Molecular characterization of *Streptococcus pyogenes* group A isolates from a tertiary hospital in Lebanon

Nathalie M. Karaky,[1] George F. Araj[2] and Sima T. Tokajian[1]

[1]Department of Natural Sciences, School of Arts and Sciences, Lebanese American University, Byblos, Lebanon

[2]Department of Pathology and Laboratory Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Correspondence
Sima T. Tokajian
stokajian@lau.edu.lb

Streptococcus pyogenes [Group A *Streptococcus* (GAS)] is one of the most important human pathogens, responsible for numerous diseases with diverse clinical manifestations. As the epidemiology of GAS infections evolves, a rapid and reliable characterization of the isolates remains essential for epidemiological analysis and infection control. This study investigated the epidemiological patterns and genetic characteristics of 150 GAS isolates from a tertiary hospital in Lebanon by *emm* typing, superantigens (SAgs) detection, PFGE and antibiotic profiling. The results revealed 41 distinct *emm* types, the most prevalent of which were *emm*89 (16 %), *emm*12 (10 %), *emm*2 (9 %) and *emm*1 (8 %). Testing for the presence of superantigens showed that *speB* (87 %), *ssa* (36 %) and *speG* (30 %) were predominant. PFGE detected 39 pulsotypes when a similarity cut-off value of 80 % was implemented. Antibiotic-susceptibility testing against seven different classes of antibiotics showed that 9 % of the isolates were resistant to clindamycin, 23 % were resistant to erythromycin and 4 % showed the macrolide–lincosamide–streptogramin B (MLS_B) phenotype. The emergence of tetracycline-resistant strains (37 %) was high when compared with previous reports from Lebanon. This study provided comprehensive evidence of the epidemiology of GAS in Lebanon, highlighting the association between *emm* types and toxin genes, and providing valuable information about the origin and dissemination of this pathogen.

Received 31 May 2013
Accepted 26 June 2014

INTRODUCTION

Group A streptococcus (GAS; *Streptococcus pyogenes*) is a common and important human pathogen responsible for a wide range of diseases, with bacteraemia being the most severe (Rantala *et al.*, 2012). The increasing number of reports published since the mid-1980s provided evidence for the severe emergence of GAS infections. Factors underlying its worldwide resurgence remain unanswered (Efstratiou, 2000).

The potency of *S. pyogenes* is attributed to several virulence factors that contribute to its pathogenic complexity. One of the most important virulence determinants, M protein encoded by the *emm* gene, facilitates adhesion and resistance to opsonophagocytosis and contributes to the overall burden of GAS infections (Staali *et al.*, 2006). Furthermore, variability in the N terminus of the M protein has been used as an epidemiological marker to characterize worldwide GAS isolates (Luca-Harari *et al.*, 2009). To date, more than 200 *emm* types and subtypes have been identified, as reported by Cole *et al.* (2011). Amongst the extracellular factors produced by GAS, bacterial superantigens (SAgs) have also gained much attention since the delineation of their mechanisms in 1989. These pyrogenic toxins trigger a substantial release of inflammatory cytokines, leading to an excessive immune response and damage to the host (Mitchell *et al.*, 2000). Hence, they are considered to be the chief contributors in the pathogenesis of GAS infections (Rivera *et al.*, 2006). The pyrogenic exotoxin A (SpeA) is a causal factor in the pathogenesis of toxin-like-shock syndrome (Musser *et al.*, 1991). Distribution of SAg genes has been used as an additional epidemiological tool to further investigate genomic heterogeneity and any probable toxin gene–disease associations (Ma *et al.*, 2009).

Analysis of the clonal relationships amongst GAS isolates remains the most practical approach to determine the epidemiology of these isolates (Chiou *et al.*, 2004).

Abbreviations: CDC, Centers for Disease Control and Prevention; CLSI, Clinical and Laboratory Standards Institute; GAS, Group A *Streptococcus*; SAg, superantigen.

One supplementary table is available with the online version of this paper.

Macrorestriction digestion of whole genomic DNA by restriction enzymes using PFGE remains the gold-standard typing technique of bacterial strains (Kidd *et al.*, 2011).

The virulence determinants associated with GAS strains worldwide are not stable. This instability emphasizes the importance of epidemiological studies targeting significant virulence factors for the identification and characterization of new emerging clones. In this study, we examined the pattern of diversity amongst and clonal distribution of 150 GAS isolates collected between years 2010 to 2011 in Lebanon. To our knowledge, this report is the first to assess the association between *emm* typing, SAg profiles, PFGE, antibiotic profiling and clinical presentations of GAS from this country.

METHODS

Clinical isolates. A systematic random sampling of 150 GAS isolates obtained from clinical specimens between August 2010 and November 2011 was kindly provided by the American University of Beirut Medical Center. The 350-bed hospital, which functions as a centre for tertiary services for inpatients, is considered the most important medical centre in Lebanon and services an expatriate population of Lebanese as well as patients from neighbouring countries. The bacterial samples were recovered from throat (95 %) and pus of skin (4 %) and wound (1 %) infections. Samples were from 67 females (45 %) and 83 males (55 %), of whom 106 (71 %) were younger than 18 years (57.3 % of whom were younger than 10 years) and 44 (29 %) were older than 18 years. The strains were confirmed to be *S. pyogenes* by β-haemolysis on blood agar plates, colony morphology (i.e. thin smooth colonies), Gram staining, negative catalase reaction, inhibition-screening test with a bacitracin disc (0.04 U) and with the use of Lancefield grouping (Oxoid). The samples were streaked on blood agar plates, were designated SN01–SN150 and stored at -80 °C.

DNA extraction, *emm* typing and superantigen detection. Purification of chromosomal DNA was performed with NucleoSpin Tissue (Macherey-Nagel) according to the manufacturer's instructions.

