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Streptococcus pyogenes emm and T types within a decade, 1996–2005: implications for epidemiology and future vaccines

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SUMMARY

Streptococcus pyogenes group A (GAS) is a primary human pathogen. We performed genetic *emm* sequence and serological T-antigen typing of 819 mostly invasive GAS isolates recovered in Israel during 1996–2005. Of the 72 *emm* types found, the six most prevalent types (1, 81, 89, 14, 28, 5) comprised 30·2% of all isolates, and *emm*-type changes were observed over the years. The predicted coverage of the 26-valent *S. pyogenes* vaccine formulated for usage in the USA was predicted to be only ~60%. On the basis of different *emm*–T antigen type associations, some Israeli strains are probably different clonal types than those found in USA. About 2% of GAS had *emm* types that were originally associated with *S. dysgalactiae* subsp. *equisimilis emm* genes. Therefore, routine *emm* typing allows meaningful GAS strain surveillance, and provides data relevant to better vaccine coverage.

Key words: *emm* typing, epidemiology, T typing, *Streptococcus pyogenes*, vaccine coverage.

INTRODUCTION

Group A streptococci (GAS) cause a variety of human infections ranging from mild, self-limited pharyngitis and impetigo to severe, sometimes life-threatening diseases such as bacteraemia, necrotizing fasciitis and toxic shock syndrome [1]. GAS also cause debilitating and life- threatening post-infectious sequelae such as nephritis and carditis. In Israel, several communities (such as ultra-orthodox Jews) have high rates of invasive GAS diseases with up to 16 cases/100 000 per year [2]. Acute rheumatic fever and post-streptoccocal arthritis are common, but the exact incidence is unknown [3]. The identification of GAS strain types has long been used for epidemiological studies and there are several strain type associations with different syndromes [4]. The M protein is a major virulence determinant of GAS and is associated with resistance to phagocytosis, adherence to cells and virulence in mouse models [5–7]. Based on the antigenic diversity of M proteins in GAS isolates, serological M typing has for decades been the principal means for strain typing. A relatively limited number of M serotypes have been described (<80), but currently M protein gene (*emm*) typing has identified more than 170 different *emm* types with over 750 subtypes [8]. In an earlier study we performed *emm* typing of M non-serotypable invasive isolates in Israel

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Table 1. *Characteristics of group A streptococci isolates according to cohort*

	Early cohort (1996–1999) $n=484$ (%)	Second cohort (2003–2005) $n=335$ (%)	P value for differences (excluding throat samples)
Throat		73	
Blood	158 (32·6)	151 (57·3)	<0·0003
Wound and soft tissue	276 (57·0)	67 (25·6)	<0·00001
Ears	7 (1·4)	18 (6·9)	0·001
Vagina and cervix	9 (1·6)	8 (2·4)	n.s.
Bone and joints	11 (2·3)	0 (0)	0·01
Other	23 (4·8)	18 (6·9)	n.s.

n.s., Not significant.

and found 59 different *emm* types [9] and a high number of different *emm* types have also been described recently in non-invasive GAS isolates from Nepal and Ethiopia [8, 10].

A 26-valent vaccine currently being evaluated for clinical use is based primarily upon the most prevalent GAS M-protein types circulating in the USA [11]. Although many vaccine candidates are M-protein-based, some researchers suggest that other targets such as the T antigen would also make good vaccine candidates [12, 13]. The heterogeneity of clonal groups in isolates sharing the same *emm* type has been frequently observed, particularly in isolates from widely separated regions and climates [8, 10]. These data indicate that the *emm* marker, while useful for predicting M-specific protective antigens, does not suffice to predict other potential variable vaccine targets within strains [13, 14].

The purpose of the current study was to determine the distribution of *emm* and T types in GAS isolates recovered in Israel during 1996–2005 [2, 9, 15] and to determine changes that occurred in the prevalence of GAS clones during those years. This distribution might have implications for the efficacy of a potential GAS vaccine.

