Distribution of *emm* type and antibiotic susceptibility of group A streptococci causing invasive and noninvasive disease

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To determine the prevalence of macrolide antibiotic and levofloxacin resistance in infections with *Streptococcus pyogenes* (group A streptococcus or GAS), strains were collected from 45 medical institutions in various parts of Japan between October 2003 and September 2006. Four hundred and eighty-two strains from patients with GAS infections were characterized genetically. Strains were classified into four groups according to the type of infection: invasive infections ($n=74$) including sepsis, cellulitis and toxic-shock-like syndrome; acute otitis media (AOM; $n=23$); abscess ($n=53$); and pharyngotonsillitis ($n=332$). Among all strains, 32 *emm* types were identified; *emm1* was significantly more common in invasive infections (39.2 %) and AOM (43.5 %) than in abscesses (3.8 %) or pharyngotonsillitis (10.2 %). *emm12* and *emm4* each accounted for 23.5 % of pharyngotonsillitis cases. Susceptibility of GAS strains to eight β -lactam agents was excellent, with MICs of 0.0005–0.063 $\mu\text{g ml}^{-1}$. Macrolide-resistant strains accounted for 16.2 % of all strains, while the percentages of strains possessing the resistance genes *erm*(A), *erm*(B) and *mef*(A) were 2.5 %, 6.2 % and 7.5 %, respectively. Although no strains with high resistance to levofloxacin were found, strains with an MIC of 2–4 $\mu\text{g ml}^{-1}$ (17.4 %) had amino acid substitutions at either Ser-79 or Asp-83 in ParC. These levofloxacin-intermediately resistant strains included 16 *emm* types, but macrolide-resistant strains were more likely than others to represent certain *emm* types.

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INTRODUCTION

Streptococcus pyogenes (group A streptococcus or GAS) is well recognized as the most common pathogen causing pharyngotonsillitis in school-age children. In addition, GAS is well known as a cause of impetigo, necrotizing fasciitis and other infections (Cunningham, 2000). In the late 1980s, toxic-shock-like syndrome caused by GAS was reported in Europe, Japan and the USA, being termed 'flesh-eating bacteria' in the popular press (Cunningham, 2000; Stevens *et al.*, 1989).

M protein encoded by the *emm* gene (Fischetti, 1989) has been exploited for M typing in epidemiological studies (Tewodros & Kronvall, 2005). M typing has progressed from identification methods using antisera to analysis of *emm* gene sequences of the N-terminal hypervariable region using the Centers for Disease Control and

Prevention (CDC) database (Beall *et al.*, 1996). Results for GAS surveillance by Rogers *et al.* (2007) demonstrated that *emm1* predominated in invasive infections compared with other noninvasive infections.

Macrolide and levofloxacin resistance have gradually increased in GAS isolates, although the isolates remain uniformly susceptible to β -lactam antibiotics. Clonal spread of levofloxacin-resistant GAS has been reported (Malhotra-Kumar *et al.*, 2005), and possible implications for public health have been pointed out.

Recently, penicillin-intermediately resistant *Streptococcus agalactiae* that has amino acid substitutions in penicillin-binding protein 2X has appeared in Japan (Kimura *et al.*, 2008). Therefore, attention to annual trends of β -lactam susceptibility of GAS is also necessary.

In this study, we aimed to clarify (i) *emm*-type distributions in invasive strains compared with noninvasive isolates; (ii) susceptibility of these strains to 11 β -lactam antibiotics, four macrolides, clindamycin and levofloxacin;

Abbreviations: AOM, acute otitis media; CDC, Centers for Disease Control and Prevention; FQ, fluoroquinolone; GAS, group A streptococcus.

and (iii) resistance genes for macrolides, lincosamides and streptogramin B (MLS_B), and levofloxacin.

