.

[b] Ciprofloxacin breakpoints are not available from NCCLS. N.D., no data.

TABLE 2. Penicillin G and clarithromycin resistance in pneumococcal isolates from 17 medical centers[a] in Germany

| Town | No. of isolates | Region[b] | Penicillin G | | Clarithromycin | |
|---|---|---|---|---|---|---|
| | | | I[c] (%) | R[c] (%) | I[c] (%) | R[c] (%) |
| Hamburg | 49 | N | 6.1 | 0 | 6.1 | 8.2 |
| Osnabrück | 64 | N | 10.9 | 0 | 6.3 | 9.4 |
| Plön | 22 | N | 4.6 | 4.6 | 4.6 | 9.1 |
| Trier | 57 | C-W | 0 | 0 | 1.8 | 7.0 |
| Wülfrath | 98 | C-W | 3.1 | 0 | 3.1 | 8.2 |
| Bonn | 47 | C-W | 6.4 | 0 | 0 | 21.3 |
| Dillingen | 43 | C-W | 14 | 0 | 9.3 | 11.6 |
| Weiden | 41 | C-W | 9.8 | 4.9 | 0 | 4.9 |
| Dortmund | 56 | C-W | 14.3 | 0 | 3.60 | 12.5 |
| Leverkusen | 109 | C-W | 5.5 | 0 | 5.5 | 8.3 |
| Aachen | 61 | C-W | 3.3 | 0 | 0 | 1.6 |
| Bad Hersfeld | 66 | E | 6.1 | 0 | 0 | 1.5 |
| Chemnitz | 18 | E | 5.6 | 0 | 0 | 11.1 |
| Berlin I | 33 | E | 12.1 | 0 | 0 | 15.2 |
| Berlin II | 13 | E | 0 | 0 | 0 | 15.4 |
| Augsburg | 89 | S | 3.4 | 0 | 1.1 | 1.1 |
| Stuttgart | 83 | S | 6.0 | 0 | 3.6 | 4.8 |

[a] Centers with less than 10 isolates ($n = 2$) were not evaluated.

[b] Centers were categorized in the following geographic regions: N, north Germany; C-W, central-western Germany; E, eastern Germany; S, southern Germany. For cumulative resistance data, see text.

[c] Breakpoints (I, R) according to NCCLS (8). Penicillin G: I, 0.1–1 mg/liter; R, ≥2 mg/liter. Clarithromycin: I, 0.5 mg/liter; R, ≥1 mg/liter.

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TABLE 3. Serotype distribution of pneumococcal respiratory tract isolates in Germany