Typing of the *emm* locus was carried out according to the recommendations of the Centers for Disease Control and Prevention (CDC) guidelines and the protocol adapted by Beall *et al.* (2000). PCR products were purified with EXOSAP-IT (USB) and both strands sequenced. Products were then purified using the DyeEx 2.0 Spin kit (Qiagen) and further sequenced using an ABI 3500 Genetic Analyzer (Applied Biosystems). After showing 98 % similarity with the *emm* locus of a CDC reference strain, sequences were compared to the CDC *S. pyogenes emm* sequence database (http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm).

Superantigens were detected according to Commons *et al.* (2008). For multiplex PCR, a Qiagen multiplex PCR kit was used. Reaction mixtures contained 1 µg of chromosomal template, 25 µl master mix with 3 mM $MgCl_2$, 5 µl primer mix (2 mM of each primer in Tris–EDTA) and RNase-free water to a final volume of 50 µl. PCR primer sets (Table S1, available in the online Supplementary Material) were selected from published papers on the basis of specificity, compatibility and ability to target fragments of streptococcal SAgs (Commons *et al.*, 2008; Proft *et al.*, 1999, 2001, 2003; Chatellier *et al.*, 2000, Smoot *et al.*, 2002) Genomic DNAs from *Staphylococcus aureus* and *Streptococcus pneumoniae* were used as negative controls. For primers yielding amplicons of very similar sizes (*speI* and *speL*, and *speJ* and *speM*), individual PCRs using the corresponding primers were performed for strains showing positive results.

Antibiotic-susceptibility testing. All isolates were tested for antibiotic resistance by the Kirby–Baüer disc diffusion and E-tests methods in accordance with standards recommended by the Clinical and Laboratory Standards Institute (CLSI, 2011). Discs and E-tests were obtained from Oxoid, UK and AB Biodisk respectively, and included cephalothin, ciprofloxacin, clindamycin, erythromycin, teicoplanin, tetracycline, trimethoprim–sulfamethoxazole, gentamicin, ampicillin and rifampicin. *S. pneumoniae* ATCC 49619 was used for quality control. Isolates showing resistance to erythromycin were further tested for inducible resistance by placing the erythromycin disc 12 mm apart from a clindamycin disc (edge to edge). Zones of inhibition were examined after incubation for 16–20 h at 35 °C to determine any blunting in the shape of the clindmaycin zone (D-test).

PFGE. Forty-three isolates, representatives of the different *emm* types previously obtained in this study, were selected to be PFGE typed following the protocol of Chiou *et al.* (2004) with some modifications. Briefly, *S. pyogenes* isolates were grown on blood agar plates incubated in 5 % CO_2 at 35 °C for 16–24 h. The standard PFGE protocol for *Listeria monocytogenes* was followed to prepare the bacterial cell suspension, determine the bacterial concentration, make

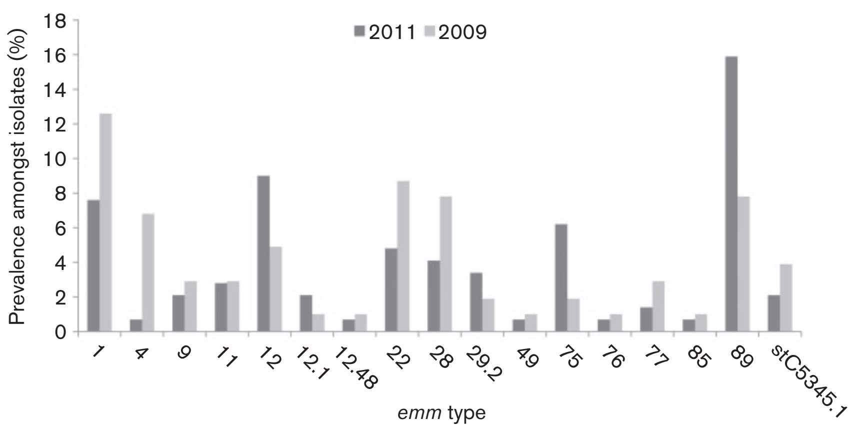


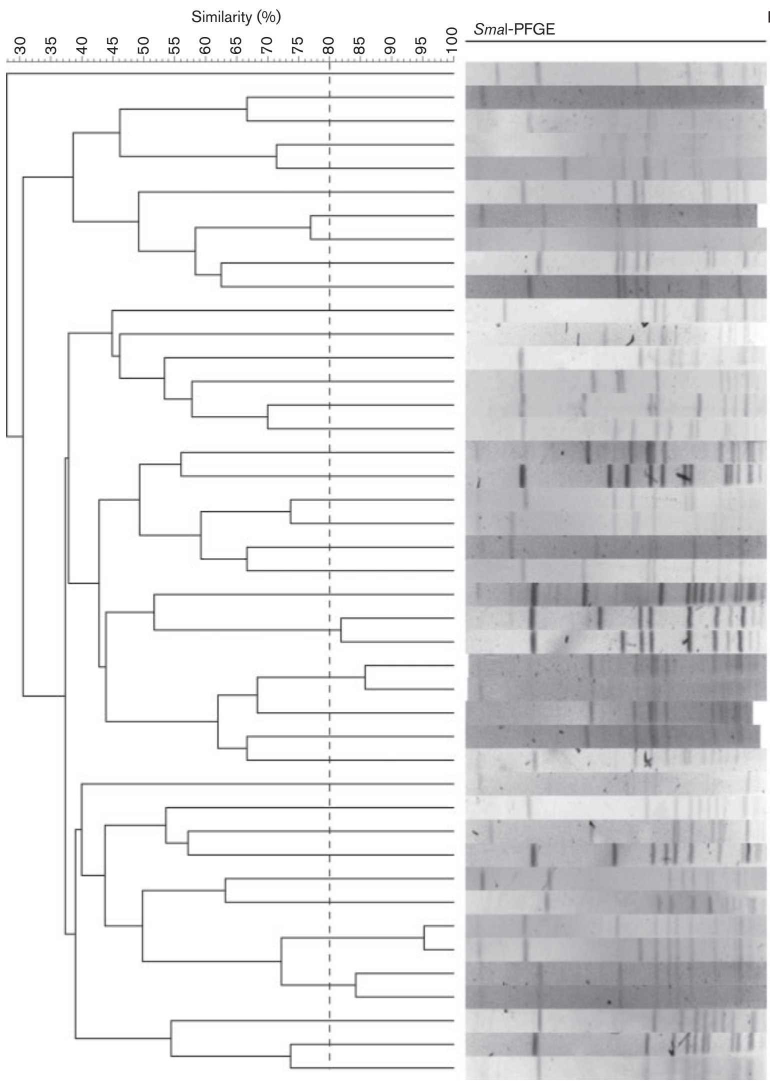
Fig. 1. Comparison of the prevalence of *emm* types obtained by Bahnan *et al.* (2011) and this study.