METHODS

Strains. GAS isolates from patients with GAS infection were collected from clinical laboratories belonging to 45 general hospitals. A total of 482 isolates were sent to our laboratory (Kitasato Institute for Life Sciences, Kitasato University) between October 2003 and September 2006. After reidentification following the *Manual of Clinical Microbiology* (Ruoff *et al.*, 2003), strains were stored at -80°C in 10% skim milk (Becton Dickinson) until subsequent testing.

For the purposes of this study, isolates were divided into four groups according to the modified classification of Rogers *et al.* (2007). The invasive infection group (total $n=74$) included septicaemia ($n=34$), cellulitis ($n=13$), septic arthritis ($n=8$), meningitis ($n=4$), necrotizing fasciitis ($n=5$), toxic-shock-like syndrome ($n=4$), pneumonia ($n=3$), mastitis ($n=2$) and pleurisy ($n=1$) isolates. In this group, isolates defined tentatively as GAS were obtained from blood, joint fluid or pleural fluid. The other GAS infections were classified in abscess ($n=53$), pharyngotonsillitis ($n=332$) and acute otitis media (AOM) ($n=23$) groups.

Characterization of the resistance mechanism. PCRs for detection of the *erm*(A), *erm*(B) and *mef*(A) genes mediating macrolide resistance were performed as described previously (Sunaoshi *et al.*, 2004).

Strains possessing the *erm*(A) gene express an inducible macrolide, lincosamide and streptogramin B resistance (iMLS_B) phenotype (Seppälä *et al.*, 1998) and strains possessing the *erm*(B) gene express a constitutive MLS_B (cMLS_B) phenotype. The iMLS_B phenotype strains by the target site modifications due to methylase activity show high resistance to 14- and 15-membered ring macrolides but are susceptible or intermediately resistant to clindamycin without induction (Giovanetti *et al.*, 1999). The cMLS_B phenotype strains by methylase activity show high resistance to all macrolides and clindamycin without induction. By contrast, strains possessing the *mef*(A) gene express a 14- and 15-membered ring macrolide resistance (M) phenotype by an active drug efflux pump (Giovanetti *et al.*, 1999; Roberts *et al.*, 1999).

For the *gyrA*, *gyrB*, *parC* and *parE* genes involved in fluoroquinolone (FQ) resistance, PCR was carried out for 40 cycles under the conditions of 94°C for 30 s, 52°C for 30 s and 72°C for 60 s. Analytical primer sets for FQ resistance, shown in Table 1, were designed to detect these genes in GAS strains (GenBank accession nos NC_002737, NC_008023, NC_008024). The PCR product was then purified using the QIAquick PCR Purification kit (Qiagen). Sequencing was performed using the BigDye Terminator Cycle Sequencing kit version 3.1 (Applied Biosystems), with assessment of results using the Applied Biosystems 3130 Genetic Analyzer.

***emm* gene typing.** Typing of the *emm* gene was performed as follows.

Extraction of template DNA was done using the established procedure (Ubukata *et al.*, 1996). In brief, one colony of GAS growing on a sheep blood agar culture plate was picked and suspended in lysis solution, which comprised 0.1 M Tris/HCl (pH 8.0), 4 µg proteinase K, 0.225% Tween 20 and 0.225% Nonidet 40. This was incubated at 60°C for 20 min and then at 90°C for 10 min.

The PCR for *emm* genotyping was carried out according to minor modifications of the method described previously by Beall *et al.*

Table 1. Primers for PCR and sequencing for quinolone resistance

Primer name	Primer sequence (5'→3')	Amplicon size (bp)
<i>gyrA</i>		
<i>gyrA</i> -s	TTTGCCAGATGTGCGTGATG	446
<i>gyrA</i> -r	TGTTAGTTGCCATCCCAACG	
<i>gyrB</i>		
<i>gyrB</i> -s	ATTGGGCAACTCAGAAGTGG	504
<i>gyrB</i> -r	GTTCTAATATGAGCGCCATCC	
<i>parC</i>		
<i>parC</i> -s	ATTATGGGAGAACGCTTCGG	442
<i>parC</i> -r	AAGCTGCTGGTAAACGGTG	
<i>parE</i>		
<i>parE</i> -s	CTCATCTAGTTCGCAAAGCC	781
<i>parE</i> -r	TTATCCTCGATCCACTGACG	

(1996). The resulting PCR fragments were purified and sequenced in the same way as described above.

