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ARTICLE

Significant decline in the erythromycin resistance of group A streptococcus isolates at a German paediatric tertiary care centre

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Abstract Group A streptococcus (GAS) is considered to be a major pathogen of bacterial tonsillopharyngitis in children. Although GAS is generally susceptible to penicillin, macrolides are often used as the second-line treatment. Over the last several decades, the rising macrolide resistance of GAS has been detected in several countries. With the current study, we aimed to determine the development of macrolide resistance at our paediatric centre. From March 2006 to May 2009, 350 GAS isolates were tested for susceptibility to erythromycin, azithromycin, clindamycin, penicillin and cefotaxime. Macrolide-resistant isolates were screened for the presence of genes related to macrolide resistance (*mefA*, *ermB*, *ermTR*, *prtF1*). In comparison to a prior study at our hospital, the erythromycin resistance rate decreased significantly from 13.6 to 2.6%. This effect may be attributable to a more restrictive use of macrolides in children in our region.

Introduction

In spite of the retained penicillin susceptibility of group A streptococcus (GAS), macrolides have been frequently used as a second-line treatment in tonsillopharyngitis over the last several decades [1] and increasing macrolide resistance rates of up to 95% have been reported [2]. Resistance of GAS to macrolides is promoted by various genes, including *mefA*, which encodes for an efflux mechanism of macrolides [3], and *erm* genes (*ermB* and *ermTR*), which encode for methylase enzymes that lead to target-side modifications [4, 5]. Furthermore, an association between erythromycin resistance and cell invasiveness, demonstrated by the presence of the fibronectin binding protein F1 (*prtF1*), has been observed [6]. In a previous study at our hospital, which comprised 301 GAS isolates collected between 1999 and 2003, an erythromycin resistance rate of 13.6% was found, and *mefA* was the predominant macrolide resistance gene [7]. Similar to other guidelines on a restrictive use of antibiotics for acute pharyngitis [1, 8], a standard operating procedure (SOP) addressing the treatment of tonsillopharyngitis in children was introduced at our hospital in 2004. This SOP clearly restricts the use of antibiotic treatment to cases with positive rapid GAS antigen test result or with the detection of GAS by culture. As penicillin is imperatively recommended as the first-line therapy, the use of erythromycin is restricted to the rare cases of penicillin allergy. The aim of the current study was to investigate the further development of GAS resistance and to analyse the distribution of macrolide resistance genes in GAS isolates at our centre.

Materials and methods

Between March 2006 and May 2009, 350 paediatric GAS isolates were collected at the Centre for Paediatrics and Adolescent Medicine, University Medical Centre Freiburg, Germany. The isolates originated predominantly from the throat swabs of children presenting with tonsillopharyngitis

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Table 1 Distribution of minimum inhibitory concentrations (MICs) for erythromycin among the 350 group A streptococcus (GAS) isolates (Etest)

Erythromycin	MIC in μg/ml												
	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19–4	6	8	12	16–192	256
No. of isolates	2	5	38	96	126	66	8	0	1	1	2	0	5

to the outpatient clinic. All isolates were initially analysed by the disc test (Kirby Bauer) for susceptibility to penicillin, cefotaxime, azithromycin, erythromycin and clindamycin. Additionally, all isolates were retested for susceptibility to erythromycin and clindamycin by the Etest (AB Biodisk, Solna, Sweden). Isolates that had been tested as intermediate or resistant to azithromycin by the disc test were retested by the Etest. In our former study, Clinical and Laboratory Standards Institute (CLSI) breakpoints had been applied [7]. In order to make the results of the previous and current study comparable, the interpretation of results was done according to the recommendations by AB bioMérieux 2009-05, which also refer to CLSI data. All isolates resistant to erythromycin, clindamycin or azithromycin were screened for the presence of *mefA*, *ermB*, *ermTR* and *prtF1* genes. Experiments were performed as published previously [9–11]. The D-test was performed on all resistant isolates. Unfortunately, two isolates failed to regrow and, therefore, could not be tested for inducible resistance. For statistical analysis, the Chi-square test was applied (software GraphPad Prism 5).

Results

Susceptibility testing

All tested GAS isolates were fully susceptible to penicillin and cefotaxime by the disc test. The overall resistance rates were 2.6% (9/350) for erythromycin and 0.9% (3/350) for clindamycin by the Etest. The resistance rate for azithromycin was determined to be 1.4% (5/349). One erythromycin-resistant isolate failed to grow when retested for azithromycin susceptibility.

While resistance testing for clindamycin displayed either high-level resistance (minimum inhibitory concentration [MIC]≥256 μg/ml) or clear susceptibility, lower-grade erythromycin resistance (MIC 6–12 μg/ml) was observed in four isolates (Tables 1 and 2). In comparison to our previous study, the overall resistance rate to erythromycin declined significantly from 13.6 to 2.6% (χ^2=27.87; p<0.0001).