METHODS

Sources of GAS

Group A streptococcal isolates were obtained from two different cohorts: 484 isolates were from a prospective, national population-based survey of invasive GAS performed in Israel during 1996–1999 (Early cohort, EC) [2, 9, 15]. The second cohort (SC) included all 335 GAS isolates sent for typing to the Israeli Ministry of Health Streptococcal Reference Laboratory during 2003–2005; this laboratory is responsible for collecting and typing all streptococci in Israel. All isolates were transported to the central laboratory in swabs and stored at −80 °C, until typing was performed. The collection comprised mainly invasive isolates voluntarily submitted by medical centres, and isolates found in epidemics during public health investigations, not necessarily associated with invasive diseases.

Characterization and typing of isolates

All isolates were validated to be GAS using streptococcal group antisera (Statens Serum Institut, Denmark). Isolates that were subsequently identified as having *emm* types which do not generally belong to *S. pyogenes* were regrouped using the PathoDx Strep grouping kit (Remel Inc., USA) and were also tested for L-pyrrolidonyl-β-naphthylamide hydrolysis –PYR reaction (Rosco Diagnostica, Denmark). T typing of the isolates was performed by slide agglutination using commercial antisera (Sevapharma, Czech Republic) by the same technician during the whole study period. Isolates not T-typable with these sera were retested with antisera produced in house [16]. *emm* gene typing was performed following the protocols of the Division of Bacterial Diseases (CDC), *S. pyogenes emm* gene sequence database [17]

Sequencing of PCR products was performed by two commercial sequencing services in Israel (Hylabs, Rehovot, and Hebrew University Sequencing Facility, Jerusalem). Nucleotide sequences of new emm type and subtypes were deposited and can be found at the CDC streptococcal database website [17].

Statistical analysis

This was performed with SPSS software (release 12.0.1; SPSS Inc. USA). χ^2 test or Fisher's exact test

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Table 2. *Comparison of* emm-*type and T-type distribution in group A streptococci isolates from the two cohorts*

	Early cohort		Second cohort			
emm types	No. of isolates (% of isolates in cohort)	T types associated with (*n*)	No. of isolates (% of isolates in cohort)	T types associated with (*n*)	*P* value for differences in no. of isolates/ *emm* type	*P* value for difference in T type distribution
1	43 (8·9)	1 (41), NT (2)	29 (8·7)	1 (28), NT (1)		
2	12 (2·5)	2 (10), **8 (1)**, NT (1)	7 (2·1)	2 (7), 28 (3), **8/25/imp19 (1)**		
3	13 (2·7)	3 (9), 3/13/B3264 (4)	5 (1·5)	3 (2), 3/13/B3264 (3), 49 (1)		
4	20 (4·1)	4 (9), 4/28 (8), **11 (1), 15/17/23 (1)**, NT (1)	12 (3·6)	4 (7), NT (5)		0·006
5	16 (3·3)	5 (8), 5/27/44 (8), **11** (1)	17 (5·1)	5/27/44 (9), 5 (2), 8/25/imp19 (1), NT (5)		0·03
6	7 (1·4)	6 (6), NT (1)	12 (3·6)	6 (1), NT (11)		0·009
8	1 (0·2)	8/25/imp19 (1)	2 (0·6)	NT (2)		
9	9 (1·9)	9 (9)	5 (1·5)	9 (5)		
11	13 (2·7)	11 (13), **3/13/B3264** (2)	9 (2·7)	11 (5), NT (4)		0·002
12	16 (3·3)	12 (16)	10 (3·0)	12 (8), NT (2)		
14*	15 (3·1)	49 (5), 14 (4), 3 (1), 3/13/B3264 (1), NT (4)	20 (6·0)	14 (11), 49 (11), NT (7)	0·017	
18	14 (2·9)	18 (9**), 3/13/B3264 (1), 3 (1)**, 15 (1), NT (2)	11 (3·3)	8 (2), 8/25/imp19 (2), NT (7)		0·002
19*	5 (1·0)	15/17/23 (1), NT (4)	4 (1·2)	NT (4)		
22	12 (2·5)	12 (9), **15/17/23 (2), 15 (1)**, NT (1)	9 (2·7)	12 (4), 3 (3), 3/13/B3264 (3), 14 (1), NT (2)		0·016
24*	1 (2·0)	15 (1)				
26*	12 (2·5)	15/17/23 (5), NT (7)	6 (1·8)	NT (6)		0·063
28	26 (5·4)	28/56 (15), 28 (9), 3/13/B3264 (1), 12 (1)	7 (2·1)	28 (4), 3/13/B3264 (1), NT (2)	0·061	
29*	8 (1·6)	28/56 (6), NT (2)	12 (3·6)	28 (1), NT (11)		0·004
30*	1 (0·2)	NT (1)	10 (3·0)	NT (10)	0·002	
31*			1 (0·3)	8/25/imp19 (1)		
33	13 (2·7)	3/13/B3264 (6), 3 (3), **28/56 (1), 8 (1)**, NT (3)	4 (1·2)	3 (1), **8/25/imp19 (1)**, NT (2)		
36*			3 (0·9)	18 (2), NT (1)		
42*	1 (0·2)	NT (1)				
43	2 (0·4)	3 (1), 3/13/B3264 (1)	2 (0·6)	NT (2)		
44	7 (1·4)	5/27/44 (6), 12 (1)	2 (0·6)	5/27/44 (1), NT (1)		
48	8 (1·6)	4/28 (5), 4 (2), 28 (1)	2 (0·6)	NT (1)		
49	1 (0·2)	49 (1)	1 (0·3)	NT (1)		
51*	1 (0·2)	14 (1)	1 (0·3)	NT (1)		
53	2 (0·4)	3/13/B3264 (1), NT (1)	22 (6·7)	1 (1), 3/13/B3264 (1), 8/25/imp19 (1), 28 (1), NT (18)	<0·00001	
58	4 (0·8)	2 (1), **6 (1), 9 (1)**, 8/25/imp19 (1), **15/17/23 (1)**	2 (0·6)	NT (2)		