agarose plugs, lyse the bacterial cells and wash the plugs. Plug slices (width, 2 mm) were digested with 10 U of *Sma*I. The bands were resolved on a 1 % agarose gel in 0.5× Tris-boric acid-EDTA (pH 8.0) and run in a CHEF-DRIII System electrophoresis apparatus (Bio-Rad) at a 120° fixed angle, fixed voltage (6 V cm^{-1}) and with pulse-time intervals ranging from 4 to 40 s for 23.5 h. Lambda ladder pulsed-field gel marker (New England BioLabs) was included in each lane for the alignment of the bands. After staining with ethidium bromide, the bands were visualized under UV (SYNGENE-G:box). Cluster analysis was performed with GelCompar II software (Applied Maths) using dice coefficient and visualized as a dendrogram by the unweighted pair group method with arithmetic mean, using average linkages with 1 % tolerance and 1 % optimization settings. A similarity cut-off of 80 % was used to define a cluster.

Statistical analysis. Statistical pair-wise analysis was undertaken with χ^2 and Fisher's exact tests by comparing observed and expected associations using Software Package for Statistical Analysis (SPSS) 22.0. A *P* value of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Knowledge of the epidemiology of GAS infections is indispensable for appropriate decision-making in the treatment of infections and for limiting their spread. In the current study, we investigated GAS-associated diseases in Lebanon, by collecting and characterizing 150 isolates using *emm* typing, toxin genes profiling, PFGE and antibiotic resistance.



PFGE Type	Sample	*emm* Type	Superantigen profile	Antibiotic resistance
1	SN 084	STC6779.0	B-smeZ	
2	SN 085	85	A-H-ssa	ERY
3	SN 063	11	B-G-smeZ	
4	SN 087	77	B-J	CLA
5	SN 017	77	B-smeZ	LEV-CLI
6	SN 041	1.57	A-B-I	
7	SN 070	1	A-B-L	CLA-TET
8	SN 031	1.11	A-B	CLA-TET
9	SN 093	13	B-ssa	
10	SN 006	1	B-smeZ	
11	SN 117	87	B-G	
12	SN 112	89.1	A-B-G	TET
13	SN 128	6	B-L	ERY-TET
14	SN 068	28	B-L-ssa	CHL-CLA-ERY-TET-CLI
15	SN 036	22	B-G	CLA-TET
16	SN 027	2	B-J-K-ssa	ERY-TET
17	SN 109	89	B-G	
18	SN 096	2	G-H-K-ssa	
19	SN 137	89	B-H-ssa	CLA
20	SN 059	28	B-L-ssa	
21	SN 080	22	A-B-ssa-smeZ	ERY
22	SN 035	22	B-G	
23	SN 127	STG652	B-K-L-ssa	
24	SN 013	STG7882.1	A-B-G	
24	SN 007	9	B-ssa	CLA-CIP-LEV-TET-CLI
25	SN 136	STC1400	B-G	
25	SN 099	89	B-G-J	
26	SN 025	89	B-L	
27	SN 075	89	G	ERY
28	SN 002	49	A-H-J-K	ERY
29	SN 111	1	A-B	CLA-TET
30	SN 149	12	B-J	ERY-CIP-TET
31	SN 034	STG7882.1	A-B-G	ERY
32	SN 021	12	B-L	CHI-CIP
33	SN 120	1.44	A-B-L	
34	SN 033	75	A-B-L	
35	SN 144	89	B-G	CLA-ERY-TET
35	SN 078	12.1	B-G-ssa	
36	SN 086	12	B-L-ssa	CLA-TET-ERY
36	SN 016	22	A-B-ssa	TET
37	SN 023	108	B-L	CLA-TET
38	SN 130	12	K-G-ssa	CLA
39	SN 022	29.2	B-K	ERY

Fig. 2. Dendrogram of PFGE clusters and genotypic relationships of *S. pyogenes* isolates. *Sma*I macrorestriction patterns were analysed using the dice coefficient and relationships amongst the isolates visualized by the unweighted pair group method, using average linkages with 1 % tolerance and 1 % optimization settings. The similarity cut-off of 80 % is indicated by the broken vertical line. PFGE groups determined by cluster analysis are numbered 1–39. *emm* types, antibiotic-resistance and superantigen profiles are also included. Capital letters in superantigen profiles indicate *spe* genotypes. CHL, chloramphenicol; CIP, ciprofloxacin; CLA, clarithromycin; CLI, clindamycin; ERY, erythromycin; LEV, levofloxacin; TET, tetracycline.

