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SHORT REPORT
Prevalent *emm* types and superantigen gene patterns of group A *Streptococcus* in Thailand

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SUMMARY

Group A *Streptococcus* (GAS) are globally distributed bacterial pathogens. We examined the *emm* genotypes, which are important indicators of virulence, of 349 clinical GAS isolates collected using two surveillance systems, i.e. Invasive Bacterial Infection Surveillance (IBIS) from 2010 to 2011 (234 isolates) and routine surveillance of clinically isolated bacteria from various hospitals during 1996–2011 (115 isolates) in Thailand. The major *emm* genotypes in IBIS samples were *emm44* (12·0%), *emm104* (6·8%), *emm22* (5·6%), and *emm81* (5·6%), whereas only one isolate (0·4%) had the *emm1* genotype, which is significantly more common in invasive cases in the Western world. In samples collected during routine surveillance, *emm238* (10·4%), *emm44* (8·7%), and *emm165* (7·0%) were dominant. The major superantigen gene profiles were similar between the groups, and 30·1% of isolates did not possess the phage-encoded superantigens (*speA, speC, speH, speI, speK, speL, speM, ssa*). Although most isolates exhibited limited gene profiles, *emm44* isolates had highly variable gene profiles (15 patterns). We conclude that *emm44* is the predominant GAS genotype in Thailand, and isolates varied in superantigen gene profiles.

Key words: *emm* typing, group A *Streptococcus*, M protein, Thailand.

Group A *Streptococcus* (GAS), *Streptococcus pyogenes*, are among the most prevalent bacterial pathogens globally, causing a variety of acute infections (e.g. pharyngitis, skin infections, toxic shock syndrome, severe necrotizing fasciitis) and post-infectious sequelae (e.g. glomerulonephritis, rheumatic fever). The relative incidence of GAS diseases varies depending on both location and season [1].

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GAS produces a range of virulence factors that contribute to the infectious process. The M protein, encoded by the *emm* gene, is one of the major virulence factors and promotes evasion of phagocytic killing. The M protein has traditionally been used for serological typing of GAS strains but this has recently been replaced by DNA sequencing of the 5′-end of the *emm* gene encoding the distal tip of the fibrillar M protein. To date, sequence typing has identified over 250 *emm* genotypes [2].

The distribution of *emm* genotypes is important for informing local and global epidemiological trends of GAS infections. A meta-analysis by Steer *et al.* [3]

showed that 25 *emm* types account for 90% of GAS in industrialized countries with the most prevalent type being *emm1* (18·3%), followed by *emm12* (11·1%), *emm28* (8·5%), *emm3* (6·9%), and *emm4* (6·9%). However, in Africa and the Pacific regions, 26 *emm* types account for about 62% of isolates, which indicates a higher diversity of *emm* types in these regions, with a correspondingly lower prevalence of those types that are commonly found in industrial countries (e.g. *emm1, emm4, emm6, emm12*).

The distribution of *emm* types has been reported for some Asian countries, such as Japan, India, and China [3], but few reports are available on *emm*-type distribution in Southeast Asian countries, including Thailand. The aim of this study was to characterize the prevalence and distribution of *emm* genotypes in Thailand using sequence typing and superantigen gene analysis.

The Invasive Bacterial Infection Surveillance (IBIS) programme was initiated in 2010 to detect various bacterial pathogens – including *S. pyogenes* – from normally sterile body sites during routine diagnosis in the Thailand hospital network. This comprised 57 provincial hospitals located in the central, eastern, northern, northeastern, and southern regions of the country. In this project, 234 GAS isolates were obtained in 2010–2011 from blood (230), synovial fluid (2), joint fluid (1), and cerebrospinal fluid (1). An additional 115 GAS isolates were collected in 1996–2011 from pus (51), blood (40), throat (14), urine (3), sputum (2), joint fluid (1), endotrachea (1), ear (1), wound (1), and an unknown source (1) using routine surveillance of pathogenic bacteria from patients collected from various hospitals in Thailand by the Department of Medical Sciences, Ministry of Public Health, Thailand. IBIS is an active surveillance programme for invasive bacterial infections while routine surveillance is a passive system based on requests from various bacterial infections.

