Epidemiology Analysis of *Streptococcus pyogenes* in a Hospital in Southern Taiwan by Use of the Updated *emm* Cluster Typing System

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emm typing is the most widely used molecular typing method for the human pathogen *Streptococcus pyogenes* (group A streptococcus [GAS]). *emm* typing is based on a small variable region of the *emm* gene; however, the *emm* cluster typing system defines GAS types according to the nearly complete sequence of the *emm* gene. Therefore, *emm* cluster typing is considered to provide more information regarding the functional and structural properties of M proteins in different *emm* types of GAS. In the present study, 677 isolates collected between 1994 and 2008 in a hospital in southern Taiwan were analyzed by the *emm* cluster typing system. *emm* clusters A-C4, E1, E6, and A-C3 were the most prevalent *emm* cluster types and accounted for 67.4% of total isolates. *emm* clusters A-C4 and E1 were associated with noninvasive diseases, whereas E6 was significantly associated with both invasive and noninvasive manifestations. In addition, *emm* clusters D4, E2, and E3 were significantly associated with invasive manifestations. Furthermore, we found that the functional properties of M protein, including low fibrinogen-binding and high IgG-binding activities, were correlated significantly with invasive manifestations. In summary, the present study provides updated epidemiological information on GAS *emm* cluster types in southern Taiwan.

Streptococcus pyogenes (group A streptococcus [GAS]) is an important Gram-positive human pathogen, which is responsible for more than 500,000 deaths per year (1). Although there is no vaccine available, several vaccine candidates, especially M protein-based vaccines, are currently in development (2–5). M protein is a bacterial surface protein and has important roles in GAS pathogenesis (6, 7). These include binding to human fibrinogen, complement regulatory proteins, and immunoglobulins; contributing to resistance to phagocytic cell clearance; and inducing vascular leakage during infection (8–15). M protein-based vaccines are constructed from the hypervariable N-terminal region (26- and 30-valent vaccine) or the conserved C-terminal proportion (J8 vaccine and StreptInCor) of the M protein and were all proven to be effective against GAS infection in animal models (2, 3, 5). In addition, phase I trials with the 30-valent and J8 vaccines are under way in North America and Australia, respectively (16).

The variable nucleotide sequence encoding the N-terminal region of the M protein is not only the antigenic target for developing GAS vaccines (17–19) but also the basis for the sequence-based *emm* typing method (20). *emm* typing is the most widely used molecular typing approach, and more than 200 different *emm* types have been reported worldwide (21–23). However, since *emm* typing is based on a small portion of the *emm* gene, this typing method provides limited information about the predicted conformational structure or functional domains of the M protein (24). *emm* pattern typing is another typing method that is based on the presence and arrangement of *emm* and *emm*-like genes in the GAS genome (25). *emm* pattern typing has been shown to correlate significantly with tissue tropism; however, only a small proportion of specific *emm* pattern strains have been extensively studied (22, 26).

A new typing method, *emm* cluster typing, which is based on the nearly complete sequence of the *emm* gene, has been proposed

(24). This typing system classifies *emm* types into 48 discrete *emm* clusters. Each *emm* cluster type contains closely related M proteins that share similar functional and structural properties. Recently, Baroux et al. (27) and Shulman et al. (28) analyzed strains isolated from the Pacific region and North America by using the *emm* cluster system and suggested that this typing system can provide additional information for GAS vaccine development. However, these studies provided limited information about the association between *emm* cluster type, specific disease manifestations, and trends in the yearly fluctuation of *emm* cluster prevalence, which are important data for GAS epidemiology.

In the present retrospective surveillance study, GAS isolates from a hospital in southern Taiwan collected between 1994 and 2008 were analyzed with the *emm* cluster typing system. Epidemiological information on prevalence and the yearly fluctuation of *emm* cluster types was provided. In addition, the association between *emm* cluster and specific disease manifestation was analyzed in this study.

