Phenotypes of macrolide resistance of group A streptococci isolated from outpatients in Bavaria and susceptibility to 16 antibiotics

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The purpose of the present study was to determine the antimicrobial resistance among Streptococcus pyogenes in Bavaria, Germany. Five hundred and forty isolates of S. pyogenes were collected from patients with tonsillopharyngitis. Of these, 425 isolates were obtained from children and 115 from adult patients. All isolates were tested for susceptibility to macrolides, clindamycin, penicillin and 10 other commonly prescribed antimicrobial agents, using broth microdilution tests. All isolates were fully susceptible to penicillin, amoxicillin and cephalosporins; 16.1% of the isolates were resistant to tetracycline. MIC90 values of erythromycin, clarithromycin, azithromycin and josamycin were 16, 4, 16 and 0.5 mg/L. The overall resistance rate of S. pyogenes to erythromycin, clarithromycin and azithromycin was 13.3%. All isolates resistant to erythromycin were also resistant to clarithromycin and azithromycin, and vice versa. Erythromycin resistance rates were higher in adult patients (19.1%) than in children (11.8%). The resistance rate to josamycin was only 1.5%, a value similar to that of clindamycin (1.1%). Among the 72 erythromycin-resistant isolates the M phenotype of macrolide resistance predominated (78%), while percentages of cMLSβ (8%) and iMLSβ (14%) phenotypes were low. Of the iMLSβ strains (n = 10), the majority were of the subtype C (n = 8). The M phenotype was associated with a low, and the iMLSβ-C phenotype with a high, rate of resistance to tetracycline. Conclusively, present data point to rising macrolide resistance among S. pyogenes in Bavaria.

Keywords: S. pyogenes, Bavaria, susceptibility, macrolide resistance, phenotypes

Introduction

In the 1980s and 1990s reports of serious diseases caused by Streptococcus pyogenes [S. pyogenes, group A streptococci (GAS)] have again drawn attention to this common pathogen.1 Up to now, S. pyogenes has remained fully susceptible to penicillin,2 the treatment of choice in patients with S. pyogenes tonsillopharyngitis. Macrolides are treatment alternatives to penicillin, valuable especially in patients allergic to β-lactams. However, due to general use of these efficient agents, emerging resistance of S. pyogenes to macrolides has been observed in many countries,3–7 and is now the focus of much interest.

Different phenotypes of macrolide resistance have been recognized, indicating the response of individual strains of S. pyogenes to different macrolides and clindamycin. Strains of the M (or NR) phenotype are resistant to 14- and 15-membered macrolides, due to the presence of mef(A) genes, conferring efflux of the antibiotic out of the bacterial cell.8 These strains are, however, susceptible to 16-membered macrolides and clindamycin. erm genes, enabling target site modification by methylation and resistance to macrolides, lincosamides and streptogramin B, confer the MLSB type of resistance.9 MLSB resistance can be constitutive (cMLSβ or CR) or inducible (iMLSβ or IR).10 iMLSβ strains are differentiated into three subtypes (iMLSβ-A, iMLSβ-B and iMLSβ-C).11
Subtype A is characterized by constitutive high resistance (MICs ≥ 128 mg/L) to 14-, 15- and 16-membered macrolides, and inducible resistance to the lincosamide clindamycin. Subtype B has constitutive high resistance to 14- and 15-membered macrolides, and inducible resistance to josamycin and clindamycin. In addition to target-site modification, strains of subtype B possess a newly discovered efflux system, different from the efflux mediated by mef(A) genes. This novel mechanism, contributing to macrolide resistance in iMLS$_{P}$-B strains, has not been found in iMLS$_{P}$-C strains that have constitutive low resistance (MICs ≤ 32 mg/L) to the 14- and 15-membered macrolides, and inducible resistance to josamycin and clindamycin.

The occurrence of macrolide resistance was shown to be connected with increased macrolide consumption. Emerging resistance rates, up to ~50%, have been reported for various countries and geographical regions. However, data on macrolide resistance of S. pyogenes in Germany are relatively scarce. In 1996 and 1997, resistance rates of 4% and 1.6% were reported for diverse German regions. In contrast to these relatively low rates, macrolide resistance rates of 12.7% were reported for Berlin in 2000 and of 7.9% for the region of Aachen in 2001.