The first 240 bases of the 5' end of the *emm* gene sequences were compared with those in the CDC *S. pyogenes emm* sequence database (<http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm>). An *emm* type showing over 98% homology with a CDC reference strain was identified as that particular *emm* type.

Antimicrobial susceptibility tests. Susceptibility testing of GAS strains was carried out by the microdilution method using cation-adjusted Mueller–Hinton broth (Becton Dickinson) supplemented with 5% lysed horse blood according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2007) and with a final inoculum of 5×10^5 c.f.u. ml⁻¹. Oral antimicrobial agents employed in this study were ampicillin, amoxicillin, cefdinir, cefpodoxime, cefditoren, cefcapen, faropenem, tebipenem, clarithromycin, azithromycin, josamycin, clindamycin, telithromycin and levofloxacin. Parenteral agents were cefotaxime, panipenem and meropenem. These antimicrobial agents were obtained from the respective pharmaceutical manufacturers.

Statistical analysis. Statistical analysis was performed using Microsoft Excel Statistics 2006 for Windows (Social Survey Research Information). The chi-square test was used to assess significance of differences involving categorical variables.

RESULTS AND DISCUSSION

Age distribution of the patients with GAS infection

Fig. 1 shows the patient age distribution in the four groups of GAS infection cases described in Methods.

Invasive cases ($n=74$) occurred mostly in patients over 20 years old (85.1%) as opposed to in children, especially in older adults between the ages of 50 and 70 years old. Important underlying conditions such as diabetes mellitus, liver dysfunction, renal dysfunction and medical treatment for cancer were noted in 56.4% of those adults. Decreased immunity in these cases may have contributed synergisti-

Fig. 1. Age distribution of patients with *Streptococcus pyogenes* infection. Patients were classified into four groups. Black columns indicate patients with invasive infections; finely stippled columns, those with AOM; open columns, those with abscess; diagonally hatched columns, those with pharyngotonsillitis.

cally with GAS virulence factors to an increased incidence of invasive infections.

Both AOM cases ($n=23$) and pharyngotonsillitis cases ($n=332$) mainly involved children between 3 and 12 years old, which accounted for 43.5 % and 67.4 %, respectively, although 20.1 % of the pharyngotonsillitis patients were 20–30 years old.

Abscess cases ($n=53$), in which GAS was isolated from either draining or nondraining localized abscesses, were distributed across all age brackets.

Statistically significant differences in age distribution were recognized between the invasive group and each of the other three groups (invasive vs AOM, $P<0.0001$; invasive vs abscess, $P=0.0003$; invasive vs pharyngotonsillitis, $P<0.0001$).

Typing for *emm*

Results of *emm* typing of GAS isolates in four groups, that is, invasive (A), AOM (B), abscess (C) and pharyngotonsillitis (D) groups, are listed in Table 2. Although GAS isolates included a variety of *emm* types, the predominant *emm* types in each group differed. In the invasive group, *emm1* was prominent, accounting for 39.2 % of cases, while other *emm* types generally accounted for 10 % of cases or fewer. Similarly, *emm1* was most frequent in the AOM group (43.5 %), followed by *emm12* (30.4 %).

In the abscess group, *emm28* and types described as ‘other’ in Table 2 accounted for 22.6 % and 26.4 % of cases each,

while *emm1* was responsible for only 3.8 %. In the pharyngotonsillitis group, *emm4* and *emm12* accounted for 23.5 % of cases each, in contrast to 10.2 % for *emm1*.

A significant difference in prevalence of *emm* types was noted between the invasive group and each of the other three groups (invasive vs AOM, $P=0.0895$; invasive vs abscess, $P=0.0013$; invasive vs pharyngotonsillitis, $P<0.0001$).

