Survey of macrolide resistance phenotypes in Swedish clinical isolates of *Streptococcus pyogenes*

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Two hundred selected Swedish clinical strains of *Streptococcus pyogenes*, identified as erythromycin-resistant and isolated between 1980 and 1988, and 37 consecutive, resistant strains from 1989–90 were examined for resistance phenotype by disc diffusion. Strains constitutively resistant to macrolides, lincosamides and streptogramin B were absent in 1980–85 but accounted for 10% in 1986–88. The majority of the isolates belonged to a recently reported, non-inducible phenotype, described as having low-level resistance to erythromycin and sensitivity to clindamycin (82% in 1980–85, 50% in 1986–88). A significant proportion of the isolates did not agree with any known phenotype and therefore were considered as having one of three novel resistance subphenotypes. Most of the 37 strains from 1989–90 belonged to a novel subphenotype.

**Introduction**

Macrolide antibiotics include many 14-, 15- and 16-membered compounds, containing a lactone ring of variable size with a variable number of sugar moieties attached. Three main mechanisms have been found to account for bacterial resistance to macrolide/lincosamide/streptogramin B (MLS) antibiotics: target site modification, enzymatic inactivation and active efflux of the drug. Resistance due to efflux has been observed in various staphylococci and was recently described in streptococci and pneumococci. Target site modification due to methylase activity, however, is considered the most common mechanism of MLS resistance among Gram-positive species.

In group A streptococci (GAS), as in several other Gram-positive species, MLS resistance is expressed constitutively or inducibly. Constitutively resistant (CR) strains are always highly resistant and the resistance extends to most MLS antibiotics. In the inducibly resistant (IR) bacteria, expression of the relevant methylase is triggered by 14- and 15-membered rather than by the 16-membered macrolides; the level of induced resistance to 16-membered compounds and lincosamides, as well as to inducing agents, is generally low. Recently, a new phenotype called 'non-inducible' (NI), conferring low-level resistance to erythromycin (MIC < 16 mg/L) and other 14- or 15-membered macrolides but being sensitive to 16-membered macrolides, has been described. In the present paper, resistance phenotypes among Swedish clinical isolates of Streptococcus pyogenes, collected during 10 years, were studied and three subphenotypes not previously described were identified.

**Materials and methods**

**Bacterial isolates**

The following strains of *S. pyogenes* thought to be resistant to erythromycin were studied with regard to resistance phenotype. Two hundred isolates randomly selected from 590 strains examined in 1980–88 (100 each in 1980–85 and 1986–88) at the Clinical Microbiology Laboratory of the Lund University Hospital and 37 consecutive strains isolated between 1989 and 1990. The criterion used at our clinical laboratory for classifying the strain as erythromycin resistant was a zone smaller than 10 mm using a disc containing 15 μg of erythromycin in disc diffusion, corresponding to a MIC of <0.5 mg/L.

**Determination of antibiotic sensitivity and resistance phenotype**

Antibiotic sensitivity was investigated by disc diffusion or by the Etest method. From each strain, five to ten colonies were inoculated on to 5% horse blood agar using a cotton-
tipped swab. Antibiotic-containing discs or E test strips (Biodisk AB, Solna, Sweden) were then applied and the plate was incubated aerobically at 37°C for 18 h before being scored. To identify the MLS resistance phenotype, paper discs containing erythromycin (15 μg) or clindamycin (15 μg), were used; different phenotypes of MLS resistance were recognized in accordance with the description given by Seppälä et al.6

T-typing of streptococci

T-typing was performed by slide agglutination according to Griffith7 using rabbit antisera from Chemapol (Prague, Czech Republic). Strains were pretreated with trypsin (Sigma, St Louis, MO, USA) 50 g/L at 37°C for 30 min.

Results and discussion

On re-examination of the S. pyogenes isolates collected in our laboratory because of erythromycin resistance and kept for several years, as many as 17% proved to be erythromycin-sensitive. Since such a high proportion of errors at the clinical laboratory can be excluded, this discrepancy was most likely due to loss of the resistance determinants during frozen storage. MLS-resistant strains of the CR phenotype were absent in the collection from 1980–85 but accounted for 10% during 1986–88. IR strains, as classically defined, were not found in our population. The majority of strains analysed (82% in 1980–85 and 50% in 1986–88) were found to agree with the NI phenotype recently described in Finland, where it also accounted for most of the resistant clinical isolates.6 This phenotype seemed to be prevalent in South Sweden several years before it was officially reported from Finland.

The NI phenotype was defined by Seppälä et al.6 as low-level resistance to erythromycin but sensitivity to clindamycin, although an erythromycin MIC >64 mg/L was noted for a single strain. Similarly, the majority of the strains designated as NI in our study showed MICs between 8 and 12 mg/L for erythromycin and appeared to be a homogenous population. The NI phenotype is now known to be due to an efflux mechanism.2

In the Swedish population of NI strains reported here, T-type 12 predominated in 1980–88, and T-type 4 was not found until 1989–90. Several outbreaks of T-type 12 erythromycin-resistant S. pyogenes were noted in Sweden in 1984–88.8 In Finland most of the NI strains, widely occurring in 1988–90, belonged to T-types 4, 11 and 28, whereas T-type 12 was rare.9 Possibly, T-type 4 NI strains spread from Finland to Sweden in the late 1980s, this type accounted for a high occurrence of erythromycin-resistant S. pyogenes in South Sweden (6–13% 1991–92).10

Furthermore, among the present strains, two variants with high-level resistance to erythromycin (MIC 64–250 mg/L) but either sensitivity or low-level resistance to clindamycin were found in an appreciable proportion. The strains exhibiting these patterns were easily identified and

Figure 1. Two new, noninducible subphenotypes of erythromycin resistance in Streptococcus pyogenes. (a) Strain 10583 (NO ni), totally resistant to erythromycin but sensitive to clindamycin. (b) Strain 10551 (NO cm low), totally resistant to erythromycin but low-level resistant to clindamycin.
Erythromycin resistance in Streptococcus pyogenes clearly distinct from those with the NI phenotype, and appear therefore to represent distinct subphenotypes (NO\text{n}i and NO\text{cmlow}) (Figure 1a and b). Since the resistance of these phenotypes could not be methylase-mediated, it might conceivably be determined by some efflux mechanism as well. In addition, total resistance to erythromycin combined with inducible resistance to clindamycin (NO\text{ind}) was found in one strain (Figure 2) which seemingly differs from the conventional, inducible phenotype.

Of the erythromycin-resistant S. pyogenes collected in 1989–90, the relative proportion of the NI phenotype was lower than in previous years (38%), whereas comparatively more strains with a ‘novel’ (NO) phenotype were found (57%). A majority of these NO strains belonged to either one of the T-types 1, 12 or 28.

The low prevalence of CR and the absence of IR strains in our population was somewhat unexpected since only these two phenotypes are classically recognized in S. pyogenes. On the other hand, the predominance of the NI, efflux-mediated phenotype, exhibiting selective erythromycin resistance, was in agreement with recent reports from Finland and the USA. Finally, three potentially new subphenotypes of MLS resistance were identified, and their resistance mechanisms are now under investigation.

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References

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