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Genetic relatedness of group A streptococci of the newly designated serotype M90 causing a food-borne outbreak and sporadic infections

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SUMMARY

Twenty-six isolates of the newly designated M90 serotype group A Streptococcus (GAS) from a large food-borne outbreak of pharyngitis in Greece and six M90 sporadic isolates from UK, were typed by pulsed-field gel electrophoresis (PFGE). Twenty-four outbreak isolates were identical and two closely related. The Greek isolates were possibly related with one UK isolate, while other sporadic isolates exhibited distinct PFGE profiles from the former isolates.

INTRODUCTION

Pharyngitis due to group A streptococcus (GAS) is one of the most common bacterial infections. The invasiveness of GAS is mainly due to the filamentous, antiphagocytic M protein that also serves as the basis for the classical serotyping scheme [1], used by many reference laboratories. The M serotyping scheme comprised serotypes M1 to M81 and a series of provisional M types. Recently an expert group [2] designated 12 provisional types as new M types 82–93. In countries where serotyping is routinely performed types M1 and M3 have been strongly associated with rapidly fulminating invasive infection [3]. However, typability of strains from regions such as West Africa or South East Asia with standard M-antisera is less readily achieved, suggesting that several M-type specificities are undefined [4, 5]. Also, GAS isolates are not routinely serotyped in many regions due to the complexity associated with the preparation and availability of antisera.

More recently various molecular techniques have been applied to the epidemiological subtyping of GAS isolates. One of the most discriminatory and reproducible methods of epidemiological typing has proven to be pulsed-field gel electrophoresis (PFGE). Previous reports have shown its high discriminatory power in the investigation of GAS outbreaks due to the most common serotypes [6, 7].

Food-borne streptococcal epidemics have been previously documented [8, 9]. During the last 20 years their incidence declined and this has been attributed to the proper use of food refrigeration, cleanliness and compulsory pasteurization of fresh milk [10, 11]. Recently, a large food-borne outbreak due to a newly designated M type (M90) occurred in a Greek boarding school [12]. To our knowledge this was the first reported outbreak due to this serotype, which in the past had been rarely isolated and only from sporadic infections. The objective of the present study was to investigate the genetic relatedness of M90 GAS isolates recovered during this outbreak compared with isolates of the same serotype collected previously in other geographic regions.

METHODS

Bacterial isolates and serotyping

One hundred and fifty-four students were affected

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with pharyngitis between 17–19 December 1997, and throat specimens were obtained from 28 students who presented to the outpatients department of the AHEPA University Hospital, Thessaloniki, Greece. Samples were transported to the Medical Microbiology Laboratory of the hospital in Amies medium, inoculated onto 5% (v/v) sheep blood agar and incubated at 37 °C in 5–7% CO_2 for 24 h. Colonies characteristic of β-haemolytic streptococci were examined for the presence of group A antigen by slide agglutination grouping (Slidex Strepto-kit, bio-Merieux, Marcy l'Etoile, France). Twenty-six GAS isolates were recovered from individual patients and serotyped by T-protein agglutination typing, determination of the presence of serum opacity factor (SOR) and M-protein typing [1, 13]. Stock cultures were maintained at −70 °C, in 16% (v/v) glycerol broth containing 5% (v/v) horse blood. All outbreak isolates belonged to the T-type 3/13/B3264 and the provisional M-type PT4931, recently designated as M90 [2]. Five M90 isolates obtained sporadically from UK patients during the last 20 years, and the M90 reference strain (NCTC 12068) isolated in 1980 in Oxfordshire, UK [14], were selected as control strains for the study. These isolates belonged to the same T-type (3/13/B3264) and were obtained from the culture collection of the PHLS Streptococcus and Diphtheria Reference Unit (Central Public Health Laboratory, Colindale, London).

Macrorestriction analysis

All GAS isolates were typed by PFGE after *Sma*I macrorestriction as previously described [15], with the addition of mutanolysin to the cell suspension before mixing with molten agarose. Electrophoresis was carried out in a CHEF DRII apparatus (Bio–Rad Laboratories, Hercules, CA) for 24 h at 200 V, with pulse times being 10–35 s. Molecular mass markers were concatameters of phage lambda DNA (New England Biolabs, Hitchin, UK). PFGE was also performed upon the control strains of the same M serotype.