Susceptibilities to β -lactam agents

All β -lactam agents showed sharp distributions for all GAS, indicating that none of these agents showed decreased efficacy against GAS.

In data not shown here, MIC₉₀ values for GAS were excellent in the following order: tebipenem (0.002 $\mu\text{g ml}^{-1}$) > cefditoren=cefcapen (0.008 $\mu\text{g ml}^{-1}$) > amoxicillin=cefdinir=cefpodoxime (0.016 $\mu\text{g ml}^{-1}$) > ampicillin=faropenem (0.031 $\mu\text{g ml}^{-1}$) for oral β -lactams; and panipenem=meropenem (0.008 $\mu\text{g ml}^{-1}$) > cefotaxime (0.016 $\mu\text{g ml}^{-1}$) for parenteral β -lactams. All isolates remained uniformly susceptible to β -lactam antibiotics.

Susceptibilities to macrolides and resistance genes

Table 3 shows the MIC ranges, MIC₅₀ and MIC₉₀ of clarithromycin, azithromycin, josamycin, telithromycin and clindamycin for GAS according to the macrolide-resistance genes identified. Of all 482 isolates, strains

Table 2. Relationship between streptococcal diseases and *emm* types of isolates

Results of chi-square tests were as follows. A:B, $P=0.0895$; A:C, $P=0.0013$, A:D, $P<0.0001$. Values in parentheses are percentages for each classified group. AOM, acute otitis media.

<i>emm</i> type	Groups classified as:				No. of isolates
	Invasive (A)	AOM (B)	Abscess (C)	Pharyngotonsillitis (D)	
1	29 (39.2)	10 (43.5)	2 (3.8)	34 (10.2)	75
3	2 (2.7)		2 (3.8)	13 (3.9)	17
4	2 (2.7)	2 (8.7)	1 (1.9)	78 (23.5)	83
6			3 (5.7)	15 (4.5)	18
11	4 (5.4)		2 (3.8)	20 (6.0)	26
12	5 (6.7)	7 (30.4)	4 (7.5)	78 (23.5)	94
28	5 (6.7)	1 (4.3)	12 (22.6)	33 (9.9)	51
49	8 (10.8)		4 (7.5)	1 (0.3)	13
58	4 (5.4)		3 (5.7)	7 (2.1)	14
75	3 (4.1)	2 (8.7)	1 (1.9)	9 (2.7)	15
87	2 (2.7)		2 (3.8)	2 (0.6)	6
89	1 (1.4)	1 (4.3)	3 (5.7)	12 (3.6)	17
Other	9 (12.2)		14 (26.4)	30 (9.0)	53
Total	74	23	53	332	482

possessing *erm*(A), *erm*(B) and *mef*(A) represented 2.5 % ($n=12$), 6.2 % ($n=30$) and 7.5 % ($n=36$), respectively.

Table 3. MIC distributions and resistance genes identified by PCR in *Streptococcus pyogenes* strains

Antimicrobial agent	MIC range ($\mu\text{g ml}^{-1}$)	MIC ₅₀	MIC ₉₀
Clarithromycin			
Resistance gene (–)	0.031–2	0.063	0.125
<i>erm</i> (A)	2–16	4	16
<i>erm</i> (B)	>64	>64	>64
<i>mef</i> (A)	2–16	8	16
Azithromycin			
Resistance gene (–)	0.125–4	0.25	1
<i>erm</i> (A)	32–>64	>64	>64
<i>erm</i> (B)	>64	>64	>64
<i>mef</i> (A)	4–32	8	32
Josamycin			
Resistance gene (–)	0.125–2	0.5	0.5
<i>erm</i> (A)	0.5–8	4	8
<i>erm</i> (B)	>64	>64	>64
<i>mef</i> (A)	0.125–0.5	0.25	0.5
Telithromycin			
Resistance gene (–)	0.008–0.25	0.031	0.063
<i>erm</i> (A)	0.063–0.125	0.063	0.063
<i>erm</i> (B)	2–>64	>64	>64
<i>mef</i> (A)	0.25–1	0.5	1
Clindamycin			
Resistance gene (–)	0.031–0.25	0.125	0.25
<i>erm</i> (A)	0.25–>64	0.5	8
<i>erm</i> (B)	>64	>64	>64
<i>mef</i> (A)	0.031–0.25	0.063	0.25