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TABLE 1 Prevalence of *emm* and *emm* cluster types among 677 isolates in southern Taiwan

Clade (<i>n</i>)	<i>emm</i> type(s) (no. of isolates)	<i>emm</i> cluster	No. of isolates (<i>n</i> = 677)	% of isolates	
				All isolates	Most frequent <i>emm</i> types ^a
X (394)	4 (107), 78 (1), 165 (13)	E1	121	17.9	29
	13 (21), 66 (2), 76 (3), 92 (15), 106 (23), 110 (1)	E2	65	9.6	15.6
	9 (1), 49 (6), 58 (7), 82 (8), 87 (11), 103 (3), 113 (22)	E3	58	8.6	13.9
	22 (22), 77 (1), 88 (4), 89 (13), 102 (17), 114 (1), 175 (1)	E4	59	8.7	14.1
	11 (43), 42 (1), 63 (1), 75 (22), 81 (23), 99 (1)	E6	91	13.4	21.8
Y (283)	1 (82), 238 (1)	A-C3	83	12.3	19.9
	12 (161)	A-C4	161	23.8	38.6
	3 (1)	A-C5	1	0.1	0.2
	115 (1)	D2	1	0.1	0.2
	123 (1)	D3	1	0.1	0.2
	33 (3), 70 (1), 80 (8), 86 (1), 93 (1), 101 (5)	D4	19	2.8	4.6
	6 (15)	M6	15	2.2	3.6
	24 (1)	M24	1	0.1	0.2
	57 (1)	M57	1	0.1	0.2

^a *emm12*, *emm4*, *emm1*, *emm11*, and *emm81* (*n* = 416).

MATERIALS AND METHODS

Bacterial isolates and disease classifications. Six hundred seventy-seven GAS isolates collected from 1994 to 2008 at National Cheng Kung University Hospital, Tainan, Taiwan, were included in this study. Among these isolates, 334 GAS isolates were collected consecutively from patients older than 20 years between 1997 and 2008 and were described in the previous study (29). An additional 276 isolates were collected from patients younger than 20 years from 1997 to 2008, and 67 isolates were collected from patients of all ages from 1994 to 1996 in this study. The diagnoses of patients were categorized as noninvasive diseases, invasive diseases, nonsuppurative sequelae, and unknown. Colonization (GAS isolated from healthy, asymptomatic carriers is defined as colonization), pharyngitis, tonsillitis, urinary tract infection, upper respiratory tract infection, and superficial infections (including genital tract infection, wound infection, erysipelas, ecthyma, impetigo, paronychia, dermatitis, folliculitis, and carbuncle) were categorized as noninvasive diseases. Cellulitis, bacteremia, sepsis, toxic shock syndrome, necrotizing fasciitis, and deep tissue infections (diseases caused by GAS in deep sterile tissues) were categorized as invasive diseases.

***emm* and *emm* cluster typing.** PCR amplification and DNA sequencing were performed according to the protocol for *emm* typing from the Centers for Disease Control and Prevention (<http://www.cdc.gov/streplab/protocol-emm-type.html>). To identify the *emm* and *emm* cluster types of clinical isolates, the nucleotide sequence of the *emm* gene was submitted to the Streptococci Group A Subtyping Request Form BLAST 2.0 server (National Centers for Disease Control, Biotechnology Core Facility Computing Laboratory, <http://www2a.cdc.gov/ncidod/biotech/strepblast.asp>). The unknown type sequence was aligned with the reference *emm* type sequences in the database of this server to determine the *emm* and *emm* cluster types.

Statistical analysis. The chi-square test with Bonferroni correction on adjusted residuals was used to estimate the association between diseases and *emm* cluster types. These analyses were performed by SPSS software version 17 (SPSS Inc., Chicago, IL). A *P* value of less than 0.05 is considered statistical significance. The theoretical vaccine coverage (VC) was calculated as $VC = (i/N) \times 100\%$. *N* indicates the total number of isolates. The *i* indicates the number of isolates belonging to vaccine-protected *emm* types. The 95% confidence interval was estimated as $VC \pm 1.96 \times [VC(1 - VC)/N]^{0.5}$.

RESULTS

Prevalence of *emm* and *emm* cluster types. In the present study, 677 isolates collected from 1994 to 2008 were analyzed. Forty-four

different *emm* types were identified, and the 5 most frequent types were *emm12* (161, 23.8%), *emm4* (107, 15.8%), *emm1* (82, 12.1%), *emm11* (43, 6.4%), and *emm81* (23, 3.4%), which were responsible for 61.4% of all isolates (Table 1). In addition, 14 *emm* clusters were identified, and *emm* clusters A-C4 (161, 23.8%), E1 (121, 17.9%), E6 (91, 13.4%), and A-C3 (83, 12.3%) accounted for 67.4% of these isolates (Table 1). *emm12*, *emm4*, and *emm1* were the 3 most common *emm* types in southern Taiwan and also belong to the most common *emm* clusters A-C4, E1, and A-C3, respectively. *emm11*, *emm81*, and *emm75*, which accounted for 13% of the total isolates, belong to *emm* cluster E6 (Table 1).