GAS isolates were identified by colony morphology, *β*-haemolysis on sheep blood agar, and bacitracin sensitivity. The *emm* gene was amplified by polymerase chain reaction using purified chromosomal DNA at a tenfold dilution followed by sequencing according to the protocol from the Centers for Disease Control and Prevention (CDC) Atlanta, GA, USA (http://www.cdc.gov/streplab/protocol-emm-type.html). A specific *emm* type of each isolate was determined if the first 180 bp of the test and reference *emm* sequences in the CDC database were identical (http://www.cdc.gov/ncidod/biotech/Strep/Strepblast.htm). Novel *emm* types or subtypes were determined by the curator of the *emm* sequence database.

Eight phage-encoded superantigen genes (*speA, speC, speH, speI, speK, speL, speM, ssa*) and three chromosomally encoded superantigen genes (*speG, speJ, smeZ*) were amplified separately using 11 previously described primer pairs [4]. The superantigen gene distribution patterns were determined by the presence or absence of GAS superantigen genes. *S. pyogenes* SSI-1 (NCBI GenBank accession no: NC_004606), MGAS9429 (NC_008021), MGAS5005 (NC_007297), MGAS10270 (NC_008022), MGAS315 (NC_004070), and MGAS8232 (NC_003485) were used to validate the detection accuracy, and the precise identification of all superantigen genes tested was confirmed.

A total of 67 *emm* types was identified in 349 GAS isolates (Fig. 1). Within the isolates collected by IBIS, 58 *emm* types were identified and the prevalence rates were as follows: *emm44* (12·0%), *emm104* (6·8%), *emm22* (5·6%), *emm81* (5·6%), and *emm75* (5·1%). The *emm1* and *emm12* genotypes, which are common in Western countries [3], represented only 0·4% and 2·6% of the total isolates, respectively. Of the isolates collected during routine surveillance, 42 *emm* types were found with prevalence rates as follows: *emm238* (10·4%), *emm44* (8·7%), *emm165* (7·0%), *emm88* (6·1%), and *emm25* (5·2%).

The chromosomally encoded superantigen genes *speG, speJ*, and *smeZ* were found in 83·8%, 26·9% and 94·0% of the IBIS isolates, respectively, while the prophage-encoded superantigen genes *speA, speC, speH, speI, speK, speL, speM*, and *ssa* occurred in 9·0%, 30·3%, 35·0%, 26·1%, 15·4%, 19·2%, 30·8%, and 7·7%, of the isolates, respectively. Seventy superantigen patterns were identified and 14 widely distributed profiles of superantigen genes accounted for 61·1% of the total isolates (Table 1). Superantigen gene profiles A, B, and D, which were devoid of phage-encoded superantigen genes, accounted for 26·5% of the isolates. Most *emm* types exhibited limited gene profiles, e.g. 13/16 *emm104* isolates were classified as profile C, and all 13 *emm22* isolates belonged to profile A. The prevalent profiles in *emm44* isolates were profiles I (six isolates), K (four isolates), and M (four isolates), and *emm*44 isolates were characterized by 15 profiles in total.

