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SHORT REPORT
Group A streptococcal infections: trend and strain *emm* typing in an area of central Italy, 1985–2002

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SUMMARY

A retrospective study of group A streptococcal (GAS) infections was performed for the period 1985–2002 in an area of central Italy. Although very severe diseases such as streptococcal toxic shock syndrome (STSS) were observed, a general increase in invasive infections was not found. Isolates of GAS were classified by M protein genotyping (*emm* typing) and analysed according to their origin from invasive and non-invasive infections. The predominant *emm* types were types 1, 4 and 12, followed by types 3, 6 and 28. During the study period the proportion of isolates of types 1 and 12 fell, while other types (3, 6, 22, 28 and 77) appeared. Isolates from invasive and non-invasive infections shared several *emm* types; however, most invasive strains belonged to five types only (types 1, 4, 12, 28 and 77), while non-invasive isolates were generally more heterogeneous.

The resurgence of severe group A *Streptococcus* (GAS) disease has been well described in the world's literature for over 15 years [1–8]. Patients may present with invasive infections characterized by bacteraemia or serious soft tissue infections. A significant proportion of patients may develop shock and multiple organ failure or streptococcal toxic shock syndrome (STSS) [9]. Such invasive infections have persisted in most geographical areas and have actually increased in incidence in some regions [10–14]. Despite effective treatment, the mortality rate for STSS still exceeds 30%, and has been reported to be greater than 60% in some studies [3, 11, 15]. The most prevalent strains associated with STSS and other severe invasive infections have been of M1 and M3 types [3, 15–17]. In Europe, an apparent increase of invasive infections has been observed in countries such as Sweden, England, Wales and Northern Ireland [11, 12, 14, 18], and this has been accompanied by a change in the incidence of the most common M types. These studies emphasize the need to reassess surveillance strategies for these infections.

In Italy, a surveillance study on invasive GAS infections was initiated by the Istituto Superiore di Sanità in 1994 but it was suspended in 1996 after observing an incidence of invasive episodes lower than those reported in other countries; after that period, to our knowledge, only scant published epidemiological information and strain characterization data are available for Italy [17, 19, 20]. This lack of data prompted us to review retrospectively the frequency of GAS infections during the period 1985–2002 at the Clinic of Infectious Diseases of the University of Siena and the distribution of M protein genotypes (*emm*) of these isolates.

The diagnosis of streptococcal infections was based on clinical criteria, the isolation of *S. pyogenes* and/or a twofold or greater increase in titre of anti-streptolysin O antibodies between acute and convalescent-phase

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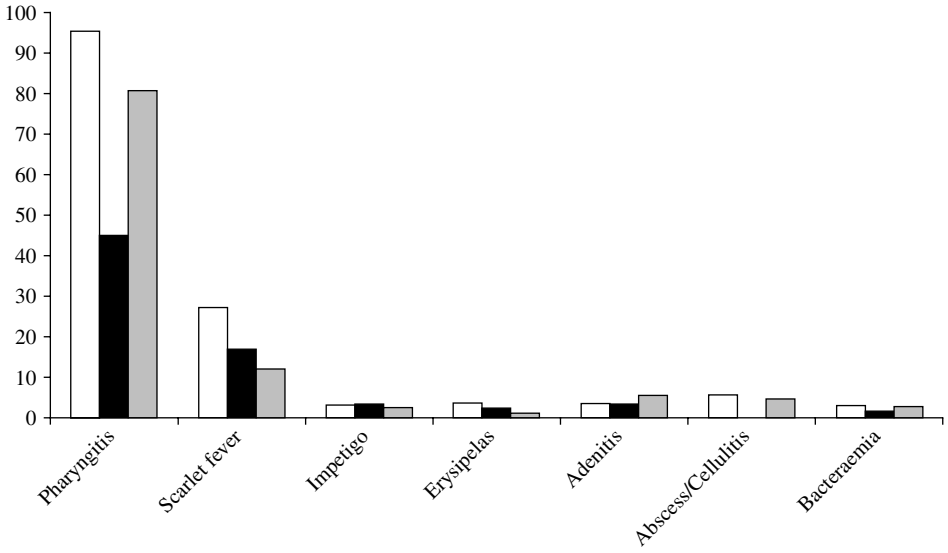