Table 1. Distribution of *emm* types according to toxin-gene and antibiotic-resistance profiles

emm type (no. of strains)	Toxin-gene profile	Antibiotic* resistance (no. of strains)								
		AZI	CHL	CIP	CLA	CLI	ERY	LEV	OFL	TET
***emm*1 (2)**	*speB–smeZ*	–	–	–	–	–	–	–	–	1
***emm*1 (3)**	*speA–speB–spe L*	–	–	–	1	–	–	–	–	1
***emm*1 (1)**	*speB–ssa*	–	–	–	–	–	–	–	–	–
***emm*1 (2)**	*speA–speB*	–	–	–	1	–	–	–	–	1
***emm*1 (2)**	*speA–speB–speJ*	–	–	–	–	–	–	–	–	–
***emm*1.11 (1)**	*speA–speB*	–	–	–	1	–	–	–	–	1
***emm*1.44 (1)**	*speA–speB–speL*	–	–	–	–	–	–	–	–	–
***emm*1.51 (2)**	*speA–speB*	–	–	–	–	–	–	–	–	1
***emm*1.57 (1)**	*speA–speB–speI*	–	–	–	–	–	–	–	–	–
***emm*2 (9)**	*speB–speG*	–	–	–	–	–	1	–	–	2
***emm*2 (2)**	*speG–speH–speK–ssa*	–	1	2	1	2	2	–	–	2
***emm*2 (1)**	*speB–speJ–speK–ssa*	–	–	–	–	–	1	–	–	1
***emm*2.2 (1)**	*speB–speH–ssa*	–	–	–	–	–	–	–	–	1
***emm*4 (1)**	*speB–speG–speL–ssa*	–	–	–	–	–	–	–	–	–
***emm*6 (2)**	*speB–speK*	–	–	–	–	–	–	–	–	1
***emm*6 (2)**	*speB–speL*	–	–	–	–	–	1	–	–	1
***emm*6 (2)**	*speB–speK–ssa*	–	–	–	–	–	–	–	–	1
***emm* 9 (3)**	*speB–ssa*	–	–	1	1	1	1	1	–	2
***emm* 11 (1)**	*speB–speG–smeZ*	–	–	–	–	–	–	–	–	1
***emm* 11 (3)**	*speB–speK*	–	–	–	–	–	–	–	–	–
***emm*12 (3)**	*speB–ssa*	–	–	–	–	–	1	–	–	–
***emm*12 (2)**	*speB–speL–ssa*	–	–	–	1	1	–	–	–	1
***emm*12 (2)**	*speG–speK–ssa*	–	–	–	2	–	–	–	–	–
***emm*12 (1)**	*speA–speB–speM–ssa*	–	–	–	–	–	–	–	–	–
***emm*12 (1)**	*speB*	–	–	–	–	–	–	–	–	–
***emm*12 (1)**	*speB–speJ*	–	–	1	–	–	1	–	–	1
***emm*12 (1)**	*speB–speL*	–	1	1	–	–	–	–	–	–
***emm*12 (1)**	*speB–speL–speK–ssa*	–	1	1	1	1	1	–	–	1
***emm*12 (1)**	*speM–ssa*	–	–	1	–	–	–	–	–	1
***emm*12 (1)**	*speB–speG–ssa*	–	–	–	–	–	–	–	–	–
***emm*12.1 (2)**	*speB*	–	–	–	–	–	1	–	1	1
***emm*12.1 (1)**	*speB–speG–ssa*	–	–	–	–	–	–	–	–	1
***emm*12.48 (1)**	*speB–speG–ssa*	–	–	–	–	–	–	–	–	1
***emm*12.7 (1)**	*speB–speG*	–	–	–	–	–	–	–	–	–
***emm*12.7 (1)**	*speB–speG–ssa*	–	–	–	–	–	–	–	–	1
***emm*13 (1)**	*speB–ssa*	–	–	–	–	–	–	–	–	–
***emm*22 (2)**	*speA–speB–ssa*	–	–	–	–	–	–	–	–	1
***emm*22 (3)**	*speB–speG*	–	–	–	1	–	–	–	–	1
***emm*22 (1)**	*speA–speB–ssa–smeZ*	–	–	–	–	–	1	–	–	–
***emm*22 (1)**	*speB–speJ–speL*	1	1	1	–	–	1	1	–	1
***emm*28 (3)**	*speB–speL–ssa*	–	2	–	1	1	2	–	–	2
***emm*28 (2)**	*speB–ssa*	–	–	–	–	–	–	–	–	–
***emm*28 (1)**	*speB–speG–ssa*	–	–	–	–	–	1	–	–	–
***emm*29.2 (3)**	*speB–speK*	–	1	–	–	–	1	–	–	–
***emm*29.2 (1)**	*speB–speG*	–	–	–	1	–	–	–	–	–
***emm*29.2 (1)**	*speB*	–	–	–	–	–	–	–	–	–
***emm*29.3 (1)**	*speG–speK*	–	–	1	–	–	1	–	–	1
***emm*49 (1)**	*speA–speH–speJ–speK–smeZ*	–	–	–	–	–	1	–	–	–
***emm*75 (1)**	*speK–speL*	–	–	–	–	–	1	–	–	–
***emm*75 (3)**	*speK–speL*	–	–	–	–	–	1	–	–	–
***emm*75 (2)**	*speB–speK*	–	–	–	1	–	1	–	–	–
***emm*75 (1)**	*speA–speB–speL*	–	–	–	–	–	–	–	–	–
***emm*76 (1)**	*speB–ssa*	–	–	–	–	–	–	–	–	1
***emm*77 (1)**	*speB–smeZ*	–	–	–	–	1	–	1	–	–

Table 1. cont.