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Table 2 (*cont.*)

emm types	Early cohort		Second cohort		*P* value for differences in no. of isolates/ *emm* type	*P* value for difference in T type distribution
	No. of isolates (% of isolates in cohort)	T types associated with (*n*)	No. of isolates (% of isolates in cohort)	T types associated with (*n*)		
59	6 (1·2)	12 (2), **1 (1)**, 11 (1), NT (2)	1 (0·3)	NT (1)		
60*	4 (0·8)	4/28 (3), 12 (1)	2 (0·6)	4 (1), NT (1)		
63			4 (1·2)	4 (1), NT (3)		
64*	17 (3·5)	3/13/B3264 (12), 3 (3), 1 (1), 8 (1)			0·001	
65/69*	5 (1·0)	8/25/imp19 (2), 2 (1), 3/13/B3264 (1), 8 (1), 15/17/23 (1), NT (1)				
66	2 (0·4)	12 (1), **28/56 (1)**				
67*	1 (0·2)	NT (1)				
68*	4 (0·8)	3/13/B3264 (4)				
71*	1 (0·2)	14 (1)				
73	7 (1·4)	3 (5), 3/13/B3264 (2)	1 (0·3)	3/13/B3264 (1)		
74	13 (2·7)	3/13/B3264 (5), 9 (5), 3 (2), 15/17/23 (1), NT (2)			0·003	
75	19 (3·9)	8/25/imp19 (12), 8 (4), 28/56 (1), NT (2)	6 (1·8)	8 (3), 8/25/imp19 (2), 5/27/44 (1)		
76	10 (2·1)	12 (10)			0·016	
77	12 (2·5)	3 (4), 3/13/B3264 (3), 4/28 (1), 9 (1), 12 (1), NT (3)	4 (1·2)	8/25/imp19 (2), 3/13/B3264 (2)		
78	3 (0·6)	11(2), 5 (1)	2 (0·6)	11 (1), NT (1)		
81	15 (3·1)	3/13/B3264 (8), 3 (2), 14 (3), 49 (1), NT (2)	24 (7·2)	8 (8), 49 (4), 8/25/imp19 (4), 14 (2), 3/13/B3264 (1), NT (7)	0·006	0·0001
82	1 (0·2)	5/27/44 (1)	10 (3·0)	5/27/44 (8), 5 (1), 11 (1), NT (1)		
83	6 (1·2)	3/13/B3264 (5), 3 (1)	4 (1·2)	12 (1), NT (3)		0·007
84*	1 (0·2)	NT (1)				
85*	3 (0·6)	3/13/B3264 (1), NT (2)	6 (1·8)	3/13/B3264 (2), 3 (1), NT (2)		
86*	3 (0·6)	3/13/B3264 (2), NT (1)				
87	7 (1·4)	28 (4), 28/56 (3)	1 (0·3)	28 (1)		
89	17 (3·5)	3/13/B3264 (9), 11 (6), 3 (2)	18 (5·4)	3/13/B3264 (15), 3 (1), NT (2)		0·012
92	5 (1·0)	**3 (2)**, **3/13/B3264 (1)**, 8/25/imp19 (1), NT (1)				
94	5 (1·0)	3/13/B3264 (4), **2 (1)**, 3 (1)	2 (0·6)	3/13/B3264 (2)		
95*	1 (0·2)	NT (1)				
102	3 (0·6)	3/13/B3264 (2), **28 (1)**	4 (1·2)	3/13/B3264 (1), 12 (1), NT (3)		
103*	2 (0·4)	NT (2)				