In the routine surveillance isolates, *speG, speJ*, and *smeZ* were identified in 83·5%, 32·2%, and 83·5%, respectively, and *speA, speC, speH, speI, speK, speL, speM*, and *ssa* in 11·3%, 20·0%, 27·8%, 9·6%, 9·6%, 8·7%, 17·4%, and 5·4%, respectively. Thirty-seven

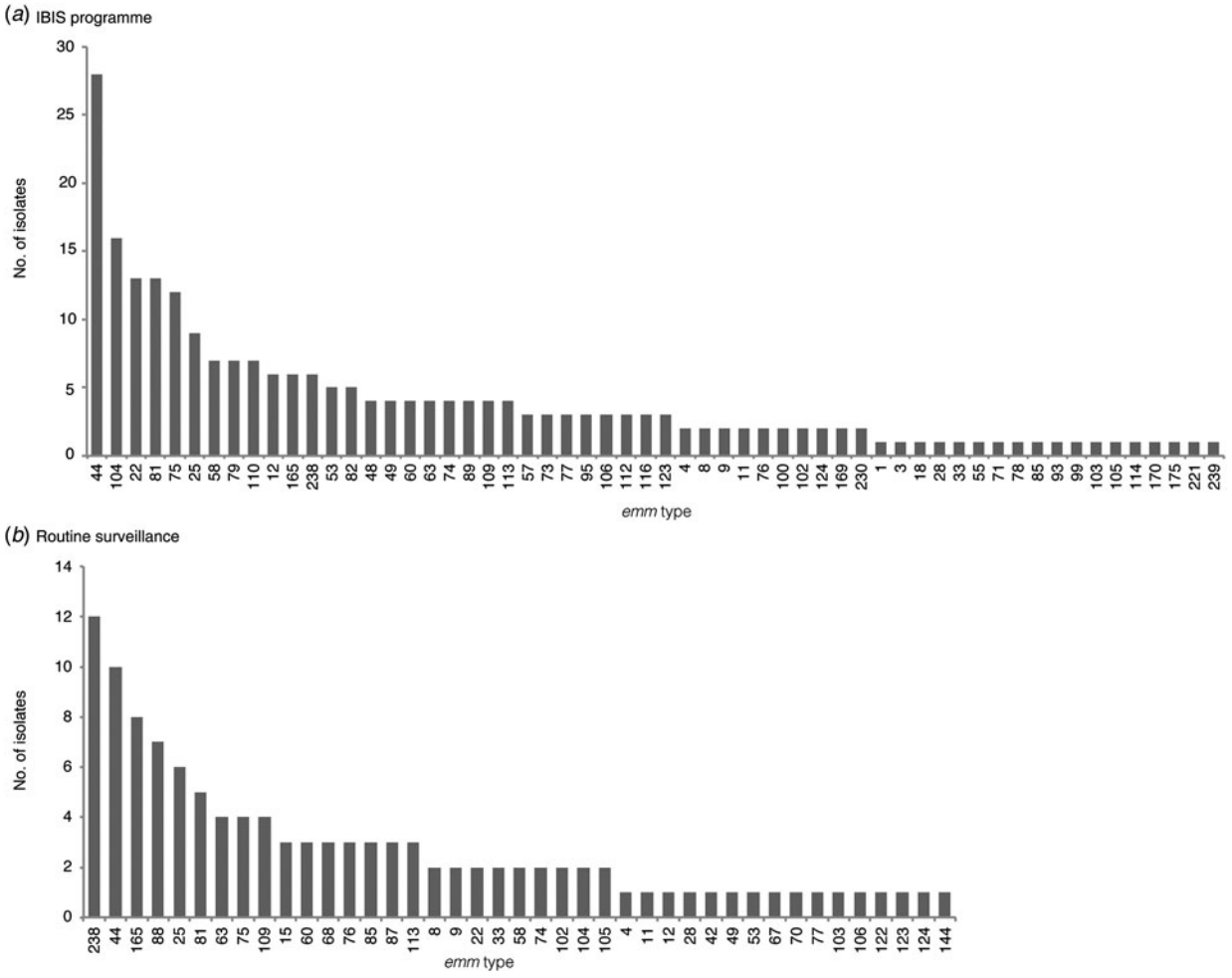

Fig. 1. Distribution of *emm* types in group A *Streptococcus* isolates in Thailand. Bars represent the number of isolates from (*a*) the Invasive Bacterial Infection Surveillance (IBIS) programme and (*b*) routine surveillance.

superantigen patterns were identified and the top seven profiles accounted for 74·8% of the total isolates and contained the major superantigen gene profiles found in the IBIS isolates. Isolates of *emm238* and *emm165* exhibited *emm*-specific profiles, whereas *emm44* isolates were variable in superantigen gene profiles (Table 1).