Trends in the yearly fluctuation of *emm* cluster type prevalence. GAS isolates were consecutively collected from 1997 to 2008. We found that isolates belonging to clade X peaked in 1997, 2004, and 2008, whereas isolates belonging to clade Y peaked in 2000, 2003, and 2006 (Fig. 1A). Similar to this trend, *emm* clusters A-C3 and A-C4, which belong to clade Y, peaked in year 2003 and years 2000 and 2006, respectively (Fig. 1B). However, *emm* clusters E1 and E6, the two most predominant *emm* clusters in clade X, showed similar trends in their yearly fluctuation from 1997 to 2008 (Fig. 1C). In further comparisons of the trends in the yearly fluctuation of different *emm* types in the same clade of *emm* clusters, we found similar trends with *emm* clusters E2 (*emm13* and *emm106*), E3 (*emm82* and *emm87*), E4 (*emm89* and *emm102*), and E6 (*emm11* and *emm81*) (see Fig. S1 in the supplemental material).

***emm* cluster type and disease manifestation.** Among 677 cases, 252, 389, and 3 cases were invasive, noninvasive, and non-suppurative sequelae, respectively (Tables 2 and 3). The clinical manifestation was unknown for 33 patients. Seventy-one percent of invasive infections were caused by clade X *emm* clusters (E1, E2, E3, E4, and E6). Among the invasive cases, deep tissue infection and cellulitis were the most common clinical manifestations (63% of invasive manifestations). Statistical analysis showed that D4 and E3 were associated with deep tissue infection ($P < 0.001$) and cellulitis ($P < 0.001$), respectively. Furthermore, *emm* cluster E2 was significantly associated with necrotizing fasciitis ($P < 0.001$). Superficial infection, pharyngitis and tonsillitis, and scarlet fever were major manifestations among noninvasive infections (93%

FIG 1 Trends in the yearly fluctuations of different *emm* cluster types. GAS isolates were consecutively collected from 1997 to 2008. The trends in the yearly fluctuations of clade X and Y strains (A), *emm* cluster types A-C3 and A-C4 (B), and *emm* clusters E1 and E6 (C) from 1994 to 2008 are shown.

[Table 3]). A-C4 was significantly associated with pharyngitis ($P < 0.001$) and scarlet fever ($P < 0.001$). In addition, E1 was associated with scarlet fever ($P < 0.001$). Among the analyzed *emm* cluster types, *emm* cluster E6 was correlated with both cellulitis (invasive manifestation, $P < 0.001$) and superficial infections (noninvasive manifestation, $P < 0.001$). *emm* clusters A-C3 and E4 were not correlated with any specific disease manifestation.

Functional classification of M protein and disease manifestations. According to the functional classification reported by Sanderson-Smith et al. (24), M proteins belonging to different *emm* clusters displayed distinct functional profiles. The binding to C4b-binding protein (C4BP) and immunoglobulin is restricted

largely to clade X, and fibrinogen binding and plasminogen binding are restricted to clade Y (24). In the present study, we first analyzed the correlation between disease manifestations and the ability of M proteins to interact with host proteins. Statistical analysis showed that the lack of fibrinogen-binding activity had a higher probability of being associated with invasive manifestations ($P < 0.001$). Among the *emm* clusters that lack fibrinogen-binding activity (D2, D3, D4, E1, E2, E4, E3, and E6), D4, E2, and E3 were correlated with invasive manifestations (Table 4). *emm* cluster E6 strains were significantly correlated with both invasive and noninvasive manifestations (Table 4). However, *emm* cluster E1 was correlated with noninvasive infection (scarlet fever). In

TABLE 2 Distribution of GAS isolates characterized by *emm* and *emm* cluster type according to clinical disease: invasive infection ($n = 252$)