Fig. 1. Number of different infections over three intervals of time. □, 1985–1990; ■, 1991–1996; ▨, 1997–2002.

sera. The diagnosis of STSS and severe GAS infections was based on clinical criteria established by the Working Group on Severe Streptococcal Infections [9]. All subjects were epidemiologically unrelated. Clinical isolates were identified as GAS by standard laboratory methods [21] and stored at −80 °C in Wilkins–Chalgren broth (Oxoid, Basingstoke, Hampshire, UK) supplemented with 20% (v/v) glycerol.

Reference strains (obtained from the CCUG Culture Collection of the Department of Clinical Bacteriology at the University of Goteborg, Sweden, and from Chiron Biocine in Siena, Italy) were: ATCC 12344, 12345, 10389, 12385, 12347, 12348, 12353; ISVT SF 130/13, SF/4, J17C, C 98/97; CCUG 30915, 12710.

Isolates were characterized by M protein genotyping, using two methods. First, PCR amplification of the M protein gene (*emm* gene) and subsequent hybridization with a panel of 10 M-specific probes in a reverse line blotting system, according to the method of Kaufhold et al. [22] modified as previously described; second, the variable M protein type-specific region of the *emm* gene was sequenced according to protocols developed by the Center for Disease Control [23]. The χ^2 or Fisher's exact test was used for statistical analysis. A P value of $<0{\cdot}05$ was considered significant.

In the study period, 315 GAS infections were identified and of these 179 were male and 136 female; 289 patients ranged in age from 3 months to 14 years and 26 patients from 15 to 63 years. An additional 13 paediatric cases of non-suppurative sequelae (12 acute glomerulonephritis and 1 rheumatic fever) were recognized. The most frequent presentation was pharyngitis (68%) followed by scarlet fever (17%); other presentations were rare. Invasive infections (STSS, bacteraemia, abscess, cellulitis, adenitis, erysipelas) together accounted for 9·5% of all cases. These occurred in previously healthy subjects and none was fatal.

Table 1. *Source of 84* S. pyogenes *clinical isolates*

Isolation site	Strains (*n*)
URT*	62
Skin	7
Blood	5
Pus	7
Soft tissue†	3
Total	84

* URT, Upper respiratory tract (15 strains from scarlet fever).
† One strain from necrotizing fasciitis.

The temporal distribution of the cases grouped by clinical presentation is shown in Figure 1 which compares cases for the periods 1985–1990, 1991–1996 and 1997–2002. An increase in the numbers of GAS cases, mainly consisting of pharyngitis, during the second half of the 1980s and during 1997–2002 was observed but this was not accompanied by a significant change in the total number of admissions to hospital (data not shown). Invasive infections occurred in the different periods without significant differences ($P=0{\cdot}762$).

A total of 84 GAS isolates was *emm* typed; isolation sites are shown in Table 1. Twenty-one isolates