Except for telithromycin, the *erm*(A) gene related to iMLS_B phenotype decreased the susceptibilities of GAS to macrolides and clindamycin 8–128-fold compared with susceptible strains. GAS with the *erm*(B) gene related to cMLS_B phenotype showed high resistance to macrolides, telithromycin and clindamycin (MIC $\geq 64 \mu\text{g ml}^{-1}$). GAS strains with the *mef*(A) gene, which mediate an M phenotype, showed slight decreases in susceptibility, from 4- to 8-fold, to clarithromycin, azithromycin and telithromycin, but maintained undiminished susceptibility to josamycin and clindamycin.

In Japan, the prevalence of macrolide resistance in GAS has remained at 5–7 % for a long time. In the survey in 2003, of the total of 533 GAS strains collected from all over the country, 0.5 % had the *erm*(A) gene, 3.2 % the *erm*(B) gene and 4.9 % the *mef*(A) gene (Sunaoshi *et al.*, 2004). Macrolide resistance appears to increase gradually.

Table 4 shows the correlation between the *emm* type and macrolide-resistance genes. Of the GAS strains typed to *emm*1, 21.3 % had the *mef*(A) gene, which was prevalent in invasive infections, in contrast to the *emm* status of strains with *erm*(A) and *erm*(B) genes.

High clindamycin resistance mediated by the *erm*(B) gene was detected at 2.7 % in invasive strains.

Susceptibility to levofloxacin and mutations of the target gene

Table 4 also shows the *emm* type distribution of GAS strains ($n=71$) which were intermediately resistant to FQ. Although no strain was identified as showing high resistance, strains with an MIC of 2–4 $\mu\text{g ml}^{-1}$ accounted for 17.4 % ($n=84$) of all isolates. Eighty-five per cent of

Table 4. Correlation of *emm* type and resistance genes of *Streptococcus pyogenes* strains

Values in parentheses are percentages for each *emm* type.

<i>emm</i> type	No. of isolates	Macrolide resistance			Fluoroquinolone resistance <i>parC</i> mutant*
		<i>erm</i> (A)	<i>erm</i> (B)	<i>mef</i> (A)	
1	75		3 (4.0)	16 (21.3)	5 (6.7)
3	17				1 (5.9)
4	83			6 (7.2)	1 (1.2)
6	18				17 (94.4)
11	26				20 (76.9)
12	94		11 (11.7)	7 (7.4)	3 (3.2)
28	51	3 (5.9)	14 (27.4)		2 (3.9)
49	13				4 (30.8)
58	14	6 (42.8)			3 (21.4)
75	15			7 (46.7)	1 (6.7)
87	6				5 (83.3)
89	17		2 (11.8)		2 (11.8)
Other	53	3 (5.7)			7 (13.2)
Total	482	12 (2.5)	30 (6.2)	36 (7.5)	71 (14.7)

*Ser-79 and Asp-83 in *ParC* encoded by *parC* changed to Phe, Ala, or Tyr and Asn.

strains with an MIC of at least $\geq 2 \mu\text{g ml}^{-1}$ ($n=71$) had an amino acid substitution at the Ser-79 or Asp-83 position in quinolone resistance-determining regions (QRDRs) encoded by *parC*. Ser-79 was changed to Phe-79, Ala-79 or Tyr-79 in 93.0 % ($n=66$), while Asp-83 was changed to Asn-83 in 7.0 % ($n=5$). There was no amino acid substitution affecting FQ resistance in *gyrA*, *gyrB* and *parE* genes.