Clade (no. of isolates; %)	<i>emm</i> type(s) (no. of isolates)	<i>emm</i> cluster	No. (%) of isolates by infection type:					
			Deep tissue infection	Cellulitis	Necrotizing fasciitis	Bacteremia	Sepsis	STSS ^a infection
X (179; 71)	4 (19), 165 (5)	E1	9 (4)	7 (3)	2 (1)	2 (1)	1 (0.4)	3 (1)
	13 (15), 76 (1), 92 (11), 106 (13)	E2	7 (3)	12 (5)	11 (4)	6 (2)	3 (1)	1 (0.4)
	49 (4), 58 (5), 82 (4), 87 (7), 103 (2), 113 (14)	E3	8 (3)	16 (6)	5 (2)	4 (2)	3 (1)	
	22 (4), 88 (1), 89 (11), 102 (9), 175 (1)	E4	9 (4)	6 (2)	6 (2)	2 (1)	2 (1)	1 (0.4)
	11 (18), 42 (1), 75 (19), 81 (14), 99 (1)	E6	16 (6)	25 (10)	4 (2)	2 (1)	5 (2)	1 (0.4)
Y (73; 29)	1 (25)	A-C3	8 (3)	3 (1)	2 (1)	3 (1)	4 (2)	5 (2)
	12 (29)	A-C4	11 (4)	6 (2)	3 (1)	4 (2)	3 (1)	2 (1)
	123 (1)	D3		1 (0.4)				
	33 (3), 80 (5), 86 (1), 93 (1), 101 (4)	D4	8 (3)	4 (2)	1 (0.4)		1 (0.4)	
	6 (3)	M6	1 (0.4)			1 (0.4)		1 (0.4)
	57 (1)	M57			1 (0.4)			
Total			77 (31)	80 (32)	35 (14)	24 (10)	22 (9)	14 (6)

^a STSS, streptococcal toxic shock syndrome.

TABLE 3 Distribution of GAS isolates characterized by *emm* and *emm* cluster type according to clinical disease: noninvasive infection (*n* = 389)

		No. (%) of isolates by infection type:						
Clade (no. of isolates; %)	<i>emm</i> type(s) (no. of isolates)	<i>emm</i> cluster	Colonization	Superficial infection	Fever	Pharyngitis and tonsillitis	Scarlet fever	UTI ^a
X (200; 51.4)	4 (85), 78 (1), 165 (8)	E1	2 (1)	22 (6)	2 (1)	25 (6)	41 (11)	2 (1)
	13 (5), 66 (2), 76 (2), 92 (4), 106 (8), 110 (1)	E2	1 (0.3)	15 (4)		5 (1)	1 (0.3)	
	9 (1), 49 (2), 58 (2), 82 (3), 87 (3), 103 (1), 113 (7)	E3		13 (3)		5 (1)	1 (0.3)	
	22 (16), 77 (1), 88 (1), 89 (1), 102 (8), 114 (1)	E4		11 (3)		9 (2)	6 (2)	2 (1)
	11 (25), 63 (1), 75 (2) 81 (9)	E6	1 (0.3)	30 (8)		3 (1)	2 (1)	1 (0.3)
Y (189; 48.6)	1 (45)	A-C3	6 (2)	3 (1)		19 (5)	17 (4)	
	12 (129)	A-C4	8 (2)	11 (3)	1 (0.3)	51 (13)	56 (14)	2 (1)
	115 (1)	D2		1 (0.3)				
	80 (1), 101 (1)	D4		1 (0.3)		1 (0.3)		
	6 (12)	M6		2 (1)		6 (2)	3 (1)	1 (0.3)
Total			18 (5)	109 (28)	3 (1)	124 (32)	127 (33)	8 (2)

^a UTI, urinary tract infection.

addition, low IgG-binding activity was associated with noninvasive infections ($P < 0.001$). However, among the *emm* clusters having low IgG-binding activity (D2, D3, D4, and A-C4), *emm* cluster D4 was correlated with invasive manifestations (Table 4; see also Table S1 in the supplemental material). Other functional classifications, such as IgA-binding and C4BP-binding activities, were not correlated with specific disease manifestations.

M protein vaccine coverage. Based on the *emm* types of the 30-valent M protein based-vaccine (30), the theoretical protective coverage for our strains was 79.8% (95% confidence interval [CI], 76.8% to 82.8%). When considering the *emm* types with cross-opsonization described previously (30), the theoretical protective

coverage was increased to 83.6% (95% CI, 80.8% to 86.4% [see Table S2 in the supplemental material]).

DISCUSSION

In the present study, we found that the most frequently isolated *emm* cluster types in southern Taiwan were A-C4, E1, E6, and A-C3. Among them, *emm* clusters A-C4 and E1 were associated with noninvasive manifestations. *emm* cluster E6 was associated with both invasive and noninvasive diseases. In addition, *emm* clusters D4, E2, and E3 were significantly associated with invasive manifestations. Furthermore, low fibrinogen-binding and high IgG-binding activities were significantly correlated with invasive

TABLE 4 Distribution of 677 GAS isolates in M protein fibrinogen-binding and IgG-binding activities by clinical disease