emm type (no. of strains)	Toxin-gene profile	Antibiotic* resistance (no. of strains)								
		AZI	CHL	CIP	CLA	CLI	ERY	LEV	OFL	TET
***emm*77 (1)**	*speB–speJ*	–	–	–	1	–	–	–	–	–
***emm*85 (1)**	*speA–speH–ssa*	–	–	–	–	–	1	–	–	–
***emm*87 (1)**	*speB–speG*	–	–	–	–	–	–	–	–	–
***emm*89 (5)**	*speB–speG*	–	1	–	1	–	1	–	–	1
***emm*89 (4)**	*speA–speB–speG*	–	1	2	2	1	2	–	–	2
***emm*89 (3)**	*speB–speJ–speG*	–	–	–	–	–	–	–	–	2
***emm*89 (3)**	*speG*	–	–	–	–	–	3	–	–	–
***emm*89 (3)**	*speB–speJ–ssa*	–	–	–	–	–	–	–	–	3
***emm*89 (2)**	*speB–speL*	–	–	–	–	1	–	1	–	1
***emm*89 (2)**	*speB–speK*	–	–	–	1	–	–	–	–	1
***emm*89 (1)**	*speB–speG–ssa*	–	–	–	–	–	1	–	–	1
***emm*89 (1)**	*speB–speH–ssa*	–	–	–	1	–	–	–	–	–
***emm*89.1 (1)**	*speA–speB–speG*	–	–	–	–	–	–	–	–	1
***emm*94 (1)**	*speB–speG–speL*	–	–	–	–	–	–	–	–	–
***emm*108 (2)**	*speB–speJ*	–	–	–	2	–	–	–	–	3
***emm*108 (2)**	*speB–speL*	–	–	–	1	–	1	–	–	1
***emm*108 (1)**	*speB–speC–speK*	–	–	–	–	–	–	–	–	–
***emm*108 (1)**	*speB–speG–smeZ*	–	–	1	–	–	–	–	–	–
***emm*108 (1)**	*speB–speG–ssa*	–	–	–	–	–	–	–	–	–
***emm*108 (1)**	*speB–ssa*	–	–	–	–	–	–	–	–	–
***emm*118 (1)**	*speB–speH–speK–smeZ*	–	–	–	–	–	–	–	–	–
st0412.0 (1)	*speA–speK*	–	–	–	–	–	–	–	–	–
st3211.0 (1)	*speB–speK*	–	–	–	–	–	–	–	–	–
st5240.0 (1)	*speA–speB*	–	–	–	1	–	1	–	–	–
stC1400 (1)	*speB–speG–smeZ*	–	–	–	–	–	–	–	–	–
stC5345.1 (2)	*speG–speK–ssa*	–	–	–	–	–	–	–	–	1
stC5345.1 (1)	*speB–speC–speK*	–	–	–	–	–	–	–	–	1
stC6779.0 (1)	*speB–smeZ*	–	–	–	–	–	–	–	–	–
stG166b.0 (1)	*speL–ssa*	–	–	–	1	–	1	–	–	1
stG652 (1)	*speB–speK–speL–ssa*	–	–	–	–	–	–	–	–	–
stG7882.1 (4)	*speA–speB–speG*	–	–	–	–	–	1	–	–	–
stGM220 (1)	*speB–speJ–ssa*	–	–	–	–	–	–	–	–	–
Non-typable (1)	*speA–speB*	–	–	–	–	–	–	–	–	–
Non-typable (1)	*speB–speG*	–	–	–	–	–	–	–	–	1
Non-typable (3)	No profile	–	1	–	–	2	1	–	–	2

*AZI, Azithromycin; CHL, chloramphenicol; CIP, ciprofloxacin; CLA, clarithromycin; CLI, clindamycin; ERY, erythromycin; LEV, levofloxacin; OFL, ofloxacin; TET, tetracycline; –, no resistance.

Occurrence of *emm* types amongst GAS isolates varies according to the geographical location, between and within individual countries (Ma *et al.*, 2009). Amongst the 150 isolates collected for this study in Lebanon, 145 showed interpretable *emm* sequences. Forty-one different *emm* types were identified, which reflects a higher heterogeneity than that reported from a previous study from Lebanon in which 28 *emm* types were identified amongst 103 GAS strains (91 % representing throat isolates) (Bahnan *et al.*, 2011). Comparing results from the two studies showed persistence but also variability in the relative occurrence of some *emm* types (Fig. 1). The two most common *emm* types recovered in this study, were *emm*89 (15 %), classified as *emm* pattern E, and *emm*12 (9 %), classified as *emm* pattern A–C (McMillan *et al.*, 2013). Both *emm* types are known to be highly associated with streptococcal toxic shock syndrome, necrotizing fasciitis, cellulitis, arthritis, puerperal sepsis and meningitis (Luca-Harari *et al.*, 2009), all of which are potentially fatal (Creti *et al.*, 2007). It is noteworthy that *emm* pattern E represents a generalist group, whose strains are associated with throat and superficial skin infections, while *emm* pattern A–C strains mainly cause throat infections (McMillan *et al.*, 2013). *emm* types not previously encountered in Lebanon [*emm*94 (1 %), *emm*108 (6 %) and *emm*118 (1 %)] were also detected in this study. Here, no statistically significant association was identified between *emm*-type groups and age (P=0.125) or gender (P=0.959) of patients. Moreover, the *emm*-type distribution in this study differed markedly from those reported by the CDC to be the prevalent GAS isolates in the Middle East (*emm*81, *emm*14, *emm*5 and *emm*6) and neighbouring countries (Nir-Paz *et al.*, 2010). These results highlight again the geographical instability within GAS *emm* types in Lebanon, and this was consistent with previous findings, which reported that the diversity of strains circulating in low-income settings far exceeds that in the high-income ones (Smeesters *et al.*, 2009; Steer *et al.*, 2009).

