Streptococcus pyogenes emm types and subtypes of isolates from paediatric asymptomatic carriers and children with pharyngitis

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SUMMARY

This study determined *emm* subtypes of *Streptococcus pyogenes* strains isolated from asymptomatic carriers and children with pharyngitis. All strains were previously investigated for fibronectin-binding genes (*prtF1* and *prtF2*) and antimicrobial susceptibility. The most significant differences between the two groups, which share only 5 of the 14 detected *emm* subtypes, concern the presence of the two more common *emm* subtypes, 12.0 (50.0% vs. 3.1%, for asymptomatic carriers and children with pharyngitis, respectively) and 1.0 (28.1% vs. 0%, for children with pharyngitis and asymptomatic carriers, respectively).

KEY WORDS: Streptococcus pyogenes, emm subtypes, Pharyngitis, Asymptomatic carrier

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Adherence to and internalization into host cells significantly contributes to the pathogenesis of *Streptococcus pyogenes* infections (Kreikemeyer *et al.,* 2004). The M protein, encoded by the *emm* gene, generally mediates these infections and has traditionally been targeted for serotyping of *S. pyogenes* isolates. However, *emm* gene sequence typing (Beall *et al.,* 1996) is now becoming the standard method and more than 150 *emm* types have already been described (McGregor *et al.,* 2004). Moreover, the knowledge of the distribution of *S. pyogenes emm* types has implications for the development of group A streptococcal vaccines (Shulman *et al.,* 2009). Other fibronectin binding proteins, such as PrtF1 and PrtF2, can also play an important role in adhesiveness and intracellular invasion of *S. pyogenes* (Beall *et al.,* 2000; Cunningham, 2000). Our previous study (Musumeci *et al.,* 2003) compared the involvement of the *prtF1* and *prtF2* genes in *S. pyogenes* that colonizes asymptomatic carriers and causes pharyngitis, and investigated the correlation between the presence of these genes and antimicrobial susceptibility phenotypes. That study showed that the proportion of *S. pyogenes* strains carrying the *prtfF2* gene was significantly higher among asymptomatic carriers (80%) than among children with pharyngitis (53%) ($P<0.05$) suggesting that the presence of the *prtF2* gene could be linked to the ability of *S. pyogenes* to persist in the throat of asymptomatic carriers.

The aim of this study was to determine the *emm* subtypes of the *S. pyogenes* strains isolated from children with pharyngitis and from asymptomatic carriers in our previous study (Musumeci *et al.,* 2003), and to correlate the *emm* subtype with the previously determined gene pattern (*prtF1* and *prtF2*) and antimicrobial susceptibility phenotype.

In this study, 62 strains of *S. pyogenes* from asymptomatic carriers (30 strains), coming from different classes of different schools, and children with pharyngitis (32 strains) were used. All the subjects of the study were aged between 7 and 11 years. Streptococci (that had been stored in rab-

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bit blood at -70°C) were grown in Todd-Hewitt broth (Difco), supplemented with 0.2% yeast extract (Oxoid), supplemented with 1.8% BactoAgar (Oxoid) and 5% (v/v) defibrinated sheep blood (Oxoid). We then detected and characterized M/*emm* subtypes in the *S. pyogenes* isolates. *emm* gene specific PCR and *emm* sequence typing were performed as described by Beall *et al.* (1998) (*emm* sequences available at http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm). In addition, to confirm the relationship between the *emm* pattern type and SOF (serum opacity factor), the *sof* gene was detected and sequenced based on PCR and sequence analysis of a variable length 450-650-base PCR fragment (Beall *et al.,* 2000). Proportional differences in the frequency of the *emm* subtypes between the two groups were analyzed by Fisher's exact test ($P<0.05$).

Results are shown in Table 1. The most frequent *emm* subtypes detected in pharyngitis isolates were: *emm*1.0 (28.1%), *emm*89.0 (21.9%), *emm*2.0 (12.5%) and *emm*28.0 (9.4%). Moreover, 4 strains (12.5%) belonging to *emm* type 6 (2 *emm*6.0 and 2 *emm*6.36), and 1 strain of a new *emm*12 subtype (*emm*12.40), were present. The *emm* gene sequence of this last strain was submitted to the CDC *Streptococcus* Laboratory (ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/emmsequ/emm12.40). In isolates from asymptomatic carriers the most frequent *emm* subtypes were: *emm*12.0 (50.0%), *emm*2.0 (10.0%) and *emm*94.0 (10.0%); while there were no *emm*1.0 strains. It is interesting to observe that both the *emm*6 type strains of this group are of the *emm*6.36 subtype. The most significant differences between the two groups, which share only 5 of the 14 detected *emm* subtypes, concern the presence of the two more common *emm* subtypes, 12.0 (50.0% vs 3.1%, for asymptomatic carriers and children with pharyngitis, respectively; $P<0.001$) and 1.0 (28.1% vs. 0%, for children with pharyngitis and asymptomatic carriers, respectively; $P=0.001$).