Disease category ^f	Total	No. (%) of isolates			
		By activity level:			
		Fibrinogen binding		IgG binding	
		Low ^b	High ^c	Low ^d	High ^e
Invasive infection ^a	249 (100)	194 (78)	55 (22)	44 (18)	205 (82)
Deep tissue infection	76 (31)	57 (23)	19 (8)	19 (7)	57 (24)
Cellulitis	80 (32)	71 (28)	9 (4)	11 (4)	69 (28)
Necrotizing fasciitis	35 (14)	29 (12)	6 (2)	4 (2)	31 (12)
Bacteremia	23 (9)	16 (6)	7 (3)	4 (2)	19 (7)
Sepsis	22 (9)	15 (6)	7 (3)	4 (2)	18 (7)
STSS infection	13 (5)	6 (2)	7 (3)	2 (1)	11 (4)
Noninvasive infection ^a	377 (100)	203 (54)	174 (46)	132 (35)	245 (65)
Colonization	18 (5)	4 (1)	14 (4)	8 (2)	10 (3)
Superficial infection	107 (28)	93 (24)	14 (4)	13 (3)	94 (25)
Fever	3 (0.8)	2 (0.5)	1 (0.2)	1 (0.3)	2 (0.5)
Pharyngitis and tonsillitis	118 (31)	48 (12)	70 (19)	52 (14)	66 (17)
Scarlet fever	124 (33)	51 (13)	73 (20)	56 (15)	68 (18)
UTI	7 (2)	5 (1)	2 (0.5)	2 (0.5)	5 (1)

^a *emm* cluster M6 is not included in this table (*n* = 12, unknown binding activity for fibrinogen and IgG).^b Low fibrinogen-binding activity included *emm* clusters E1, E2, E3, E4, E6, D2, D3, and D4.^c High fibrinogen-binding activity included *emm* clusters A-C3, A-C4, and M57.^d Low IgG-binding activity included *emm* clusters A-C4, D3, and D4.^e High IgG-binding activity included *emm* clusters E1, E2, E3, E4, E6, A-C3, and M57.^f Abbreviations: STSS, streptococcal toxic shock syndrome; UTI, urinary tract infection.

manifestations, suggesting that fibrinogen-binding and IgG-binding activities of M protein are potential risk factors for invasive infections.

In comparison with the surveillance results from North America (2000 to 2007, GAS isolates recovered from patients with pharyngitis in the United States) (28), three of our most frequently isolated *emm* clusters (A-C3, A-C4, and E1) are also the most prevalent *emm* clusters in the United States. However, different *emm* cluster types were found among isolates from southern Taiwan and the Pacific region (27). In North America, *emm* cluster E2, which includes 15 different *emm* types, accounted for only 0.2 to 0.5% of the isolates (28). However, in the present study, 9.6% of isolates were *emm* cluster E2, and this *emm* cluster type was significantly associated with necrotizing fasciitis. *emm* cluster A-C5, which includes one of the most important types, *emm3*, was only 0.1% of the isolates from southern Taiwan. Furthermore, M95, one of the most frequent *emm* clusters in the Pacific Region (27), was absent in southern Taiwan. These results support the suggestion by Shulman et al. (28) that some *emm* cluster types are restricted to certain geographic areas. In addition, identical *emm* cluster types may also be associated with different disease manifestations. For example, *emm* cluster D4 was associated with skin infection (noninvasive manifestation) and deep tissue infection (invasive manifestation) in North America (28) and southern Taiwan, respectively.

Dale et al. (2) indicated that the structural similarities among M proteins used in the multivalent M protein-based vaccine may contribute to the cross-protection against nonvaccine M proteins. By definition, M proteins included in the same *emm* cluster share >70% sequence identity (24). Therefore, most of the M proteins in the same *emm* cluster type show cross-protection in rabbits (24). According to these reports, we proposed that *emm* types that belong to the same clade of *emm* cluster should have similar trends in yearly fluctuation if cross-protection did occur. However, results from clade X and clade Y were inconsistent. The trends in the yearly fluctuation in prevalence of the two most prevalent *emm* cluster types of clade Y, A-C3 and A-C4, were completely different (Fig. 1B). In contrast, *emm* clusters E1 and E6, which belong to clade X, showed similar yearly fluctuations in prevalence (Fig. 1C). In addition, compared to the trends in yearly fluctuation between the two most prevalent *emm* types in the *emm* clusters E2, E3, E4, and E6, all the types showed similar yearly fluctuations from 1997 to 2008 (see Fig. S1 in the supplemental material). These results suggest that the cross-protection may provide pressure to restrict fluctuations in the prevalence of clade X strains. Although these results are not representative, due to the isolates being collected from a single hospital, our results still provide a hypothesis to explain the possible mechanism of cross-protection between different *emm* types.