In order to evaluate antimicrobial resistance among S. pyogenes in Bavaria, 540 isolates from outpatients in 20 communities were collected. All isolates were tested for susceptibility to 16 antimicrobial agents, and the erythromycin-resistant strains were examined for their phenotypes of macrolide resistance.

### Materials and methods

**Bacterial strains**

A total of 540 isolates of S. pyogenes were collected from outpatients by general practitioners and paediatricians in 20 communities in Bavaria, Germany, from December 1999 to December 2000. Of these, 425 isolates were obtained from children and 115 from adult patients. All isolates were recovered from throat swabs of patients with symptoms of tonsillopharyngitis. Presence of S. pyogenes was detected by rapid immunoassay (Abbott, Chicago, IL, USA). The swabs were sent to our laboratory, where strains were isolated and identified by standard laboratory methods. The isolates were stored at −180°C, and subcultured on blood agar before susceptibility testing.

**Susceptibility testing**

MICs were determined of erythromycin, clarithromycin (Abbott, Vienna, Austria), azithromycin (Pfizer, Latina, Italy), penicillin, amoxicillin, ceftriaxone, josamycin (Biochemie, Kundl, Austria), clindamycin, linezolid (Pharmacia & Upjohn, Crawley, UK), cefaclor, vancomycin (Lilly, Giessen, Germany), cefuroxime (Eli Lilly, Vienna, Austria), cefpodoxime (Sankyo, Vienna, Austria), ciprofloxacin (Bayer, Leverkusen, Germany), levofloxacin (Aventis Pharma, Bridgewater, NJ, USA) and tetracycline (Sigma, St Louis, MO, USA). The broth microdilution method was used according to the recommendations of the NCCLS. Mueller–Hinton broth (Merck, Darmstadt, Germany), supplemented with 3% lysed horse blood, was used as liquid growth medium. Panels were inoculated with bacteria to achieve a final concentration of 5 × 10$^5$ cfu/mL. The antibiotic solutions were prepared from two-fold log$_2$ dilutions, and tested at final concentrations ranging from 0.004 to 256 mg/L. *Streptococcus pneumoniae* ATCC 49619 was used as a quality control strain. After 20 h of incubation in ambient air, MICs were recorded as the lowest concentration permitting no visible growth. MIC breakpoints suggested by the NCCLS were used for penicillin (susceptible, ≤ 0.12 mg/L; resistant, ≥ 4 mg/L), erythromycin, clarithromycin, clindamycin (susceptible, ≤ 0.25 mg/L; resistant, ≥ 1 mg/L), ampicillin (susceptible, ≤ 0.25 mg/L; resistant, ≥ 8 mg/L), ceftriaxone, azithromycin (susceptible, ≤ 0.5 mg/L; resistant, ≥ 2 mg/L), vancomycin (susceptible, ≤ 1 mg/L), ciprofloxacin and tetracycline (susceptible, ≤ 2 mg/L; resistant, ≥ 8 mg/L). MIC breakpoints suggested by the French Society for Microbiology were used for josamycin (susceptible, ≤ 1 mg/L; resistant, ≥ 4 mg/L). In the absence of established NCCLS breakpoints, the breakpoints of ceftriaxone were applied tentatively to cefaclor, cefpodoxime and cefuroxime, the breakpoints of ciprofloxacin were applied to levofloxacin, and the breakpoints of ampicillin were applied to amoxicillin. The NCCLS quality control limit of linezolid MICs for *S. pneumoniae* ATCC 49619 (0.5–2 mg/L) was used for determination of linezolid susceptibility.

**Determination of the phenotypes of macrolide resistance**

The phenotypes and subtypes of macrolide resistance were determined by a triple-disc diffusion test, as described by Giovanetti et al.