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Table 2 (*cont.*)

emm types	Early cohort		Second cohort		*P* value for differences in no. of isolates/ *emm* type	*P* value for difference in T type distribution
	No. of isolates (% of isolates in cohort)	T types associated with (*n*)	No. of isolates (% of isolates in cohort)	T types associated with (*n*)		
106*			11 (3·3)	5/27/44 (5), 3/13/B3264 (4), 3 (3), 5 (2), NT (1)	0·00001	
108*	1 (0·2)	28/56 (1)				
113*	1 (0·2)	3/13/B3264 (1)				
114	1 (0·2)	NT (1)				
117*	2 (0·4)	11 (1), 12 (1)				
118	17 (3·5)	3/13/B3264 (10), 3 (6), NT (1)	4 (1·2)	3/13/B3264 (3), 8 (1)	0·057	
123*	1 (0·2)	3 (1), 9 (1)				
124*			2 (0·6)	NT (2)		
ST6735*			2 (0·6)	8 (1), 8/25/imp19 (1)		
ST221*	3 (0·6)	3 (1), 3/13/B3264 (2)				
St1815*	1 (0·2)	8 (1)				
ST5282*	1 (0·2)	3 (1)				
STN165*	1 (0·2)	3/13/B3264 (1)				

Only *P* values $<0·07$ are shown.
T types in bold represent those not commonly associated with the relevant *emm* type (16, 21). Some isolates had more than one T type by the serological assay, both types are given.
* Rare in the USA ($<0·1$% of isolates according to [20]).

were used to assess differences in proportions where required, and the Mann–Whitney *U* test was used for non-parametric comparisons. A two-sided *P* value of $<0·05$ was considered significant. Comparisons were made between the two cohorts of the study regarding the *emm* and T types of isolates, We also compared the associated clinical source and the geographical regions in Israel by allocating patients into four distinct geographical regions.

RESULTS

A total of 484/530 GAS isolates were available for *emm* typing from the EC and all 335 isolates from the SC. Table 1 shows the characterization of isolates according to source. Since the SC was formed from voluntary submission of strains to the central laboratory, there was possible selection bias resulting from underreporting and selection for epidemics and more severe cases. A main difference between the two cohorts was that in the SC 21·8% ($n=73$ isolates) were throat samples and 78·2% were from invasive disease while all EC isolates were from invasive disease. Data regarding the region in Israel were missing for 57 isolates (17%) in the SC and in 80 isolates (16·5%) of the EC, due to lack of specified patient origin. The number of isolates in the EC according to region in Israel was 71 (Northern), 100 (Central), 85 (Southern) and 157 (Jerusalem) while the corresponding numbers in the SC were: three (Northern); 98 (Central), 106 (Southern) and 62 (Jerusalem). This might suggest a lower submission rate from the northern part of Israel or might reflect a lower incidence of GAS disease in that area.