Genotypic levofloxacin-intermediately resistant GAS strains belonged to 16 *emm* types, although *emm6* and *emm11* were prominent at 94.4 % and 76.9 %, respectively, as described previously (Orscheln *et al.*, 2005). This finding suggests that genotypically FQ-intermediately resistant GAS is selected under the pressure of exposure to FQs including levofloxacin. *In vitro* experiments have indicated that all *emm* types seem equally prone to induction of FQ resistance (Billal *et al.*, 2007).

Recently, we isolated a GAS strain, showing high resistance to FQ, from an adult patient (29 years old) with pharyngotonsillitis in September 2007. The strain possessed amino acid substitutions in QRDRs of both *gyrA* and *parC*, which had already been reported in several countries (Malhotra-Kumar *et al.*, 2005; Reinert *et al.*, 2004; Richter *et al.*, 2003; Rivera *et al.*, 2005; Yan *et al.*, 2000).

According to the market research for oral antibiotics (Fujita *et al.*, 2007) in Japan, oral FQs, including four respiratory FQs, have been prescribed for adult outpatients aged ≥ 15 years at the highest rate of 50 %, followed by oral cephalosporin antibiotics at the rate of 43 %, which is higher than that for penicillins. The status of the current usage of FQ causes concern that the incidence of GAS and *Streptococcus pneumoniae* strains possessing high FQ resistance might increase in the near future.

On the other hand, macrolides to which 14-membered ring macrolides and azalides belong have been widely prescribed to reduce inflammation in patients with diffuse pan-bronchiolitis, chronic bronchitis and chronic sinusitis at low doses for long periods, in addition to being used in the treatment of community-acquired respiratory tract infections. Such long-term non-chemotherapeutical usage of macrolides may result in a decrease in usefulness of macrolides for infectious disease and be related to selection and spread of macrolide resistance in *S. pneumoniae* and *S. pyogenes*.

In conclusion, continuous molecular epidemiological surveillance of GAS is necessary to ensure proper use of antimicrobial agents.

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REFERENCES

- Beall, B., Facklam, R. & Thompson, T. (1996). Sequencing *emm*-specific PCR products for routine and accurate typing of group A streptococci. *J Clin Microbiol* **34**, 953–958.
- Billal, D. S., Fedorko, D. P., Yan, S. S., Hotomi, M., Fujihara, K., Nelson, N. & Yamanaka, N. (2007). *In vitro* induction and selection of fluoroquinolone-resistant mutants of *Streptococcus pyogenes* strains with multiple *emm* types. *J Antimicrob Chemother* **59**, 28–34.
- CLSI (2007). *Performance Standards for Antimicrobial Susceptibility Testing*, 17th Informational Supplement, M100-S17. Wayne, PA: Clinical and Laboratory Standards Institute.