To our knowledge, this is the first nationwide survey to assess the distribution of *emm* genotypes and superantigen genes of clinical GAS isolates in Thailand. In total, 67 *emm* types were identified, the most prevalent type being *emm44*, which is rare in industrialized countries. Interestingly, *emm1* and *emm12* isolates, which are the most common in Western countries, are rare in Thailand. These findings suggest that GAS infections in Thailand are caused by diverse types of *S. pyogenes* and that the *emm* genotype distribution is distinctly different from those found in North America, Europe, North Africa, and East Asia [3].

In both surveillance systems the percentage of GAS isolates over all the bacterial isolates was 1·1% (115/10 896) in routine surveillance, and 8·3% (234/2826) in IBIS. GAS were isolated from various body sites (invasive and non-invasive) and over different time periods (IBIS 2010–2011; routine 1996–2011) and therefore, direct comparisons of type distributions between isolates from the two surveillance systems were not possible. However, *emm44*-type GAS were preferentially isolated in both systems, indicating that this type has been dominant in Thailand for decades. Interestingly, in the routine surveillance isolates, only a single representative from blood of 40 isolates (2·4%) was *emm44*. On the other hand, 7/50 non-sterile pus isolates (14·0%) and 2/14 throat swab isolates (14·3%) were *emm44*, respectively. Thus,

Table 1. *Characterization of superantigen gene patterns and emm types of group A* Streptococcus *isolates in Thailand*

	Superantigen profile											IBIS (2010–2011)				Routine surveillance (1996–2011)			
Profile	*speA*	*speC*	*speG*	*speH*	*speI*	*speJ*	*speK*	*speL*	*speM*	*smeZ*	*ssa*	No. of isolates	*emm44*	*emm104*	*emm22*	No. of isolates	*emm238*	*emm44*	*emm165*
A	–	–	+	–	–	–	–	–	–	+	–	35	0	0	13	17	0	0	0
B	–	–	–	–	–	–	–	–	–	+	–	16	0	0	0	13	0	0	8
C	–	–	–	–	–	–	–	+	+	+	–	13	0	13	0	1	0	0	0
D	–	–	+	–	–	+	–	–	–	+	–	11	0	0	0	15	8	0	0
E	–	–	+	+	–	+	–	–	–	+	–	10	0	0	0	6	0	1	0
F	–	+	+	–	–	–	–	–	–	+	–	10	0	0	0	6	0	0	0
G	–	–	+	–	–	–	+	–	+	+	–	8	0	0	0	1	0	0	0
H	–	+	+	+	+	–	–	–	–	+	–	8	6	0	0	1	0	0	0
I	–	–	+	–	–	–	–	+	+	+	–	6	0	1	0	0	0	0	0
J	+	–	+	–	–	–	–	–	–	+	–	6	0	0	0	5	0	0	0
K	–	+	+	+	+	–	–	–	–	–	–	5	4	0	0	1	0	0	0
L	–	+	+	+	+	–	–	–	–	+	+	5	0	0	0	0	0	0	0
M	–	+	+	+	+	+	–	–	–	+	–	5	4	0	0	1	0	0	0
N	+	+	+	–	–	–	–	–	–	+	–	5	0	0	0	5	0	0	0
Other												91	14	2	0	43	4	9	0

IBIS, Invasive Bacterial Infection Surveillance.

emm44 GAS might not have been rare in non-invasive GAS in Thailand and corroborates its relative high frequency reported from non-invasive skin infections in Asia [3]. Consequently, our finding that 12% of IBIS isolates in 2010–2011 were of *emm44* GAS is noteworthy.

