Study on the variability of the mef-msr locus expressing the efflux-mediated macrolide resistance in Streptococcus pyogenes

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INTRODUCTION
Over the last few years, macrolide resistance among Group A streptococci has risen steeply and continuously in most of the world (Robinson DA et al., 2006). Macrolide resistance in *Streptococcus pyogenes* is related to two fundamental mechanisms: target modification and active antibiotic efflux (Seppala H et al., 1993). The first is due to the production of an enzyme with methylase activity encoded by erm class genes that catalyzes a modification of 23S rRNA (Weisblum B, 1995). This mechanism results in reduced antibiotic affinity for the target which gives rise to a phenotype denominated MLSB (cross-resistance to all macrolides, to lincosamides and to streptogramin B).

The second mechanism established so far is active macrolide efflux due to protein pumps (Sutcliffe J et al., 1996). This confers low-level resistance (MICs, 1 to 16 mg/L) to 14-membered (e.g. erythromycin) and 15-membered macrolides (azithromycin), but not to 16-membered macrolides, lincosamides and streptogramin B (or their analogues). This is denominated “M phenotype”.

The proteins responsible for active macrolide efflux are membrane-associated and are encoded by genes of the mef and msr families. Seven variants of the mef gene have been described in *S.pyogenes* (the principal is mef(A)), four of them giving rise to a separate subclass.

Genetically, mef is always associated with the msr gene. The two genes are found in conjugative transposons and/or temperate bacteriophages (Varaldo PE et al., 2009). Investigations of their expression in *S. pneumoniae* have demonstrated that both genes are essential for the active macrolide efflux mechanism (Ambrose KD et al., 2005).

However, studies on both the variability of their association and the correlation of this variability to the expressed M phenotype are still lacking in *S.pyogenes*.

THE STUDY
The sequence of the mef(A)-msr(D) region of twenty-nine mef(A)-positive *S.pyogenes* strains was assessed and compared.
These strains were collected between 1997 and 2004 from symptomatic patients, showed a M-phenotype, and MICs for erythromycin ranging from 4 to 32 mg/L. They belonged to 12 different emm-types among the most prevalent in Italy (emm1, 2, 4, 6, 8, 12, 18, 22, 48, 75, 89, 94). Cluster and multivariate analysis indicated that the sequence polymorphisms of mef(A)-msr(D) locus correlate with and are predictive of the level of resistance (P<0.05).

In order to demonstrate the inducible nature of the erythromycin resistance in GAS, the strain m46 (harbouring the phage Φ-m46.1 that carries the mef(A)) was induced with a sub-inhibitory concentration of erythromycin for 2h; then the culture was treated with increasing, but always sub-lethal, concentrations of erythromycin and the growth followed for 24 hours.

The results showed that erythromycin resistance in GAS is induced by the presence of sub-inhibitory concentrations of erythromycin. The difference between induced and non-induced cultures was striking during the exponential phase and at the lower sub-MIC concentrations of the challenger (2, 4, 8 mg/L of erythromycin). In parallel, the expression of mef(A) after erythromycin induction was investigated by RT-PCR. The results clearly demonstrated that the mRNA specific for mef(A) is more abundant in the induced than in the non-induced samples.

In the S.pyogenes reference strain m46, the expression of the mef(A)-msr(D) locus was analyzed by Northern blot. Total RNAs extracted from cells grown in erythromycin-uninduced and induced conditions were hybridized with a random primer-labeled probe specific for mef(A). A main product of about 3,000 nt in size was detected, only in induced cells. An msr(D) specific probe hybridized with the same RNA band. Therefore, msr(D) and mef(A) are co-transcribed in a single bicistronic mRNA.

To understand if Φ-m46.1 could participate in dissemination of the erythromycin and tetracycline resistance phenotype in S.pyogenes strain m46 was induced with 0.2 mg/L of mitomycin C and the supernatant was filtered and treated with DNase and RNase. The phage suspension was used to infect the susceptible recipients (belonging to 12 different emm-types) and lysogenic clones were selected on plates with 4 mg/L of erythromycin.

69% of susceptible strains acquired the erythromycin and tetracycline resistance when infected with purified Φm46.1. All of the emm1, emm12, emm75, emm18, and emm94 and a fraction of the emm3, emm4, emm5, and emm6 strains were lysogenized and converted to the erythromycin and tetracycline-resistant phenotype.

No lysogenic clones were isolated from the emm77, emm78, and emm89 recipients.
In conclusion in *S. pyogenes*, the increase in MICs for erythromycin could be overall explained by the accumulation of mutation within the sequence of the mef(A)-msr(D) region. Similarly to the case of pneumococci, efflux-mediated macrolide resistance in *S. pyogenes* is inducible. In agreement with previous genetic data suggesting that the product of both genes contributes to the overall resistance to macrolides, mef(A) and msr(D) are co-transcribed as a single bicistronic mRNA.

Results obtained in this project were presented in part in:


REFERENCES