| Serotype | No. (%) of all strains | Subgroup of strains | | | | | |
|---|---|---|---|---|---|---|---|
| | | No. (%) of children[a] | No. (%) of children with otitis media | No. (%) of adults[b] | Strains with resistance [no. (%)] to: | | |
| | | | | | Penicillin G[c] | Clarithromycin | Tetracycline |
| 1 | 4 (1.0) | 3 (0.9) | 3 (4.3) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) |
| 3 | 15 (3.6) | 14 (4.3) | 5 (7.2) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) |
| 4 | 4 (1.0) | 4 (1.2) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 5 | 2 (0.5) | 2 (0.6) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 6A | 41 (9.9) | 37 (11.4) | 4 (5.8) | 2 (2.7) | 5 (8.1) | 3 (4.4) | 3 (3.3) |
| 6B | 51 (12.3) | 38 (11.7) | 5 (7.2) | 14 (18.9) | 17 (27.4) | 19 (27.9) | 23 (25.0) |
| 7F | 3 (0.7) | 3 (0.9) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 8 | 3 (0.7) | 2 (0.6) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 9A | 3 (0.7) | 2 (0.6) | 1 (1.4) | 1 (1.4) | 1 (1.6) | 0 (0) | 0 (0) |
| 9L | 3 (0.7) | 1 (0.3) | 0 (0) | 2 (2.7) | 0 (0) | 0 (0) | 1 (1.1) |
| 9N | 4 (1.0) | 3 (0.9) | 0 (0) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) |
| 9V | 8 (1.9) | 7 (2.2) | 2 (2.9) | 1 (1.4) | 4 (6.5) | 1 (1.5) | 0 (0) |
| 10A | 10 (2.4) | 9 (2.8) | 1 (1.4) | 1 (1.4) | 1 (1.6) | 0 (0) | 0 (0) |
| 11A | 13 (3.1) | 10 (3.1) | 1 (1.4) | 3 (4.1) | 1 (1.6) | 0 (0) | 0 (0) |
| 11C | 1 (0.2) | 1 (0.3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 12F | 3 (0.7) | 2 (0.6) | 0 (0) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) |
| 13 | 3 (0.7) | 1 (0.3) | 0 (0) | 2 (2.7) | 1 (1.6) | 0 (0) | 0 (0) |
| 14 | 17 (4.1) | 15 (4.6) | 5 (7.2) | 2 (2.7) | 1 (1.6) | 8 (11.8) | 2 (2.2) |
| 15A | 6 (1.4) | 3 (0.9) | 2 (2.9) | 1 (1.4) | 2 (3.2) | 1 (1.5) | 2 (2.2) |
| 15B | 9 (2.2) | 7 (2.2) | 2 (2.9) | 2 (2.7) | 2 (3.2) | 0 (0) | 0 (0) |
| 15C | 5 (1.2) | 5 (1.5) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 15F | 5 (1.2) | 3 (0.9) | 0 (0) | 1 (1.4) | 1 (1.6) | 1 (1.5) | 1 (1.1) |
| 16F | 3 (0.7) | 3 (0.9) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 17F | 5 (1.2) | 5 (1.5) | 0 (0) | 0 (0) | 2 (3.2) | 0 (0) | 0 (0) |
| 18B | 1 (0.2) | 0 (0) | 0 (0) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) |
| 18C | 7 (1.7) | 7 (2.2) | 4 (5.8) | 0 (0) | 0 (0) | 1 (1.5) | 0 (0) |
| 19A | 13 (3.1) | 9 (2.8) | 4 (5.8) | 4 (5.4) | 7 (11.3) | 4 (5.9) | 4 (4.3) |
| 19B | 1 (0.2) | 1 (0.3) | 0 (0) | 0 (0) | 0 (0) | 1 (1.5) | 1 (1.1) |
| 19F | 76 (18.3) | 55 (17.0) | 15 (21.7) | 14 (18.9) | 7 (11.3) | 11 (16.2) | 29 (31.5) |
| 21 | 3 (0.7) | 1 (0.3) | 0 (0) | 1 (1.4) | 2 (3.2) | 0 (0) | 1 (1.1) |
| 22F | 3 (0.7) | 3 (0.9) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 23A | 3 (0.7) | 3 (0.9) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 23F | 58 (14.0) | 42 (13.0) | 8 (11.6) | 13 (17.6) | 6 (9.7) | 15 (22.1) | 19 (20.7) |
| 24A | 1 (0.2) | 0 (0) | 0 (0) | 0 (0) | 1 (1.6) | 1 (1.5) | 1 (1.1) |
| 24F | 2 (0.5) | 1 (0.3) | 0 (0) | 1 (1.4) | 0 (0) | 0 (0) | 1 (1.1) |
| 28A | 1 (0.2) | 1 (0.3) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 31 | 1 (0.2) | 1 (0.3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 33C | 1 (0.2) | 1 (0.3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 33F | 2 (0.5) | 2 (0.6) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 34 | 1 (0.2) | 1 (0.3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 35C | 1 (0.2) | 1 (0.3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 35F | 3 (0.7) | 2 (0.6) | 0 (0) | 0 (0) | 1 (1.6) | 0 (0) | 0 (0) |
| 38 | 3 (0.7) | 3 (0.9) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 46 | 1 (0.2) | 0 (0) | 0 (0) | 1 (1.4) | 0 (0) | 1 (1.5) | 1 (1.1) |
| Rough | 12 (2.9) | 10 (3.1) | 1 (1.4) | 3 (4.1) | 0 (0) | 1 (1.5) | 3 (3.3) |
| Total | 415 (100) | 324 (100) | 69 (100)[d] | 74 (100) | 62 (100) | 68 (100) | 92 (100) |

[a] Children <5 years of age.
[b] Individuals >18 years of age.
[c] Strains include penicillin-intermediate and -resistant isolates.
[d] Coverage of 23-valent vaccine, 81.1%

TTG ATG (positions 978 to 958), and the sequences (*mef*) 5′ AGT ATC ATT AAT CAC TAG TGC 3′ (positions 57 to 77), and 5′ GTA ATA GAT GCA ATC ACA GC 3′ (positions 551 to 532) were chosen. Pneumococcal strains were serotyped by Neufeld's Quellung reaction using type and factor sera provided by the Statens Serum Institut, Copenhagen, Denmark (9).