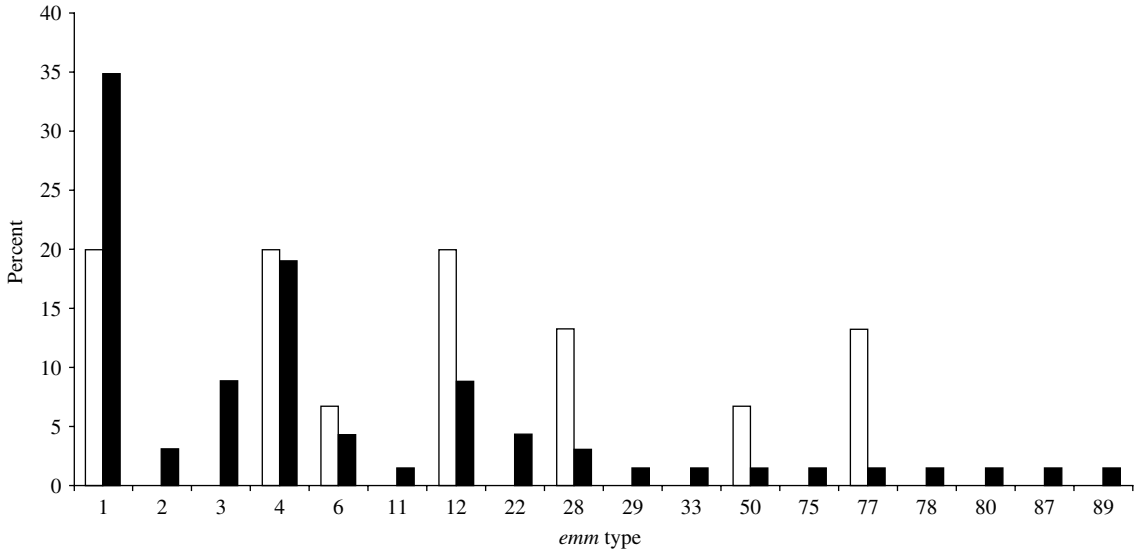

Fig. 2. *Emm*-type distribution in *S. pyogenes* clinical isolates from invasive (□) and non-invasive (■) infections.

(25%) failed to hybridize with any of the oligonucleotides used and were further analysed by *emm* gene sequencing. Figure 2 shows that 18 different *emm* types were detected. The most common was type 1 (32% of total isolates), followed by type 4 (19%) and type 12 (11%), eight types were represented by single isolates only. The distribution of *emm* types over time and in different clinical conditions was analysed. Table 2, shows the types detected in the periods 1985–1990, 1991–1996, and 1997–2002. Type 1, which was prevalent until the mid-1990s, significantly decreased afterwards and type 12 also declined over time; type 4 was relatively stable and types 3, 22 and 77 appeared only recently in the study period.

Fifteen *emm* types were found among isolates from the upper respiratory tract and seven types among skin isolates. Isolates from blood belonged to four types only (1, 12, 28 and 77) and the isolate associated with necrotizing fasciitis was of type 6. The distribution of *emm* types among invasive and non-invasive infections is shown in Figure 2. Most invasive strains belonged to types 1, 4 and 12 (each type representing ~20% of the total invasive isolates), followed by types 28 and 77 (13% each of the total invasive isolates). It is noteworthy that the *emm* 12 invasive strains were isolated during the early years of the study. The isolate from the case of STSS was of type 28 but this was not found in earlier isolates and was rare among non-invasive isolates. Types 1 and 4 were also more frequent for non-invasive infections and isolates from these cases were generally more heterogeneous. Traditionally, specific M types, such as M1 and M3 have been associated with the pathogenesis of GAS disease owing to their relatively high frequency in invasive infections and contribution to high mortality rates [2, 3, 16]. However, their prevalence among invasive episodes might only be a reflection of their overall prevalence within a community [5, 10, 24].

