Association between Resistance to Erythromycin and the Presence of the Fibronectin Binding Protein F1 Gene, \textit{prtF1}, in \textit{Streptococcus pyogenes} Isolates from German Pediatric Patients

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A total of 301 German pediatric group A streptococcus isolates were screened for the presence of macrolide resistance and the fibronectin binding protein F1 gene (\textit{prtF1}) encoding an adhesin and cell invasiveness protein. The \textit{prtF1} gene was present significantly more often among macrolide-resistant isolates. The majority of these were not clonally related.

Group A streptococcal (GAS) (\textit{Streptococcus pyogenes}) tonsillopharyngitis is one of the most common bacterial infections in children. Resistance to penicillin has not been described, but many European countries have recently experienced an increase in macrolide resistance (2, 6, 17). Macrolide resistance mechanisms include target site modification, leading to resistance to macrolides, lincosamides, and streptogramin B (MLS phenotype), or an efflux mechanism mediating resistance only to 14- and 15-membered macrolides (M phenotype) (11). Recently, an association between erythromycin resistance and cell invasiveness, as demonstrated by the presence of the fibronectin binding protein F1, encoded by the \textit{prtF1} gene, has been observed (8, 14). In addition, Cocuzza et al. recently demonstrated selection for \textit{prtF1}-positive strains after \(\beta\)-lactam therapy (5). Here, we investigated pediatric GAS strains in order to generate data on antibiotic resistance rates in the pediatric population of a southwestern region of Germany. Moreover, the association between the \textit{prtF1} gene and macrolide and tetracycline resistance was investigated.

From June 1999 through January 2003, 301 GAS isolates were collected at the Department of Pediatrics and Adolescent Medicine, University Hospital Freiburg, Freiburg, Germany, from in- and outpatients \(<16\) years old. GAS were grown mostly from throat swabs. Duplicate isolates from the same patient were excluded. All isolates were tested for susceptibility to erythromycin, clindamycin, penicillin G, and cefaclor by the double-disk method (20) and screened for the presence of \textit{ermA}, \textit{ermB}, \textit{ermTR}, and \textit{prtF1} genes as previously described (2, 9, 16). Pulsed-field gel electrophoresis (PFGE) was performed on the DNA of macrolide-resistant strains after digestion with the enzymes Smal and SfiI (New England Biolabs, Frankfurt, Germany). Clonal relatedness was defined as a similarity coefficient higher than 80%. For statistical analysis, the chi-square test and the Mantel-Haenszel test for linear trends were applied.

All GAS isolates were susceptible to penicillin and cefaclor. The overall rate of resistance to erythromycin was 13.6% (41/301 isolates). A trend toward reduced erythromycin resistance over time in this area of Germany was observed (Mantel-Haenszel test; \(P = 0.028\)). The distribution of resistance rates over the study period is shown in Fig. 1. For tetracycline, an overall resistance rate of 31.9\% (\(n = 96\)) was found; an additional 142 isolates showed intermediate resistance. Only six isolates were resistant to clindamycin, and all of these were also resistant to erythromycin. The MICs are given in Table 1. Macrolide efflux, encoded by \textit{mef} genes, was found to be the most common mechanism detected among erythromycin-resis-
tant isolates. Methyltransferase, encoded by \textit{ermB} and \textit{ermTR} genes, played a minor role (Table 2).

The genetic basis for three isolates of the M phenotype was not elucidated. However, possible mechanisms, such as mutations in 23S rRNA and ribosomal protein L4, have been described in GAS (3, 12), but they were not investigated in this study.

To explore the clonal relatedness of the 41 erythromycin-resistant strains, PFGE was performed. The DNAs of 35 strains were available for PFGE. Analysis of SmaI-digested genomic DNAs of 19 out of 41 erythromycin-resistant GAS isolates revealed 10 different PFGE patterns, which did not indicate clonal relatedness (Fig. 2a). The DNAs of 16 isolates, all carrying the \textit{mefA} gene, could not be restricted by SmaI despite repeated attempts. The DNAs of these strains could be digested with SfiI and yielded six different PFGE patterns. Ten of these isolates (62.5%) belonged to two clones (Fig. 2b), which might indicate some clonal spread of the strains. The existence of 16 different PFGE profiles, however, illustrates the overall genetic diversity of the macrolide-resistant GAS isolates. Interestingly, all isolates that could not be typed with