SAgs play an even more important role than *emm* types in disease manifestations (Rantala *et al.*, 2012). In this study, *speB* was detected in 87 % of the isolates followed by *ssa* (36 %) and *speG* (30 %), findings that were consistent with their worldwide geographical distribution, reflecting a homogeneous spectrum of circulating clones (Commons *et al.*, 2008). No significant association was observed between SAg profile distribution and age or gender (P=0.379 and 0.627, respectively). Seven SAg-gene profiles were detected, with '*speB–speG*' (11 %) predominating and being mainly associated with *emm*12 and *emm*89, a combination linked to puerperal sepsis and cellulitis (Luca-Harari *et al.*, 2009). However, in contrast to other studies (Commons *et al.*, 2008; Meisal *et al.*, 2010; Ma *et al.*, 2009; Rantala *et al.*, 2012), specific restriction to SAg profiles amongst individual *emm* types was not detected. A high incidence *of speA* was seen in almost all *emm*1 types and subtypes (*emm*1.11, 1.44, 1.51 and 1.57), an association that is often involved in severe infections, namely chronic renal failure (Luca-Harari *et al.*, 2008). *speI* (1 %) was found in a single isolate, along with *speA* and *speB*, and was typed as *emm*1.57. A similar finding was previously reported by Meisal *et al.* (2010) in Norway, suggesting that *emm*1.57 may have been carried by travellers from Norway to Lebanon. The low prevalence of s*peI* may be attributable to the potential of the gene to be lost during phage integration into the genome (Commons *et al.*, 2008).

All isolates, except for five (12 %), were typable using PFGE by *Sma*I and exhibited 39 distinct banding patterns with four groups containing more than one isolate when a similarity cut-off value of 80 % was used. This further demonstrated the high genetic heterogeneity amongst the collected samples. The diversity of PFGE patterns also varied amongst the same *emm* types, with the most prevalent *emm* types, *emm*89 and *emm*12, being observed amongst six (pulsotypes 17, 19, 25, 26, 27 and 35) and four (pulsotypes 30, 32, 36 and 38) distant pulsotypes, respectively. No correlation was established amongst SAg profiles and PFGE types, where similar SAg profiles were found in different *emm* types and pulsotypes; the predominant SAg profile *speA-speB-speG* was observed in pulsotypes 12, 24 and 31 (Fig. 2).

Antimicrobial resistance is considered a serious drawback in the management of patients with infectious diseases; however, penicillin G is still the antibiotic of choice for treating GAS infections (Ciftci *et al.*, 2003; Imohl *et al.*, 2010; Ndiaye *et al.*, 2009). All isolates tested were susceptible to penicillin G, ampicillin, gentamicin, teicoplanin, vancomycin and quinupristin/dalfopristin, while 23 % were resistant to erythromycin. This level of resistance, however, is relatively high compared with levels reported from Italy (4 %), Tunisia (5 %) and Argentina (<1 %) (Ardanuy *et al.*, 2010; Creti *et al.*, 2007; Gracia *et al.*, 2009; Hraoui *et al.*, 2011). Erythromycin-resistant *S. pyogenes* strains increased from 10 to 23 % when compared with results reported for strains from Lebanon by Bahnan *et al.* (2011). Erythromycin resistance amongst *S. pyogenes* isolates observed in Europe is highly variable, ranging from 2 % in UK and 10 % in Sweden to 17 % in Finland (Ciftci *et al.*, 2003; Mzoughi *et al.*, 2004; Hraoui *et al.*, 2011; Jasir *et al.*, 2001). Three isolates showed resistance to tetracycline and clindamycin, suggesting a link to multi-resistant European clones. One of the isolates was of type *emm*28, a predominant type in invasive post-partum *S. pyogenes* infections (Mihaila-Amrouche *et al.*, 2004). In addition, 13 % of the isolates were resistant to clindamycin; of those, 5 % were also resistant to erythromycin, 1 % showed the $iMLS_B$ phenotype and 3 % the $cMLS_B$ phenotype, with the resistance being conferred through either *erm*A or *erm*B genes. However, only 2 % of the studied strains were macrolide resistant but clindamycin susceptible and showed the M phenotype, having an efflux mechanism of macrolide resistance through the *mefA* gene (Villaseñor-Sierra *et al.*, 2012). Resistance to tetracycline (37 %) was also unexpectedly high, despite its decreased usage due to a worldwide upsurge of resistance, but this was in line with the findings of Nielsen *et al.* (2004) and Ndiaye *et al.* (2009). Since the new generation of quinolones represents a second option for patients allergic to β-lactams, rates of macrolide–quinolone co-resistance have become a major challenge (Ardanuy *et al.*, 2010). In our study, a variable rate of resistance was detected for quinolones: 13 % to ciprofloxacin (a second-generation quinolone) and 5 % to levofloxacin (a third-generation quinolone). Table 1 summarizes the distribution of *emm* types according to toxin-gene profile and antibiotic resistance.

To our knowledge this study is the first that combines *emm* typing, SAg and antibiotic-resistance profiling for the characterization of *S. pyogenes* isolated in Lebanon. A wide diversity of *emm* types was observed amongst all tested isolates, which emphasizes the need to implement infection-control measures to limit the spread of GAS diseases. The present study also revealed the role and distribution of SAg genes amongst GAS isolates and changes in antibiotic-susceptibility patterns. Access to cost-effective typing techniques remains crucial for the continuous surveillance of GAS epidemiology in Lebanon, and hence for the implementation of operational control measures. Further studies that include a larger number of samples, as well as access to patients' clinical histories, are important to keep track of the prevailing types, determine their genetic relationships and define the contribution of global spread on the heterogeneity of circulating *emm* types.

ACKNOWLEDGEMENTS

This study was supported and funded by the School of Arts and Sciences Research and Development Council at the Lebanese American University. The technical assistance of Ms. Houda Harastani and Ms. Maya Farah throughout the study was highly appreciated. We thank the staff of the clinical microbiology section at AUB-MC for their cooperation in sample collection. All authors hereby declare that no conflict of interest exists.