Interaction with fibrinogen is important for GAS pathogenesis. Bacterial surface M or M-related proteins have been shown to bind to human fibrinogen (9, 15). Binding to fibrinogen contributes to preventing the deposition of complement on the bacterial surface and is important for GAS to evade phagocytic clearance (10, 12, 14). Furthermore, M protein released from the GAS surface can form complexes with fibrinogen and directly contributes to vascular leakage during infection (13, 15). Anderson et al. (8) further showed that the binding of M protein to fibrinogen reduces GAS adherence to human pharyngeal keratinocytes. Although bacterial adhesion ability is considered to be critical for

initial infection, the decrease of adhesion to local tissue is also related to disseminated infection (31). In addition, M and M-related proteins also bind to human IgG to confer resistance to phagocytosis (11, 32). In the present study, we found that both IgG and fibrinogen binding are significantly correlated with disease manifestations. However, among 8 *emm* cluster types (D2, D3, D4, E1, E2, E3, E4, and E6) that lack fibrinogen-binding activity (24), three of them were associated with invasive diseases. In addition, *emm* cluster E1, which has low fibrinogen-binding and high IgG-binding activities, was associated with scarlet fever (non-invasive manifestation). Therefore, although low fibrinogen-binding and high IgG-binding activities are statistically correlated with invasive manifestations, whether these properties could be used as risk factors to predict invasive GAS infections still needs to be further verified.

The advantage of the *emm* cluster typing system is to group several *emm* types into a single cluster type, which can add more information in GAS molecular epidemiology. For example, *emm* types in the *emm* cluster A-C3 included *emm1*, *emm163*, *emm227*, *emm238*, and *emm229*. However, among these *emm* types, *emm1* is the most prevalent type and others are rarely found. The same situation is also found in *emm* cluster A-C4 (the *emm12* type is the most prevalent type) and E1 (the *emm4* type is the most prevalent type). Therefore, when analyzing the correlation between disease manifestations and *emm* clusters (especially for A-C3, A-C4, and E1), types *emm1*, *emm12*, and *emm4* contribute significantly in the statistical analysis, which is the major limitation of this typing system.

As a complementary molecular typing tool, the *emm* cluster typing system has been shown to provide additional information about the functional and structural properties of M protein and could be a useful tool to identify vaccine antigens (24, 27, 28). However, basic epidemiology information, including the yearly fluctuation in prevalence of *emm* cluster types and the correlation between disease and *emm* cluster types, was absent. In the present study, we provide this epidemiology information from 677 isolates collected from 1994 to 2008 in southern Taiwan. We suggest that accumulating the information from both *emm* and *emm* cluster typing systems would provide further help in monitoring and preventing GAS infections worldwide.

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REFERENCES

- Carapetis JR, Steer AC, Mulholland EK, Weber M. 2005. The global burden of group A streptococcal diseases. *Lancet Infect Dis* 5:685–694. [http://dx.doi.org/10.1016/S1473-3099\(05\)70267-X](http://dx.doi.org/10.1016/S1473-3099(05)70267-X).
- Dale JB, Fischetti VA, Carapetis JR, Steer AC, Sow S, Kumar R, Mayosi BM, Rubin FA, Mulholland K, Hombach JM, Schödel F, Henao-Restrepo AM. 2013. Group A streptococcal vaccines: paving a path for