A total of 72 different *emm* types were identified in the two cohorts (Table 2). We found 17 new *emm* subtypes and one new *emm* type in 28 of the isolates in both cohorts; in the larger EC, there were only two new subtypes in three isolates (*emm* types 26·1, 26·2), and one new type (stN165). The 17 new subtypes were all represented in the SC ($P<0·001$) and 15 were unique to this cohort (5·52, 5·53, 6·41, 6·43, 19·8, 30·3, 30·4, 30·5, 30·6, 30·7, 30·8, 30·9, 31·2, 36·3, 53·7). However, it should be noted, that types *emm5* and *emm6* characteristically display a large number of subtypes based upon the overlap of the subtype-determining region with unstable tandem repeats [18]. Many of the isolates of these new subtypes (15/28)

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Table 3. *GAS isolates with* emm *types normally associated with* S. dysgalactiae *subsp*. equisimilis

emm (*n*)	Associated T types (*n*)	Identification (*n*)
stG6 (2)	2 (2)	*S. pyogenes* (1), non-*S. pyogenes* (1)
stG245 (2)	3/13/B3264 (1), 25 (1)	*S. pyogenes* (1), non-*S. pyogenes* (1)
stG480 (3)	4 (2), NT (1)	*S. pyogenes* (2), non-*S. pyogenes* (1)
stG485 (7)	2 (3), 4 (1), 8 (1), 8/25/imp19 (1), 25 (1), 28 (1), 28/56 (1)	*S. pyogenes* (6), non-*S. pyogenes* (1)
stG653 (1)	4 (1)	*S. pyogenes* (1)
stG840 (1)	4 (1)	*S. pyogenes* (1)
stC36 (1)	3/13/B3264 (1)	*S. pyogenes* (1)
stC839 (1)	2 (1), 25 (1)	*S. pyogenes* (1)

Isolates were considered to be *S. pyogenes* on the basis of a positive PYR test

were found in the Jerusalem area, mainly from a hospital serving an ultra-orthodox Jewish community, or from a particular hospital in central Israel that also serves an ultra-orthodox community. The other major source for new subtypes (8/28 isolates) was the major hospital of the Southern region of Israel which serves a large Bedouin population. Data regarding ethnicity of patients was not available to us.

The six most prevalent *emm* types were 1 (72 isolates), 81 (39), 89 (35), 14 (35), 28 (33), 5 (33) which comprised 30·2% of all isolates. The distribution of *emm* types differed between the cohorts ($P<0{\cdot}001$). Table 2 shows that the nine most common types (39·4% of all isolates) in the EC were *emm* 1, 28, 4, 75, 64, 89, 118, 5 and 12, while in the SC the leading nine types (49·6% of all isolates) were 1, 53, 81, 14, 89, 5, 4, 6 and 29. Only 60·1% of the isolates in the EC and 56·4% in the SC are included in the current 26-valent GAS vaccine. When throat isolates were excluded from the SC this did not significantly change *emm*-type distributions and the nine most common types were: 1 (8·3%), 53 (8·3%), 81 (6·8%), 5 (5·7%), 89 (4·9%), 106 (4·2%), 6 (3·8%), 18 (3·8%) and 14 (3·4%) with 53·4% coverage by the 26-valent vaccine. In order to control potential sampling bias error in the SC we compared *emm* changes in Jerusalem and the Southern region in which the submission of samples was similar to the method applied during the EC. In both regions a significant change in *emm*-type distribution was observed in invasive isolates between the EC and SC ($P<0{\cdot}005$ for each region).

Some of the *emm* types that were encountered in this survey (e.g. 14, 19, 26, 29, 64 and others; Table 2) were not commonly reported in the USA. However, many of the *emm* types had T-type associations that were previously known [19, 20] although several new associations were noted (e.g. *emm26* and *emm81*), which have not been previously noted in the USA. Some variation of *emm*/T-type associations was observed between the two cohorts tested (Table 2).

We had previously reported that some M-type serologically defined GAS isolates may harbour *emm* gene types first described in other streptococcal species [9]. In this study we found 18 isolates that were previously grouped as GAS by serological methods, but sequence typing revealed that they harbour an *emm* type associated with group G or group C streptococci rather than *S. pyogenes* (Table 3). Interestingly, all but one of these isolates were T-typable and 14 were considered to be *S. pyogenes* on the basis of a positive PYR test.