### Results

#### β-Lactams, fluoroquinolones, linezolid, vancomycin and tetracycline

Table 1 gives the results of susceptibility testing of 540 *S. pyogenes* isolates in vitro, and shows the MIC range and the calculated MIC$_{50}$ and MIC$_{90}$ values of antibiotics tested. All isolates were fully susceptible to penicillin and to amoxicillin. The penicillin concentrations at which 50% and 90% of the strains were inhibited (MIC$_{50}$ and MIC$_{90}$) were 0.015 and 0.03 mg/L. Evidently, the strains were also susceptible to cefuroxime, cefpodoxime and ceftriaxone with MIC$_{90}$s of
Antimicrobial resistance in *S. pyogenes*

Based on comparison of MIC<sub>50</sub> and MIC<sub>90</sub> values, cefaclor (MIC<sub>90</sub> 0.25 mg/L) was four- to eight-fold less potent than the other cephalosporins tested. All strains were also fully susceptible to linezolid, vancomycin and the two fluoroquinolones, ciprofloxacin and levofloxacin. Analysing susceptibilities to β-lactams, fluoroquinolones, vancomycin and linezolid, and josamycin and clindamycin mentioned below, no substantial differences were found between the strains derived from adults and children.

Eighty-eight strains (16.1%) were resistant to tetracycline. The MIC<sub>50</sub> of tetracycline was 0.5 mg/L, and the MIC<sub>90</sub> was 128 mg/L. For tetracycline, comparable resistance rates were observed in strains originating from children (15.8%) and from adults (17.4%), the difference not being significant ($P = 0.222$ by χ<sup>2</sup> test).

Macrolides and clindamycin

Among 540 isolates, 72 (13.3%) were resistant to erythromycin. MIC<sub>50</sub> and MIC<sub>90</sub> values of erythromycin were 0.06 and 16 mg/L. The strains resistant to erythromycin were also resistant to clarithromycin and azithromycin and vice versa. In contrast to previous data,<sup>17,21</sup> the rate of erythromycin, clarithromycin and azithromycin resistance was significantly higher ($P = 0.039$ by χ<sup>2</sup> test) in strains derived from adults (19.1%) than in strains derived from children (11.8%). MICs of the 16-membered macrolide josamycin ranged from 0.12 to >256 mg/L. The MIC<sub>90</sub> (0.5 mg/L) indicates the low resistance rate to josamycin (nine isolates, 1.7%). Six isolates (1.1%) were constitutively resistant and two isolates (0.4%) intermediately resistant to clindamycin. All strains resistant or intermediately resistant to josamycin or clindamycin were also resistant to the 14- and 15-membered macrolides.

### Table 1. Susceptibility of 540 isolates of *S. pyogenes* to 16 antibiotics

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (mg/L)</th>
<th>Percentage of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.008–256</td>
<td>0.06</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.008–256</td>
<td>0.06</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.06–256</td>
<td>0.25</td>
</tr>
<tr>
<td>Josamycin</td>
<td>0.12–256</td>
<td>0.25</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.008–256</td>
<td>0.06</td>
</tr>
<tr>
<td>Penicillin</td>
<td>≤0.004–0.12</td>
<td>0.015</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>≤0.004–0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>≤0.004–0.25</td>
<td>0.12</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>≤0.004–0.5</td>
<td>0.015</td>
</tr>
<tr>
<td>Cepodoxime</td>
<td>≤0.004–0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>≤0.004–0.12</td>
<td>0.015</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.06–2</td>
<td>1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.06–2</td>
<td>0.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25–2</td>
<td>1</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>0.03–256</td>
<td>0.5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.12–1</td>
<td>0.25</td>
</tr>
</tbody>
</table>

<0.12 mg/L. Based on comparison of MIC<sub>50</sub> and MIC<sub>90</sub> values, cefaclor (MIC<sub>90</sub> 0.25 mg/L) was four- to eight-fold less potent than the other cephalosporins tested. All strains were also fully susceptible to linezolid, vancomycin and the two fluoroquinolones, ciprofloxacin and levofloxacin. Analysing susceptibilities to β-lactams, fluoroquinolones, vancomycin and linezolid, and josamycin and clindamycin mentioned below, no substantial differences were found between the strains derived from adults and children.