- accelerated development. *Vaccine* 31(Suppl 2):B216–B222. <http://dx.doi.org/10.1016/j.vaccine.2012.09.045>.
3. Good MF, Batzloff MR, Pandey M. 2013. Strategies in the development of vaccines to prevent infections with group A *Streptococcus*. *Hum Vaccin Immunother* 9:2393–2397. <http://dx.doi.org/10.4161/hv.25506>.
 4. Waddington CS, Snelling TL, Carapetis JR. 2014. Management of invasive group A streptococcal infections. *J Infect* 69(Suppl 1):S63–S69. <http://dx.doi.org/10.1016/j.jinf.2014.08.005>.
 5. Postol E, Alencar R, Higa FT, Freschi de Barros S, Demarchi LMF, Kalil J, Guilherme L. 2013. StreptInCor: a candidate vaccine epitope against *S. pyogenes* infections induces protection in outbred mice. *PLoS One* 8:e60969. <http://dx.doi.org/10.1371/journal.pone.0060969>.
 6. Bisno AL, Brito MO, Collins CM. 2003. Molecular basis of group A streptococcal virulence. *Lancet Infect Dis* 3:191–200. [http://dx.doi.org/10.1016/S1473-3099\(03\)00576-0](http://dx.doi.org/10.1016/S1473-3099(03)00576-0).
 7. Smeesters PR, McMillan DJ, Sriprakash KS. 2010. The streptococcal M protein: a highly versatile molecule. *Trends Microbiol* 18:275–282. <http://dx.doi.org/10.1016/j.tim.2010.02.007>.
 8. Anderson EL, Cole JN, Olson J, Ryba B, Ghosh P, Nizet V. 2014. The fibrinogen-binding M1 protein reduces pharyngeal cell adherence and colonization phenotypes of M1T1 group A *Streptococcus*. *J Biol Chem* 289:3539–3546. <http://dx.doi.org/10.1074/jbc.M113.529537>.
 9. Uchiyama S, Andreoni F, Zürcher C, Schilcher K, Ender M, Madon J, Matt U, Ghosh P, Nizet V, Schuepbach RA, Zinkernagel AS. 2013. Coiled-coil irregularities of the M1 protein structure promote M1-fibrinogen interaction and influence group A *Streptococcus* host cell interactions and virulence. *J Mol Med* 91:861–869. <http://dx.doi.org/10.1007/s00109-013-1012-6>.
 10. Li Y, Courtney HS. 2011. Promotion of phagocytosis of *Streptococcus pyogenes* in human blood by a fibrinogen-binding peptide. *Microbes Infect* 13:413–418. <http://dx.doi.org/10.1016/j.micinf.2010.12.008>.
 11. Courtney HS, Li Y. 2013. Non-immune binding of human IgG to M-related proteins confers resistance to phagocytosis of group A streptococci in blood. *PLoS One* 8:e78719. <http://dx.doi.org/10.1371/journal.pone.0078719>.
 12. Carlsson F, Sandin C, Lindahl G. 2005. Human fibrinogen bound to *Streptococcus pyogenes* M protein inhibits complement deposition via the classical pathway. *Mol Microbiol* 56:28–39. <http://dx.doi.org/10.1111/j.1365-2958.2005.04527.x>.
 13. Herwald H, Cramer H, Mörgelin M, Russell W, Sollenberg U, Norrby-Teglund A, Flodgaard H, Lindbom L, Björck L. 2004. M protein, a classical bacterial virulence determinant, forms complexes with fibrinogen that induce vascular leakage. *Cell* 116:367–379. [http://dx.doi.org/10.1016/S0092-8674\(04\)00057-1](http://dx.doi.org/10.1016/S0092-8674(04)00057-1).
 14. Courtney HS, Hasty DL, Dale JB. 2006. Anti-phagocytic mechanisms of *Streptococcus pyogenes*: binding of fibrinogen to M-related protein. *Mol Microbiol* 59:936–947. <http://dx.doi.org/10.1111/j.1365-2958.2005.04977.x>.
 15. Macheboeuf P, Buffalo C, Fu C-Y, Zinkernagel AS, Cole JN, Johnson JE, Nizet V, Ghosh P. 2011. Streptococcal M1 protein constructs a pathological host fibrinogen network. *Nature* 472:64–68. <http://dx.doi.org/10.1038/nature09967>.
 16. Moreland NJ, Waddington CS, Williamson DA, Sriskandan S, Smeesters PR, Proft T, Steer AC, Walker MJ, Baker EN, Baker MG, Lennon D, Dunbar R, Carapetis J, Fraser JD. 2014. Working towards a group A streptococcal vaccine: report of a collaborative Trans-Tasman workshop. *Vaccine* 32:3713–3720. <http://dx.doi.org/10.1016/j.vaccine.2014.05.017>.
 17. Smeesters PR, Mardulyn P, Vergison A, Leprieux R, Van Melder L. 2008. Genetic diversity of group A *Streptococcus* M protein: implications for typing and vaccine development. *Vaccine* 26:5835–5842. <http://dx.doi.org/10.1016/j.vaccine.2008.08.037>.