DISCUSSION

S. pyogenes (GAS) is a leading human pathogen [12, 21] and its wide antigenic diversity is a consequence of allelic variation in the M protein/*emm* gene. In this study we have presented the spectrum of GAS isolates in Israel based on two consecutive nationwide cohorts, an early cohort of all invasive isolates collected in Israel in the late 1990s and the other more recent isolates voluntarily submitted to the state Streptococcus Reference Laboratory. This study suggests that M serotyping previously performed in Israel was inaccurate. For example, type M3 was previously thought to be the most prevalent (26%) in Israel [9]; but *emm* gene typing performed on the same set of strains (i.e. EC), revealed a frequency of only 2·6% A similar change in prevalence was observed for other widespread M types such as M2 and M28. Thus, *emm*

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typing enabled us to describe a precise resolution of GAS strains in Israel.

One of the prominent features from both cohorts was the large diversity of *emm* types found in a small country, which has a population of only seven million and a geographical size of 22145 km². Other studies [8, 10, 22] suggested similar *emm*-type diversity in GAS found in Nepal, Ethiopia and India. Studies from the UK, USA and Japan [23–25] showed a smaller array of *emm* types, suggesting that higher diversity is probably characteristic of specific geographic regions. The high *emm*-type diversity in Israel may be due to immigration of people from geographically diverse locations contributing GAS strains predominant in several different countries. For example, st221 has never been observed in USA isolates from the CDC's national invasive disease surveillance; yet was originally described from GAS recovered in Ethiopia [26], from which there has been considerable immigration to Israel since the 1990s. Nevertheless, some of the prevalent *emm* types in Israel are rarely found in European countries with close proximity to Israel such as Cyprus and Greece [19]. This fact coupled with our finding of several new *emm*/T-type associations, might suggest the presence of endemic Israeli virulent clones.

The current 26-valent M-protein-based vaccine under development includes types accounting for 56–60% of the isolates described in this study in both cohorts, which is similar to the coverage by the vaccine of Ethiopian isolates [10]. However, this is lower than the 80% coverage of USA or Japanese isolates [8, 25] and exceeds the coverage of 19% in Nepalese isolates [8] Therefore, effective M-type-specific protein-based vaccines may need to be tailored according to the specific epidemiology of the country. In addition, the changing *emm*-type distribution over time (Table 2) might suggest that a continuous adjustment of the vaccine constituents would be necessary even when the strain coverage is higher. Alternatively, there may be an advantage to other vaccine approaches, such as pilus proteins and the T antigen.

A limitation of our study is that unlike the EC, the SC, was not a systematic sampling of GAS isolates but comprised more severe or invasive cases (e.g. 262/335 submissions). Moreover, there was no routine submission of throat isolates; thus, the main reason for submitting such isolates was the occasional intriguing cases, clusters or epidemics investigated by local health officers. Therefore, the 'non-invasive' specimens may represent unique or perhaps more virulent strains. Taken together with the EC performed in the late 1990s, this study gives a partial but probable representative description of the GAS strains associated with invasive infections in Israel.

We found significant differences in both proportions of certain *emm* types and the specific T types within the same *emm* type in the two sets of isolates. This phenomenon has already been documented using serological M- and T-typing many years ago [27]. It was later suggested [20, 28] and demonstrated by genetic testing of isolates [8, 28] that changes of the T type and serum opacity factor within the same *emm* type represent different clonal types. Thus, T-type variation within the same *emm* type potentially indicates intra-*emm* type clonal diversity (e.g. types *emm4*, *emm18*, *emm81*, *emm89*). This phenomenon suggests the emergence and spread of novel GAS clones in Israel. Similar phenomenon of changing epidemiology of *emm* types in *S. pyogenes* has recently been reported in Sweden [29].

Another interesting observation is the finding of 18 isolates associated with *emm* types normally associated with *S. dysgalactiae* subsp. *equisimilis*, yet having *S. pyogenes* T antigen types and the group A carbohydrate. We [9] and others [30] have previously reported this phenomenon and *emm* typing of all serotypable isolates enabled us to detect such strains here.

To conclude, routine *emm* typing allowed meaningful GAS strain surveillance. We found the ultra-orthodox Jewish population in Israel, which is known to have an extremely high incidence of GAS causing serious infections [2], to have a high rate of new *emm* subtypes. These data indicate the need for a vaccine that is tailored against a broad array of different strains and is also adaptable to changes in the epidemiology of *S. pyogenes emm* types.

DECLARATION OF INTEREST

None.

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