Distribution of macrolide resistance genes

Eight out of nine macrolide-resistant isolates tested positive for macrolide resistance-encoding genes. Three isolates carried *mefA*, four isolates carried *ermB* and one isolate carried *ermTR*. None of the isolates was simultaneously positive for two macrolide resistance genes, but seven of these eight isolates carried *prtF1* as well. One isolate was positive for *prtF1* only and negative for the three other genes. Only one resistant isolate showed the iMLB phenotype when performing the double-disc test. This resistant isolate was the only one to carry *ermTR* (Table 3).

Discussion

The resistance rates of GAS vary considerably among different countries [12]. In Germany, data from the National Reference Centre showed a distribution of macrolide resistance of between 4.2 and 13.6% in the time period from 1999 to 2007 [13], but precise data referring to children are lacking. In our current study, we noticed a significant decline in erythromycin resistance at our paediatric centre during the time period from 2006 to 2009 as compared to 1999 to 2003. As early as in 1995, Seppälä et al. reported on a relationship between macrolide consumption and the resistance rate of GAS [14]. While a double-blind, placebo-controlled study demonstrated that, particularly new-generation macrolides, such as azithromycin and clarithromycin, are prone to induce resistance [15], a high use of macrolides remains a common practice in many areas with resistance rates reported of over 70% [16]. Given the numerous examples in the literature which highlight the association between resistance development and antibiotic consumption, we hypothesise that the introduction of our SOP, which proposes a uniform and

Table 2 Distribution of MICs for clindamycin among the 350 GAS isolates (Etest)

Clindamycin	MIC in μg/ml								
	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19–192	256
No. of isolates	2	4	19	49	169	91	13	0	3

Table 3 Macrolide resistance in nine GAS isolates: distribution of macrolide resistance genes and MICs for three different macrolides

Resistance (no. of isolates)	No. of isolates	*prtF1*	*mefA*	*ermB*	*ermTR*	MIC in μg/ml		
						EM	CM	AM
EM (3)	1	−	−	+	−	256	0.125	n.t.
	1	+	−	−	−	256	0.064	n.t.
	1	+	+	−	−	8	0.047	n.t
EM-CM (1)	1	+	−	+	−	256	256	1
EM-AM (3)	1	+	+	−	−	6	0.032	24
	1	+	+	−	−	12	0.047	24
	1	+	−	−	+	12	0.064	256
EM-CM-AM (2)	2	+	−	+	−	256	256	256

AM = azithromycin; CM = clindamycin; EM = erythromycin; n.t. = not tested

restrictive approach of antibiotic therapy for tonsillopharyngitis at our hospital, may have contributed to the decreasing macrolide resistance observed at our centre. However, as there is a lack of data on the precise regional antibiotic consumption in children in Germany, unfortunately, we cannot directly relate numbers of antibiotic prescriptions to the development of macrolide resistance. It is, yet, of particular interest that the overall antibiotic prescription rates for children in Germany are lowest in Southern Germany, where our hospital is located [17].

In spite of the presumed association with antibiotic consumption, the underlying mechanism driving resistance development in different regions remains debatable. It has been suggested that changes in resistance in a given population may be partly due to the spreading of resistant clones [18]. In contrast to our previous study, where *mefA* was found to be the predominant resistance mechanism, there was no marked difference in the frequency of *mefA* as compared to *ermB* in the current study. Although this might reflect a change in *emm* genotypes, no definitive conclusions can be drawn, since we did not investigate *emm* genotypes and the clonal relatedness of resistant isolates in detail. While some authors [19] suggest a link between the clonal distribution and macrolide consumption, the lack of data on the precise local antimicrobial consumption in different areas again presents a major obstacle for the final interpretation of resistance trends. It, therefore, seems highly advisable to implement measures that enable researchers and physicians to gain access to detailed regional antibiotic consumption data.

Conclusion

In summary, this study demonstrates that susceptibility testing remains crucial for the surveillance of the macrolide resistance of group A streptococcus (GAS). As rising resistance rates may be indicative for an overuse of antibiotics, declining resistance most probably can be attributed to a more reasonable antibiotic prescription policy. The spread and disappearance of resistant clones, potentially driven by antibiotic selection pressure, may present the molecular basis of the changing resistance rates of GAS. Still, the lack of data on regional antibiotic consumption presents a dilemma for the definitive interpretation of resistance development. Therefore, prospective surveillance of local antibiotic consumption should be initiated. The reporting of declining resistance rates following educative measures will hopefully encourage paediatricians and general practitioners to continue, or even increase, their efforts for the restrictive use of antibiotics in children.

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Conflict of interest The authors declare that they have no conflict of interest.

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