REFERENCES

Ardanuy, C., Domenech, A., Rolo, D., Calatayud, L., Tubau, F., Ayats, J., Martín, R. & Liñares, J. (2010). Molecular characterization of macrolide- and multidrug-resistant *Streptococcus pyogenes* isolated from adult patients in Barcelona, Spain (1993–2008). *J Antimicrob Chemother* **65**, 634–643.

Bahnan, W., Hashwa, F., Araj, G. & Tokajian, S. (2011). *emm* typing, antibiotic resistance and PFGE analysis of *Streptococcus pyogenes* in Lebanon. *J Med Microbiol* **60**, 98–101.

Beall, B., Gherardi, G., Lovgren, M., Facklam, R. R., Forwick, B. A. & Tyrrell, G. J. (2000). emm and sof gene sequence variation in relation to serological typing of opacity-factor-positive group A streptococci. *Microbiol* **146**, 1195–1209.

Chatellier, S., Ihendyane, N., Kansal, R. G., Khambaty, F., Basma, H., Norrby-Teglund, A., Low, D. E., McGeer, A. & Kotb, M. (2000). Genetic relatedness and superantigen expression in group A streptococcus serotype M1 isolates from patients with severe and nonsevere invasive diseases. *Infect Immun* **68**, 3523–3534.

Chiou, C. S., Liao, T. L., Wang, T. H., Chang, H. L., Liao, J. C. & Li, C. C. (2004). Epidemiology and molecular characterization of *Streptococcus pyogenes* recovered from scarlet fever patients in central Taiwan from 1996 to 1999. *J Clin Microbiol* **42**, 3998–4006.

Ciftci, E., Doğru, U., Guriz, H., Aysev, A. D. & Ince, E. (2003). Antibiotic Susceptibility of *Streptococcus pyogenes* strains isolated from throat cultures of children with tonsillopharyngitis. *J Ank Med Sch* **25**, 15–20.

CLSI (2011). *Performance standards for antimicrobial susceptibility testing*; 20th Informational Supplement M100-S20. Wayne, PA: Clinical and Laboratory Standards Institute.

Cole, J. N., Barnett, T. C., Nizet, V. & Walker, M. J. (2011). Molecular insight into invasive group A streptococcal disease. *Nat Rev Microbiol* **9**, 724–736.

Commons, R., Rogers, S., Gooding, T., Danchin, M., Carapetis, J., Robins-Browne, R. & Curtis, N. (2008). Superantigen genes in group A streptococcal isolates and their relationship with *emm* types. *J Med Microbiol* **57**, 1238–1246.

Creti, R., Imperi, M., Baldassarri, L., Pataracchia, M., Recchia, S., Alfarone, G. & Orefici, G. (2007). *emm* types, virulence factors, and antibiotic resistance of invasive *Streptococcus pyogenes* isolates from Italy: what has changed in 11 years? *J Clin Microbiol* **45**, 2249–2256.

Efstratiou, A. (2000). Group A streptococci in the 1990s. *J Antimicrob Chemother* **45** (Suppl), 3–12.

Gracia, M., Díaz, C., Coronel, P., Gimeno, M., García-Rodas, R., Rodríguez-Cerrato, V., del Prado, G., Huelves, L., Ruiz, V. & other authors (2009). Antimicrobial susceptibility of *Streptococcus pyogenes* in Central, Eastern, and Baltic European countries, 2005 to 2006: the cefditoren surveillance program. *Diagn Microbiol Infect Dis* **64**, 52–56.

Hraoui, M., Boutiba-Ben Boubaker, I., Doloy, A., Samir, E., Ben Redjeb, S. & Bouvet, A. (2011). Epidemiological markers of *Streptococcus pyogenes* strains in Tunisia. *Clin Microbiol Infect* **17**, 63–68.

Imöhl, M., Reinert, R. R., Ocklenburg, C. & van der Linden, M. (2010). Epidemiology of invasive *Streptococcus pyogenes* disease in Germany during 2003–2007. *FEMS Immunol Med Microbiol* **58**, 389–396.

Jasir, A., Tanna, A., Efstratiou, A. & Schalén, C. (2001). Unusual occurrence of M type 77, antibiotic-resistant group A streptococci in southern Sweden. *J Clin Microbiol* **39**, 586–590.

Kidd, T. J., Grimwood, K., Ramsay, K. A., Rainey, P. B. & Bell, S. C. (2011). Comparison of three molecular techniques for typing *Pseudomonas aeruginosa* isolates in sputum samples from patients with cystic fibrosis. *J Clin Microbiol* **49**, 263–268.

Luca-Harari, B., Ekelund, K., van der Linden, M., Staum-Kaltoft, M., Hammerum, A. M. & Jasir, A. (2008). Clinical and epidemiological aspects of invasive *Streptococcus pyogenes* infections in Denmark during 2003 and 2004. *J Clin Microbiol* **46**, 79–86.

Luca-Harari, B., Darenberg, J., Neal, S., Siljander, T., Strakova, L., Tanna, A., Creti, R., Ekelund, K., Koliou, M. & other authors (2009). Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol* **47**, 1155–1165.

Ma, Y., Yang, Y., Huang, M., Wang, Y., Chen, Y., Deng, L., Yu, S., Deng, Q., Zhang, H. & other authors (2009). Characterization of *emm* types and superantigens of *Streptococcus pyogenes* isolates from children during two sampling periods. *Epidemiol Infect* **137**, 1414–1419.

McMillan, D. J., Drèze, P. A., Vu, T., Bessen, D. E., Guglielmini, J., Steer, A. C., Carapetis, J. R., Van Melderen, L., Sriprakash, K. S. & Smeesters, P. R. (2013). Updated model of group A *Streptococcus* M proteins based on a comprehensive worldwide study. *Clin Microbiol Infect* **19**, E222–E229.