As noted above the isolation of *emm44* GAS has been documented in Asia [3, 5], and spread of an *emm44* tetracycline-resistant GAS clone has been reported in France [6]. However, these have rarely been associated with invasive infections [3, 7]. It is widely held that invasive infection is mostly associated with epidemic M1T1 clones that have acquired novel virulence genes coded by prophages or other mobile genetic elements in industrialized countries [8]. By contrast, in Thailand the M1T1 clone is rare, irrespective of surveillance method, and *emm44* GAS is the type most associated with invasive GAS infection. Since the distribution of epidemic pathogens often show a particular geographical pattern, we examined the relationship between specific *emm* type and the region from which they were obtained by IBIS. The geographical distribution of *emm44* GAS isolates was: central (7·7%); east (6·5%); north (12·9%); northeast, (18·3%); and south (8·0%). This suggests a higher prevalence of this genotype in the north/northeast regions of Thailand.

We also identified a number (10·4%) of GAS of *emm238* (previously termed ST11014) in routine surveillance isolates; this type is noted to be rare in several countries [9, 10]. By contrast, *emm1* and *emm12*, which are associated with invasive infection in Western countries [3], were observed far less frequently (0·4% and 2·6%, respectively) in this study. These findings indicate that the *emm* types of GAS cause widespread infections in a population-specific manner, as previously suggested [11].

Superantigens are associated with disease conditions such as scarlet fever and streptococcal toxic shock syndrome [12]. Of 11 superantigen genes known in GAS, *speA, speC, speH, speI, speK, speL, speM*, and *ssa* are located in the prophage region of the genome [12], while the rest are confined to chromosomal regions. This indicates that these genes are mainly acquired via horizontal gene transfer and their variation results from the acquisition or loss of prophages. In this study, we identified 75 unique superantigen gene profiles, suggesting the presence of diverse subclones of the GAS isolates in addition to the observed variation in *emm* type. All isolates possessed at least one of the three chromosomally encoded superantigen genes, *speG* (83·2%), *speJ* (28·6%), and *smeZ* (91·2%). Although these frequencies are in agreement with those reported for GAS isolates in other countries [12], the detection rates of the phage-encoded genes differed substantially from other studies; these genes were identified in <4% of isolates in Norway and Australia [4, 12], whereas about 30% of the isolates from Thailand possessed them.

Most of the isolates of a given *emm* type were associated with a single gene profile, with some variation that was likely due to the loss or acquisition of superantigen genes [4, 13]. Most isolates in this study showed *emm*-specific gene profiles, with a few exceptions. However, the *emm44* GAS were not characterized by dominant *emm* profiles using either isolation method, and included 15 gene patterns. This suggests that *emm44* GAS in Thailand might be able to acquire various phages, including the virulent phage related to invasive infection and their sensitivity to phage infection might differ from other *emm*-type GAS isolates. Clustered Regularly Interspaced Palindromic Repeat (CRIPR)/Cas system is known to be an anti-phage system in bacteria, which functions as adaptive immunity against foreign genetic elements including bacteriophages. We have previously reported that the CRIPR/Cas locus in *S. pyogenes* regulates prophage inhibition [14], and so it is therefore possible that these loci in *emm44* GAS are attenuated or diminished, resulting in a variety of prophage integrations.

In conclusion, GAS *emm* types in Thailand exhibited a distribution pattern that differs from the patterns observed in other countries, and *emm44* was prevalent. Although *emm44* tended to be isolated from non-sterile specimens in routine surveillance and is known to be a non-invasive type in other Asian regions, a number of invasive *emm44* GAS were identified in 2010–2011 IBIS isolates in Thailand. Furthermore, this genotype exhibited highly variable superantigen gene profiles. These findings not only add value to diagnosis and treatment for GAS infection in Thailand but also reveal the benefit and importance of an active surveillance system for this pathogen.

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DECLARATION OF INTEREST

None.

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