Strains were isolated from the following sources: nasopharyngeal swabs ($n$ = 443 [46.1%]), ear swabs ($n$ = 181 [18.3%]), sputum ($n$ = 110 [11.5%]), throat swabs ($n$ = 99 [10.3%]), bronchial secretions or lavages ($n$ = 35 [3.7%]), sinus punctures ($n$ = 28 [2.9%]), eye swabs ($n$ = 31 [3.2%]), and other sources ($n$ = 34 [2.8%]). Pneumococci were predominantly isolated from infants and young children ≤5 years of age ($n$ = 358 [37.3% of cases]) and from children aged 5 to 10 years ($n$ = 136 [14.2% of cases]). Of the isolates, 93.4% were found to be susceptible to penicillin G, and 6.2% were penicillin intermediate. Three strains (0.3%) were highly resistant to penicillin (MIC ≥ 2 mg/liter). Reduced sensitivity to clarithromycin was detected in 10.6% of the strains (Table 1). In children <5 years of age ($n$ = 358) the following resistance rates were observed:

penicillin G, 7.6% (intermediate and resistant strains); amoxicillin-clavulanic acid, 1.1%; cefuroxime, 2.0%; and clarithromycin, 9.2% (intermediate and resistant strains). In centers where more than 10 isolates were included in the study ($n$ = 17), the combined percentages of intermediate and resistant strains varied between 0 and 14.7% and 1.5 and 21.3% for penicillin G and clarithromycin, respectively (Table 2). Centers were categorized as belonging to one of four geographic regions in Germany. The resistance rates (intermediate and resistance strains) observed for penicillin G and clarithromycin respectively in these regions were as follows: northern Germany, 8.3 and 14.6%; central to western Germany, 6.6 and 12.1%; eastern Germany, 6.9 and 7.7%; and southern Germany, 4.6 and 5.1%.

Serotyping of penicillin nonsensitive strains showed pneomococcal serotypes 6B (27.4%), 19A (11.3%), 19F (11.3%), and 23F (9.7%) to be predominant. Clarithromycin-resistant ($n$ = 73) strains were analyzed for the underlying resistance determinants. Of these strains, 54 (74.0%) and 15 (20.5%) belonged to the *erm* and *mef* types of resistance, respectively. Four strains showed no PCR product in repeated assays. Of 126 tetracycline-resistant strains, 64 were characterized for underlying resistance mechanisms. Of these strains, 62 of 64 (96.9%) belonged to the *tetM* genotype. Only two of 64 strains (3.3%) showed the *tetO* resistance genotype. Of the 92 tetracycline-resistant strains available for serotyping, 31.5% belonged to the pneumococcal serotype 19F, 20.7% to the serotype 23F, and 25% to the serotype 6B. A total of 415 strains were serotyped. In order of decreasing frequency, 19F (18.3%), 23F (14.0%), 6B (12.3%), and 6A (9.9%) were the predominant serotypes. Separate analyses were performed for serotype distribution in certain age groups and for antibiotic-resistant strains. Among 324 isolates from children who were ≤5 years of age, serotypes 19F (17.0%), 23F (13.0%), and 6B (11.7%) were the leading types. (Table 3).

The resistance rate to penicillin G found in respiratory tract infections was highly comparable to that reported from pneumococcal invasive disease in both children (15) and adults (11). One remarkable finding of the present investigation is that resistance rates showed pronounced variations between geographic regions and study centers within Germany. Major differences in beta-lactam resistance between children and adults have been reported in many other countries, and young age has been shown to be a risk factor for beta-lactam-resistant pneumococcal infection (2). In Germany only slight differences in beta-lactam resistance rates between children and adults were recorded. As in many other countries, macrolide resistance has now overcome the level of beta-lactam resistance in Germany (1). In Germany macrolide resistance is mainly due to the high prevalence of pneumococci expressing an rRNA methylase ($MLS_B$ phenotypes [74% of macrolide-resistant strains]). The prevalence of these resistance genotypes is subject to major variations between countries (3, 6). In four strains the resistance determinant could not be identified. These strains need further investigation and may possess a yet unknown resistance determinant or may have mutations in 23S rRNA and ribosomal protein L4 (13). Levofloxacin resistance is extremely rare in Germany, and only one serotype 6B strain exhibiting an MIC of 8 mg/liter was isolated in this study, which is the first to offer data on the serotype distribution of noninvasive pneumococcal disease in Germany. Among 324 strains from children <5 years of age, the coverage of the seven-valent pneumococcal conjugate vaccine was 51.9% (66.0% for the seven-valent vaccine plus cross-reactive serotypes 6A and 19A).

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