At least one strain of each of the most common *emm* subtypes (12.0, 1.0, 89.0, 2.0, 6.36) was not positive for *prtF1* and/or *prtF2*. Only strains belonging to *emm*12.0 and *emm*89.0 were more frequently positive for both *prtF1* and *prtF2* (87.5 and 55.6%, respectively) than negative for one or both genes. Strains belonging to *emm*1.0 and *emm*2.0 were more frequently negative for both *prtF1* and *prtF2* (44.4 and 57.1%, respectively) than positive for one or both genes. In asymptomatic carriers, the *emm*12.0 strains were more frequently positive for *prtF2* than the remaining

TABLE 1 - *Characteristics of Streptococcus pyogenes isolates analysed in this study.*

emm subtype (total no. of strains)	*No. of strains*	*sofI*	*prtF1**	*prtF2**	*Susceptibility phenotype**
Pharyngitis					
1.0 (9)	2	-	+	+	cMLS
	1	-	+	+	EryS
	1	-	+	-	cMLS
	1	-	-	+	EryS
	2	-	-	-	cMLS
	2	-	-	-	EryS
2.0 (4)	1	+	+	+	EryS
	1	+	-	+	EryS
	2	+	-	-	EryS
6.0 (2)	2	-	+	+	cMLS
6.36 (2)	1	-	+	+	cMLS
	1	-	+	-	cMLS
12.0 (1)	1	+	+	+	EryS
12.40 (1)	1	+	+	+	cMLS
28.0 (3)	1	+	+	+	M
	1	+	+	-	cMLS
	1	+	-	+	cMLS
44/61.0 (1)	1	+	+	-	EryS
89.0 (7)	1	+	+	+	cMLS
	2	+	+	+	iMLS-A
	1	+	+	+	M
	2	+	+	-	cMLS
	1	+	+	-	iMLS-A
NT (2)	1	-	+	-	iMLS-C
	1	-	-	-	iMLS-B
Asymptomatic carriers					
2.0 (3)	1	+	+	-	cMLS
	2	+	-	-	cMLS
4.0 (1)	1	+	+	+	cMLS
6.36 (2)	1	-	+	-	EryS
	1	-	-	-	EryS
11.0 (1)	1	+	-	+	EryS
12.0 (15)	4	+	+	+	cMLS
	9	+	+	+	M
	1	+	-	+	EryS
	1	+	-	-	EryS
44/61.0 (1)	1	+	+	+	EryS
77.0 (1)	1	+	+	+	EryS
87.0 (1)	1	+	+	+	cMLS
89.0 (2)	1	+	+	+	cMLS
	1	+	-	+	EryS
94.0 (3)	1	+	+	+	EryS
	2	+	-	+	EryS

*Musumeci et al., 2003.

strains with other *emm* subtypes (93.3% vs. 66.7%, respectively; *P*=0.076).

Correlation with antimicrobial susceptibility phenotypes showed that 13 of the 16 (81.3%) *emm*12.0, 5 of the 9 (55.6%) *emm*1.0, and all the 8 (100%) *emm*89.0 strains, were erythromycin resistant. In particular, in asymptomatic carriers, erythromycin resistance was detected in 13 of the 14 (92.9%) *prtF2*-positive *emm*12.0, and in 3 of the 10 (30.0%) *prtF2*-positive strains belonging to the other *emm* subtypes.

As expected, all the *emm*1 and *emm*6 isolates were *sof* negative, while the strains belonging to the other *emm* types were *sof* positive (Table 1).

Different studies have revealed that there were significant differences in *emm* type distribution by clinical disease state, by region and, at individual regions, by year to year (Lorino *et al.,* 2006; Creti *et al.,* 2007; Schulman *et al.,* 2009; Steer *et al.,* 2009; Siljander *et al.,* 2010). However, comparison of data from other surveys from different national or international regions with our results indicated that the *emm* types always detected were *emm*1, *emm*4, *emm*6, *emm*12, *emm*28, and *emm*89 (Creti *et al.,* 2005; Mencarelli *et al.,* 2005; Baldassarri *et al.,* 2007; Creti *et al.,* 2007; Darenberg *et al.,* 2007; Schulman *et al.,* 2009; Steer *et al.,* 2009), while *emm*3, frequently reported in these surveys, did not appear in both groups of our study.

With regard to the isolation source, Creti *et al.* (2005) found that the *emm*12 type was by far the most common *emm* type in pharyngitis, and, together with the *emm*22 type, the most common in asymptomatic carrier. Moreover, Creti *et al.* (2007) demonstrated that in 11 years *emm*1 strains were responsible for about 20% of invasive *S. pyogenes* infections. In the present study *emm*12 type was highly prevalent in asymptomatic carriers, while *emm*1 constituted 28% of the isolates from pharyngitis, but was not detected in isolates from asymptomatic carriers.

In the previous study (Musumeci *et al.,* 2003), it was suggested that *prtF2*-bearing strains might be better colonizers of the human host and lead to throat carriage.

Baldassarri *et al.* (2007) found in a number of *emm* types of isolates from invasive diseases (including *emm*12) a strong association between *prtF1*/*prtF2* and the *erm* gene, and hypothesized that strains carrying the *prtF2* gene, alone or in combination with other genes, could be more efficient in intracellular survival.

Our study showed that in asymptomatic carriers 50% of the isolates belonged to the *emm*12.0 subtype, and 87% of these carried both genes (*prtF1*/*prtF2*) encoding internalization-associated proteins and was erythromycin-resistant. Thus, it could be hypothesized that the strains of this subtype have a greater cell penetration and intracellular survival ability. In order to better understand the mechanisms of asymptomatic carrier status further evaluation of larger bacterial populations is required to confirm this hypothesis.

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