RESULTS AND DISCUSSION

Twenty-four of the outbreak isolates had an identical macrorestriction profile. The remaining two isolates (251 and 260) exhibited two-band differences compared with the prevalent PFGE pattern and with each other (Fig. 1). These band differences were reproducible in subsequent runs from different subcultures. By the broadly accepted criteria for interpretation of PFGE banding patterns [16], these two isolates were characterized as closely related or subtypes of the outbreak strain. The M90 reference strain, isolated in the UK many years ago, was possibly related to the Greek isolates differing by five bands from the common outbreak pattern [16]. The remaining five UK isolates exhibited PFGE patterns with more than six band differences from those of the Greek isolates and the reference strain and were considered as genetically unrelated. Among these five isolates, one (745) differed by more than nine bands from the others (789, 794, 808 and 809) which formed a common cluster with 1–3 band differences from each other. Isolates 808 and 809 had identical patterns (Fig. 1).

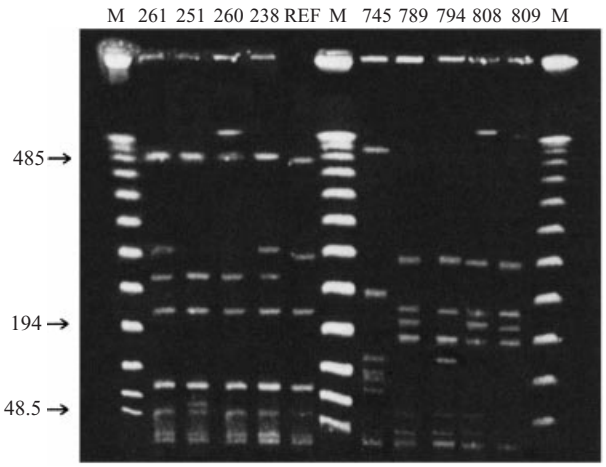

Fig. 1. Macrorestriction patterns of M90 GAS. Lanes: M, molecular mass markers; 261 and 238, outbreak strains with the common outbreak pattern; 251 and 260, outbreak strains with subtypes of the outbreak pattern; REF, reference M90 strain; 745, 789, 794, 808 and 809, M90 strains isolated in the UK.

This food-borne GAS outbreak was one of the largest documented. The high proportion of individuals simultaneously affected (64% of students) might indicate a high invasiveness of this streptococcal serotype. It is quite likely that the emergence of a 'new' M type within a population with probable low immunity levels against this serotype influenced the spread of disease and the high attack rate [17]. Data on the prevalence of the various GAS serotypes causing infections in Greece are generally unavailable and thus it cannot be excluded that M90 strains are circulating more often in this country than in others, where GAS serotyping is regularly performed and this

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type is rarely isolated. The latter possibility could be supported by the finding that two of the outbreak isolates were closely related but not indistinguishable from the common pattern and from each other. This observation might imply that GAS of this serotype were circulating within this community or externally for a longer period, during which a single genetic event (resulting in two-band differences among two outbreak isolates) has possibly occurred. Also, the possibility that this genetic event occurred during the outbreak cannot be excluded.

GAS strains have a high A+T content (61–65 mol%) [18] and lack natural transformation systems, being relatively stable to genetic events. Nevertheless, remarkable variations between isolates of the same serotype have also been observed in PFGE studies describing GAS isolates of other serotypes [7, 15]. The minor band differences between the M90 reference strain and the Greek isolates possibly resulted after two random genetic events and are surprising, taken into consideration that other UK isolates, four of them belonging to the same cluster, had distinct genomic profiles. The above genetic similarities indicate that one UK and the Greek isolates might be descendents of a common parental strain, which had possibly disseminated from UK to overseas or vice versa. As Greece is the Eastern gateway of the European Union, epidemics of uncommon invasive GAS strains would emerge as a significant threat due to low population immunity. The possible spread of this unusual serotype in our region as well as in other parts of Greece is currently under investigation.

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