Table 2. *Variation over time of* emm *types of* S. pyogenes *clinical isolates*

	Number (%) of strains		
emm gene	1985–1990	1991–1996	1997–2002
*emm*1	5 (35·7%)	20 (43·4%)	2 (8·2%)
*emm*2	0	1 (2·2%)	1 (4·2%)
*emm*3	0	1 (2·2%)	5 (20·8%)
*emm*4	2 (14·4%)	9 (19·6%)	5 (20·8%)
*emm*6	0	3 (6·5%)	1 (4·2%)
*emm*11	0	0	1 (4·2%)
*emm*12	5 (35·7%)	3 (6·5%)	1 (4·2%)
*emm*22	0	0	3 (12·5%)
*emm*28	0	3 (6·5%)	1 (4·2%)
*emm*29	0	1 (2·2%)	0
*emm*33	0	1 (2·2%)	0
*emm*50	1 (7·1%)	1 (2·2%)	0
*emm*75	1 (7·1%)	0	0
*emm*77	0	0	3 (12·5%)
*emm*78	0	1 (2·2%)	0
*emm*80	0	1 (2·2%)	0
*emm*87	0	1 (2·2%)	0
*emm*89	0	0	1 (1·4%)
Total	14	46	24

The present study attempted to survey clinical and epidemiological characteristics of GAS infections

over almost two decades in an area of central Italy and to study the distribution of M types over time and in different clinical conditions. To our knowledge, recently published epidemiological information concerning Italy is at present lacking. We observed a trend towards an increase in the numbers of streptococcal disease during the second half of the 1980s and at the beginning of the 21st century but this was not accompanied by a general increase in invasive infections, unlike some other European areas [12, 14, 18]. This trend might be partially explained by the fact that cases were mainly paediatric and presenting most often as pharyngitis. However, severe streptococcal infections have been reported with increasing frequencies in many areas even during paediatric age [4, 25]. The most serious paediatric infection we observed was an abdominal abscess due to streptococcal superinfection during varicella consistent with the widely reported view that children with varicella are clearly at a greater risk of GAS invasive infections [25].

M protein genotyping showed that *emm* types 1, 3, 4, 6, 12, 28 were the most frequent and our findings are in accordance with other studies from various areas, which indicate the wide distribution of the M types we observed [10, 19, 22, 26]. The distribution of types appeared to vary from year to year and during the study period the proportion of isolates of types 1 and 12 fell, while types 3, 6, 22, 28, 77 emerged. Changes in prevalent M types may occur frequently during spread among susceptible populations, often within 1 or 2 years. Additionally, changes in prevalence of several different M types often occur concomitantly within the same population [24]. In recent years, a lower proportion of *emm1* strains has also been reported in Sweden [16, 18]. However, comparison of our data with those of other countries is difficult because most of the data available in the literature concern isolates sampled only during the 1990s; moreover, the *emm* typing of isolates collected during the current European surveillance programme on GAS invasive disease is still ongoing, and data are not yet fully available [14].

Like others [8, 13, 26] we also found that invasive and non-invasive isolates shared several *emm* types which indicates that many circulating strains were potentially pathogenic and probably induced invasive episodes in proportion to their spread in the population [8, 26, 27]. The exceptions were *emm* types 12, 28, 77 isolates, which were more common among invasive than non-invasive infections; the same types have been frequently found in invasive infections from other areas [2, 3, 10, 18]. The relative importance of any individual M type in the proportion of invasive disease it causes is likely to result from a combination of the relative frequency of strains of this type circulating within a community, the invasiveness of these strains, and the degree of individual and population-level immunity to the strains. The importance of host immune status against streptococcal virulence factors has been addressed by fundamental studies reporting a link between levels of antibodies directed against M antigen or streptococcal pyrogenic exotoxins and the disease frequency and severity [16]. Furthermore, other host factors – in particular pre-existing chronic disease, advanced age, impaired immune status – as well as other GAS virulence factors are known to be important factors in the progression of streptococcal infections from harmless colonization or surface infection to severe invasive episodes [16, 28].

In conclusion, the present study provides epidemiological information for central Italy, and further underscores the complexity of the dynamic epidemiology of group A streptococci and of the possible interaction between bacterial virulence factors and host factors. Continuous studies of trends in invasive and non-invasive GAS disease incidence, clinical manifestations and strain characterization are warranted to monitor trends in disease incidence and to improve therapeutic and preventive strategies for these infections which will be with us for many years to come.

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