Meisal, R., Andreasson, I. K. G., Høiby, E. A., Aaberge, I. S., Michaelsen, T. E. & Caugant, D. A. (2010). *Streptococcus pyogenes* isolates causing severe infections in Norway in 2006 to 2007: *emm* types, multilocus sequence types, and superantigen profiles. *J Clin Microbiol* **48**, 842–851.

Mihaila-Amrouche, L., Bouvet, A. & Loubinoux, J. (2004). Clonal spread of *emm* type 28 isolates of *Streptococcus pyogenes* that are multiresistant to antibiotics. *J Clin Microbiol* **42**, 3844–3846.

Mitchell, D. T., Levitt, D. G., Schlievert, P. M. & Ohlendorf, D. H. (2000). Structural evidence for the evolution of pyrogenic toxin superantigens. *J Mol Evol* **51**, 520–531.

Musser, J. M., Hauser, A. R., Kim, M. H., Schlievert, P. M., Nelson, K. & Selander, R. K. (1991). *Streptococcus pyogenes* causing toxic-shock-like syndrome and other invasive diseases: clonal diversity and pyrogenic exotoxin expression. *Proc Natl Acad Sci U S A* **88**, 2668–2672.

Mzoughi, R., Bouallègue, O., Selmi, H., Ben Said, H., Essoussi, A. S. & Jeddi, M. (2004). Group A streptococci in children with acute pharyngitis in Sousse, Tunisia. *East Mediterr Health J* **10**, 488–493.

Ndiaye, A. G., Sarr, N. D. C., Thiam, K. & Boye, C. S. (2009). *In vitro* activity of antimicrobial agents against *Streptococcus pyogenes* isolates from patients with acute tonsillopharyngitis in Dakar, Senegal. *Microbiol Ins* 2, 25–29.

Nielsen, H. U., Hammerum, A. M., Ekelund, K., Bang, D., Pallesen, L. V. & Frimodt-Møller, N. (2004). Tetracycline and macrolide co-resistance in *Streptococcus pyogenes*: co-selection as a reason for increase in macrolide-resistant *S. pyogenes? Microb Drug Resist* **10**, 231–238.

Nir-Paz, R., Korenman, Z., Ron, M., Michael-Gayego, A., Cohen-Poradosu, R., Valinsky, L., Beall, B. & Moses, A. E. (2010). *Streptococcus pyogenes emm* and T types within a decade, 1996–2005: implications for epidemiology and future vaccines. *Epidemiol Infect* **138**, 53–60.

Proft, T., Moffatt, S. L., Berkahn, C. J. & Fraser, J. D. (1999). Identification and characterization of novel superantigens from *Streptococcus pyogenes. J Exp Med* **189**, 89–102.

Proft, T., Arcus, V. L., Handley, V., Baker, E. N. & Fraser, J. D. (2001). Immunological and biochemical characterization of streptococcal pyrogenic exotoxins I and J (SPE-I and SPE-J) from *Streptococcus pyogenes*. *J Immunol* **166**, 6711–6719.

Proft, T., Webb, P. D., Handley, V. & Fraser, J. D. (2003). Two novel superantigens found in both group A and group C *Streptococcus*. *Infect Immun* **71**, 1361–1369.

Rantala, S., Vähäkuopus, S., Siljander, T., Vuopio, J., Huhtala, H., Vuento, R. & Syrjänen, J. (2012). *Streptococcus pyogenes* bacteraemia, *emm* types and superantigen profiles. *Eur J Clin Microbiol Infect Dis* **31**, 859–865.

Rivera, A., Rebollo, M., Miró, E., Mateo, M., Navarro, F., Gurguí, M., Mirelis, B. & Coll, P. (2006). Superantigen gene profile, *emm* type and antibiotic resistance genes among group A streptococcal isolates from Barcelona, Spain. *J Med Microbiol* **55**, 1115–1123.

Smeesters, P. R., McMillan, D. J., Sriprakash, K. S. & Georgousakis, M. M. (2009). Differences among group A streptococcus epidemiological landscapes: consequences for M protein-based vaccines? *Expert Rev Vaccines* **8**, 1705–1720.

Smoot, L. M., McCormick, J. K., Smoot, J. C., Hoe, N. P., Strickland, I., Cole, R. L., Barbian, K. D., Earhart, C. A., Ohlendorf, D. H. & other authors (2002). Characterization of two novel pyrogenic toxin superantigens made by an acute rheumatic fever clone of *Streptococcus pyogenes* associated with multiple disease outbreaks. *Infect Immun* **70**, 7095–7104.

Staali, L., Bauer, S., Mörgelin, M., Björck, L. & Tapper, H. (2006). *Streptococcus pyogenes* bacteria modulate membrane traffic in human neutrophils and selectively inhibit azurophilic granule fusion with phagosomes. *Cell Microbiol* **8**, 690–703.

Steer, A. C., Law, I., Matatolu, L., Beall, B. W. & Carapetis, J. R. (2009). Global *emm* type distribution of group A streptococci: systematic review and implications for vaccine development. *Lancet Infect Dis* **9**, 611–616.

Villaseñor-Sierra, A., Katahira, E., Jaramillo-Valdivia, A. N., Barajas-García, Mde. L., Bryant, A., Morfín-Otero, R., Márquez-Díaz, F., Tinoco, J. C., Sánchez-Corona, J. & Stevens, D. L. (2012). Phenotypes and genotypes of erythromycin-resistant *Streptococcus pyogenes* strains isolated from invasive and non-invasive infections from Mexico and the USA during 1999-2010. *Int J Infect Dis* **16**, e178–e181.