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### Table 2. Phenotypes of macrolide resistance

<table>
<thead>
<tr>
<th>Phenotype of macrolide resistance</th>
<th>No. of strains (%)</th>
<th>Of these, no. resistant to tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>56 (78)</td>
<td>3</td>
</tr>
<tr>
<td>cMLSB</td>
<td>6 (8)</td>
<td>2</td>
</tr>
<tr>
<td>iMLSB&lt;sub&gt;A&lt;/sub&gt;</td>
<td>2 (3)</td>
<td>0</td>
</tr>
<tr>
<td>iMLSB&lt;sub&gt;B&lt;/sub&gt;-C</td>
<td>8 (11)</td>
<td>8</td>
</tr>
</tbody>
</table>
Discussion

*S. pyogenes* has remained fully susceptible to penicillin for decades, despite the continued use of this agent as first-line antibiotic in the treatment of infections caused by this pathogen.2,22 This is also confirmed by the present results in vitro. Apparently, reported clinical failures do not indicate a reduction in susceptibility, but reflect rather the influence of other factors, such as lack of patient compliance, reinfection, β-lactamase production by adjacent bacteria or intracellular persistence of bacterial cells.23–25 In addition to penicillin, amoxicillin and other β-lactam antibiotics are fully active against *S. pyogenes*, offering therapeutic alternatives for the treatment of group A streptococcal tonsillopharyngitis. Lately, the use of amoxicillin as first-line antibiotic has been discussed for patients with tonsillopharyngitis caused by *S. pyogenes*.26

The rate of macrolide resistance in Bavaria is the highest among *S. pyogenes* in Germany to date, and raises concern. Considering previously reported resistance rates from different regions in Germany, with values of 4% in 1993,15 1.6% in 1997,16 12.7% in 1998,17 7.9% in 200118 and the presently determined rate of 13.3%, it appears that there is a trend towards higher rates of macrolide resistance in Germany. Experience from other countries has shown that macrolide resistance rates can increase rapidly.22 Rising resistance rates have been connected to prior extensive use of macrolides within a population.13,14 In pneumococci isolated from invasive diseases in Germany between 1992 and 2000, macrolide resistance has increased dramatically from 3.0% to 15.3%.28 Analysis of macrolide consumption during the study period, indicated that the occurrence of erythromycin resistance was highly correlated to increased use of macrolides.28 Apparently, the trend towards higher levels of macrolide resistance observed among pneumococci in Germany also applies to *S. pyogenes*, thereby raising questions as to appropriate countermeasures.

Experience from Finland has demonstrated that restricted use of macrolides can result in a decrease in macrolide resistance rates.29 After reduction of macrolide consumption from 2.4 defined daily doses in 1991 to 1.38 daily doses in 1992, erythromycin resistance steadily decreased, from 16.5% in 1992 to 8.6% in 1996. A survey of antibiotic use in the European Union in 1997 attested a macrolide and lincosamide consumption of 2.54 defined daily doses per 1000 inhabitants per day in Germany.30 Therefore, restricted clinical use of macrolides should be envisaged in order to maintain satisfactory efficacy of these important antibiotics. Furthermore, verification of the presence of *S. pyogenes* on the site of infection by microbiological methods, or rapid tests prior to therapy, are recommended to avoid inappropriate antibiotic treatment of viral throat infections.

It has been observed worldwide that distribution and predominance of phenotypes of macrolide resistance in *S. pyogenes* differ widely.3,7,11,31–34 In Berlin, for example, the majority (55%) of erythromycin-resistant isolates displayed the iMLS_B phenotype.17 Our present data show that the M phenotype predominates in Bavaria (78%). Apparently, this result is similar to that observed in Aachen, where 71% of the resistant isolates were of the M phenotype.18 Thus in Bavaria, or in the region of Aachen, 16-membered macrolides, potent against strains of the M phenotype, might yield better results in the treatment of diseases caused by *S. pyogenes* than 14- and 15-membered macrolides.

Conclusively, it can be stated that effective measures will be necessary in the future to prevent a further rise in macrolide resistance, and to avoid the loss of these important antimicrobial agents in the treatment of respiratory tract infections.

References

Antimicrobial resistance in S. pyogenes