 18. Steer AC, Batzloff MR, Mulholland K, Carapetis JR. 2009. Group A streptococcal vaccines: facts versus fantasy. *Curr Opin Infect Dis* 22:544–552. <http://dx.doi.org/10.1097/QCO.0b013e328332bbfe>.
 19. Steer AC, Magor G, Jenney AWJ, Kado J, Good MF, McMillan DJ, Batzloff M, Carapetis JR. 2009. *emm* and C-repeat region molecular typing of beta-hemolytic streptococci in a tropical country: implications for vaccine development. *J Clin Microbiol* 47:2502–2509. <http://dx.doi.org/10.1128/JCM.00312-09>.
 20. Facklam RF, Martin DR, Lovgren M, Johnson DR, Efstratiou A, Thompson TA, Gowan S, Kriz P, Tyrrell GJ, Kaplan E, Beall B. 2002. Extension of the Lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: *emm103* to *emm124*. *Clin Infect Dis* 34:28–38. <http://dx.doi.org/10.1086/324621>.
 21. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. 2009. Global *emm* type distribution of group A streptococci: systematic review and implications for vaccine development. *Lancet Infect Dis* 9:611–616. [http://dx.doi.org/10.1016/S1473-3099\(09\)70178-1](http://dx.doi.org/10.1016/S1473-3099(09)70178-1).
 22. McMillan DJ, Drèze PA, Vu T, Bessen DE, Guglielmini J, Steer AC, Carapetis JR, Van Melder L, Sriprakash KS, Smeesters PR. 2013. Updated model of group A *Streptococcus* M proteins based on a comprehensive worldwide study. *Clin Microbiol Infect* 19:E222–E229. <http://dx.doi.org/10.1111/1469-0691.12134>.
 23. Beall B, Facklam R, Thompson T. 1996. Sequencing *emm*-specific PCR products for routine and accurate typing of group A streptococci. *J Clin Microbiol* 34:953–958.
 24. Sanderson-Smith M, De Oliveira DMP, Guglielmini J, McMillan DJ, Vu T, Holien JK, Henningham A, Steer AC, Bessen DE, Dale JB, Curtis N, Beall BW, Walker MJ, Parker MW, Carapetis JR, Van Melder L, Sriprakash KS, Smeesters PR, M Protein Study Group. 2014. A systematic and functional classification of *Streptococcus pyogenes* that serves as a new tool for molecular typing and vaccine development. *J Infect Dis* 210:1325–1338. <http://dx.doi.org/10.1093/infdis/jiu260>.
 25. Hollingshead SK, Readdy TL, Yung DL, Bessen DE. 1993. Structural heterogeneity of the *emm* gene cluster in group A streptococci. *Mol Microbiol* 8:707–717. <http://dx.doi.org/10.1111/j.1365-2958.1993.tb01614.x>.
 26. Bessen DE, Lizano S. 2010. Tissue tropisms in group A streptococcal infections. *Future Microbiol* 5:623–638. <http://dx.doi.org/10.2217/fmb.10.28>.
 27. Baroux N, D'Ortenzio E, Amédéo N, Baker C, Ali Alsuwayyid B, Dupont-Rouzeyrol M, O'Connor O, Steer A, Smeesters PR. 2014. The *emm*-cluster typing system for group A *Streptococcus* identifies epidemiologic similarities across the Pacific region. *Clin Infect Dis* 59:e84–e92. <http://dx.doi.org/10.1093/cid/ciu490>.
 28. Shulman ST, Tanz RR, Dale JB, Steer AC, Smeesters PR. 2014. Added value of the *emm*-cluster typing system to analyze group A *Streptococcus* epidemiology in high-income settings. *Clin Infect Dis* 59:1651–1652. <http://dx.doi.org/10.1093/cid/ciu649>.
 29. Chiang-Ni C, Wu AB, Liu CC, Chen KT, Lin YS, Chuang WJ, Fang HY, Wu JJ. 2011. Emergence of uncommon *emm* types of *Streptococcus pyogenes* among adult patients in southern Taiwan. *J Microbiol Immunol Infect* 44:424–429. <http://dx.doi.org/10.1016/j.jmii.2011.04.005>.
 30. Dale JB, Penfound TA, Chiang EY, Walton WJ. 2011. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. *Vaccine* 29:8175–8178. <http://dx.doi.org/10.1016/j.vaccine.2011.09.005>.
 31. Nyberg P, Sakai T, Cho KH, Caparon MG, Fässler R, Björck L. 2004. Interactions with fibronectin attenuate the virulence of *Streptococcus pyogenes*. *EMBO J* 23:2166–2174. <http://dx.doi.org/10.1038/sj.emboj.7600214>.
 32. Nordenfelt P, Waldemarson S, Linder A, Mörgelin M, Karlsson C, Malmström J, Björck L. 2012. Antibody orientation at bacterial surfaces is related to invasive infection. *J Exp Med* 209:2367–2381. <http://dx.doi.org/10.1084/jem.20120325>.