\begin{table}
\caption{MICs for penicillin, cefaclor, erythromycin, and clindamycin}
\begin{tabular}{|c|c|c|}
\hline
Antibiotic & MIC (\mu g/ml) & \% Resistant \\
& 50\% & 90\% \\
\hline
Penicillin G & 0.008 & 0.012 & 0 \\
Cefaclor & 0.064 & 0.094 & 0 \\
Erythromycin & 0.064 & 4 & 18.6 \\
Clindamycin & 0.094 & 0.125 & 2.0 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\caption{Association of macrolide resistance genotypes and the presence of the \textit{prtF1} gene and resistance phenotypes}
\begin{tabular}{|c|c|c|c|}
\hline
Genotype & No. of isolates & \textit{prtF1} positive & \textit{prtF1} negative \\
& & M & MLS \\
\hline
\textit{mefA} & 11 & 11 & 22 \\
\textit{ermB} & 7 & 3 & 10 \text{cMLS} \\
\textit{ermTR} & 0 & 4 & 2 \text{iMLS}; 2 \text{cMLS} \\
\textit{mefA}/\textit{ermB} & 1 & 0 & 1 \text{iMLS} \\
\textit{ermB}/\textit{ermTR} & 0 & 1 & 1 \text{iMLS} \\
Unknown & 1 & 2 & 3 \\
\hline
\end{tabular}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{PFGEpatterns.png}
\caption{(a) Representative SmaI PFGE patterns of 19 macrolide-resistant GAS isolates. The number of isolates belonging to each PFGE pattern and the phenotype of macrolide resistance are given on the right. Clonal relatedness was defined as a similarity coefficient higher than 80\%. The molecular size marker is shown below. (b) Representative SfiI PFGE patterns of 16 macrolide-resistant, \textit{mefA}-carrying GAS isolates. Six different PFGE patterns were detected. \text{cMLS}, constitutive MLS; \text{iMLS}, inducible MLS phenotype.}
\end{figure}
SmaI carried the mefA gene. Bingen et al. (4) made similar observations in French GAS isolates. Still, the results of the French study, implying the spread of a limited number of macrolide-resistant strains in France, apparently are not transferable to Germany.

The present study from Germany shows a stable prevalence of erythromycin resistance in the first 6-month time interval of the study period. More recently, a slight decrease in erythromycin resistance was even observed (Fig. 1). The overall rate of resistance to erythromycin in this study was 13.6%, which is lower than those reported from other European countries (1, 7, 19) but in accordance with a study from the German National Reference Center for Streptococci, which found a resistance rate of 13.7% (18). Interestingly, there was a trend toward decreasing resistance in this study. The reason for this observation remains unclear. Perhaps enhanced educational activities in our region to restrict the use of macrolide antibiotics might account for this hopeful trend. Resistance to erythromycin has been very uncommon among GAS in the United States. Compared to European countries, macrolide resistance rates remained remarkably low until recently, when a resistance rate of 48%, due to the spread of a single clone (13) in the Pittsburgh area in the 2000–2001 season, was reported (10). This fact, together with the observation from Italy of an association between erythromycin resistance and the ability to enter human respiratory cells, demands attention (8, 21). Such strains are likely to have selective advantages by escaping β-lactam antibiotics intracellularly and macrolides by resistance, and therefore, clonal spread may be facilitated. In this study, the prtF1 gene, encoding an adhesin important for internalization of GAS in epithelial cells, was found in a total of 92 isolates. It was found more frequently in erythromycin-resistant (20/41) than in erythromycin-susceptible (72/260) isolates (48.7% versus 27.7%; P < 0.05). In contrast, the presence of the prtF1 gene was clearly associated with tetracycline susceptibility: the prtF1-resistant ones (17/96) (36.6% versus 17.7%; P < 0.01). This is a novel and interesting observation, but its explanation remains elusive.

Our data confirm the results obtained in previous studies from Italy showing that there was a statistically significant association between erythromycin resistance and the presence of the prtF1 gene. The prevalence of macrolide-resistant GAS carrying prtF1, however, was markedly smaller in this study than in the original study (48.7% versus 89%) (5, 8, 21). The ability of GAS to enter pharyngeal cells may enable them on the one hand to avoid host defenses and on the other hand to escape the action of antibiotics like β-lactams, which are confined to the extracellular space (6, 21). Data presented by Cocuzza et al. indicate that eradication failure in children treated with β-lactams was associated with a positive differential selection pressure for prtF1-positive strains (5). Strains combining erythromycin resistance and the ability to enter human respiratory tract cells may therefore be able to escape both β-lactam and macrolide antibiotics, the first antibiotic class by virtue of intracellular location and the second by virtue of resistance. This might have facilitated the enormous spread of macrolide resistance in certain countries (21). Interestingly, we could not find an association between phenotype and presence of the prtF1 gene, as described by Cocuzza et al. (5). In our study, no difference in prtF1 positivity could be detected between macrolide-resistant isolates belonging to the M or MLS phenotype (Table 2).

In summary, erythromycin resistance rates in pediatric patients in Germany have declined over the study period, but the average resistance rate is above 10%. This is of interest when considering the significant emergence of macrolide resistance in other parts of the world. The simultaneous presence of the prtF1 gene might be an additional hint of the possible threat of a combination of virulence and antibiotic resistance genes in GAS.

REFERENCES


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