- Cunningham, M. W. (2000). Pathogenesis of group A streptococcal infections. *Clin Microbiol Rev* **13**, 470–511.
- Fischetti, V. A. (1989). Streptococcal M protein: molecular design and biological behavior. *Clin Microbiol Rev* **2**, 285–314.
- Fujita, T., Kawaguchi, T. & Fujii, Y. (2007). The closeout report: database construction of oral antimicrobial agents using the results from drug use investigations. *Jpn J Antibiot* **60**, 265–307 (in Japanese).
- Giovanetti, E., Montanari, M. P., Mingoia, M. & Varaldo, P. E. (1999). Phenotypes and genotypes of erythromycin-resistant *Streptococcus pyogenes* strains in Italy and heterogeneity of inducibly resistant strains. *Antimicrob Agents Chemother* **43**, 1935–1940.
- Kimura, K., Suzuki, S., Wachino, J. I., Kurokawa, H., Yamane, K., Shibata, N., Nagano, N., Kato, H., Shibayama, K. & Arakawa, Y. (2008). First molecular characterization of group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother* **52**, 2890–2897.
- Malhotra-Kumar, S., Lammens, C., Chapelle, S., Mallentjer, C., Weyler, J. & Goossens, H. (2005). Clonal spread of fluoroquinolone non-susceptible *Streptococcus pyogenes*. *J Antimicrob Chemother* **55**, 320–325.
- Orscheln, R. C., Johnson, D. R., Olson, S. M., Presti, R. M., Martin, J. M., Kaplan, E. L. & Storch, G. A. (2005). Intrinsic reduced susceptibility of serotype 6 *Streptococcus pyogenes* to fluoroquinolone antibiotics. *J Infect Dis* **191**, 1272–1279.
- Reinert, R. R., Lutticken, R. & Al-Lahham, A. (2004). High-level fluoroquinolone resistance in a clinical *Streptococcus pyogenes* isolate in Germany. *Clin Microbiol Infect* **10**, 659–662.
- Richter, S. S., Diekema, D. J., Heilmann, K. P., Almer, L. S., Shortridge, V. D., Zeitler, R., Flamm, R. K. & Doern, G. V. (2003). Fluoroquinolone resistance in *Streptococcus pyogenes*. *Clin Infect Dis* **36**, 380–383.
- Rivera, A., Rebollo, M., Sanchez, F., Navarro, F., Miro, E., Mirelis, B. & Coll, P. (2005). Characterisation of fluoroquinolone-resistant clinical isolates of *Streptococcus pyogenes* in Barcelona, Spain. *Clin Microbiol Infect* **11**, 759–761.
- Roberts, M. C., Sutcliffe, J., Courvalin, P., Jensen, L. B., Rood, J. & Seppälä, H. (1999). Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* **43**, 2823–2830.
- Rogers, S., Commons, R., Danchin, M. H., Selvaraj, G., Kelpie, L., Curtis, N., Robins-Browne, R. & Carapetis, J. R. (2007). Strain prevalence, rather than innate virulence potential, is the major factor responsible for an increase in serious group A streptococcus infections. *J Infect Dis* **195**, 1625–1633.
- Ruoff, K. L., Whiley, R. A. & Beighton, D. (2003). *Streptococcus*. In *Manual of Clinical Microbiology*, pp. 405–421. Edited by P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Tenover & R. H. Tenover. Washington, DC: American Society for Microbiology.
- Seppälä, H., Skurnik, M., Soini, H., Roberts, M. C. & Huovinen, P. (1998). A novel erythromycin resistance methylase gene (*ermTR*) in *Streptococcus pyogenes*. *Antimicrob Agents Chemother* **42**, 257–262.
- Stevens, D. L., Tanner, M. H., Winship, J., Swartz, R., Ries, K. M., Schlievert, P. M. & Kaplan, E. (1989). Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *N Engl J Med* **321**, 1–7.
- Sunaoshi, K., Nakayama, E., Kobayashi, R., Suzuki, E., Tajima, T. & Ubukata, K. (2004). Antibiotic susceptibility and T type identification of *Streptococcus pyogenes* isolated from pediatric outpatients with pharyngotonsillitis. *Jpn J Chemother* **52**, 401–407.
- Tewodros, W. & Kronvall, G. (2005). M protein gene (*emm* type) analysis of group A beta-hemolytic streptococci from Ethiopia reveals unique patterns. *J Clin Microbiol* **43**, 4369–4376.
- Ubukata, K., Asahi, Y., Yamane, A. & Konno, M. (1996). Combinational detection of autolysin and penicillin-binding protein 2B genes of *Streptococcus pneumoniae* by PCR. *J Clin Microbiol* **34**, 592–596.
- Yan, S. S., Fox, M. L., Holland, S. M., Stock, F., Gill, V. J. & Fedorko, D. P. (2000). Resistance to multiple fluoroquinolones in a clinical isolate of *Streptococcus pyogenes*: identification of *gyrA* and *parC* and specification of point mutations associated with resistance. *Antimicrob Agents Chemother* **44**, 3196–3198.