

# Research/Recherche

## A comparison of group A streptococcal serotypes isolated from the upper respiratory tract in the USA and Thailand: implications

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*Characterization of group A beta-haemolytic streptococci in upper respiratory tract isolates from the USA and Thailand revealed that whereas 80% of the U.S. isolates could be M or opacity factor (OF) typed, <20% of the Thai isolates could be characterized with the available typing sera (P <0.001). There was also a statistically significant difference observed in the percentage of strains that could be characterized by the T-agglutination pattern (93% in the USA vs 61% in Thailand, P <0.001). Even among the identifiable strains, marked differences in the distribution of the recovered serotypes were noted between the two countries. These results show that there are a significant number of as yet unidentified group A streptococcal strains in parts of the world where streptococcal infections and their sequelae are important public health problems. They further imply that such findings must be taken into consideration in the future when designing possible streptococcal vaccines for worldwide use.*

### Introduction

Published reports from countries in North America, Europe and other parts of the world since the mid-1980s have reemphasized the virulence of group A beta-haemolytic streptococci and their importance as a human pathogen (1-7). A temporal association between the prevalence of specific group A serotypes in the population and these severe infections, with suppurative and non-suppurative sequelae, has been suggested (8). While the association of specific serotypes of group A streptococci with clinical syndromes (e.g., acute nephritis and, perhaps to a lesser extent, rheumatic fever) has been noted in the past, the desirability and necessity for extending

such observations have become important for two reasons. The first is the need to define the virulence factors of group A strains which have a pathogenetic role in these infections and their sequelae; the second is to identify those serotypes which might be candidates for inclusion in a group A streptococcal vaccine for use in public health programmes globally for prevention of these infections.

Essential to accomplishing these two objectives is the careful epidemiological assessment of group A streptococcal infections, and the identification of the responsible serotypes (9). Most of the available data from published reports have originated from countries with established reference laboratories, which have the experience and the necessary typing sera to completely characterize group A streptococcal isolates. Many of these reports, particularly the recent experience with severe group A streptococcal infections, originated in the western hemisphere (1-4, 10, 11).

The question now is whether the group A serotypes isolated from patients in the western hemisphere accurately reflect all the important serotypes frequently associated with severe disease, and whether, therefore, these western hemisphere isolates are sufficiently representative to address the

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objectives posed above. This question is important because the incidence of rheumatic fever and the incidence of severe systemic infections have been decreasing in most industrialized countries during the past three or four decades, while in many developing countries the group A streptococcal infections and their sequelae are still common and of significant public health concern (12).

We are unaware of recent studies comparing streptococcal serotyping data from the western hemisphere with those from another part of the world. Whatever regional comparisons that could be made were based on separate studies in different laboratories, usually with different batteries of available typing sera and sometimes utilizing different laboratory techniques. We have therefore compared the percentage typability and serotype distributions of recent group A streptococcal upper respiratory tract isolates from the USA and from Thailand. Our findings are reported below.

## Materials and methods

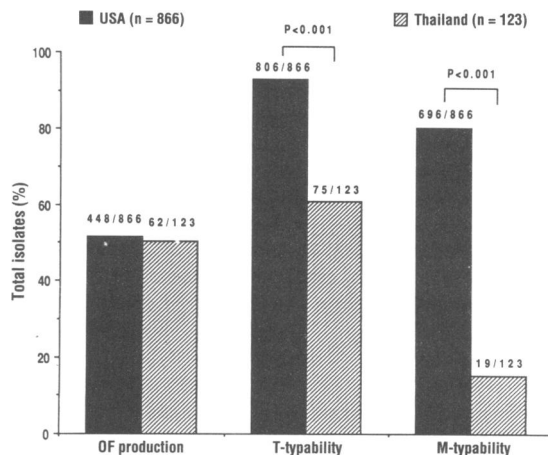
The serotype distributions of 866 group A streptococcal upper respiratory tract isolates, collected during 1988–90 from 31 states in the USA, and 123 isolates from the throats of children in Bangkok, Thailand, were compared. The origin of the 866 strains in the USA has been described (8); the Thai strains were collected in 1989 as part of a three-year study in schoolchildren in Bangkok.

All 989 isolates were characterized by bacitracin sensitivity, by serological grouping, by T-agglutination pattern, and by M type and/or opacity factor (OF) type using previously described methods (13–15). The following M-typing antisera were used: types 1–6, 8, 12, 14, 15, 17–19, 23–26, 29–33, 36–41, 43, 47, 49, 51–53, and 55–57. Detection of streptococcal serum opacity factor (OF) production and opacity factor serotyping of group A streptococci were also performed (11). OF antisera used included types 2, 4, 9, 11, 22, 25, 28, 48, 49, 58–64, 66, 68, 73, 75–78, and 81. Thus, a total of 57 M/OF-typing sera were available. T-typing sera from all recognized T antigens were also available. The indicated M serotype refers to either M protein and/or serum opacity factor. Typing of isolates from the two countries was carried out by staff in the same laboratory.

## Results

The results of this comparison are shown in Fig. 1. There was no difference in the percentages of strains from the two countries with regard to their ability to produce opacity factor (52% of the U.S. strains com-

Fig. 1. Comparison of selected characteristics (types) of upper respiratory tract group A streptococcal isolates from the USA and Thailand. Many more of the isolates from Thailand could not be identified by available typing sera, although the percentages of opacity factor (OF) producers were similar in both groups.



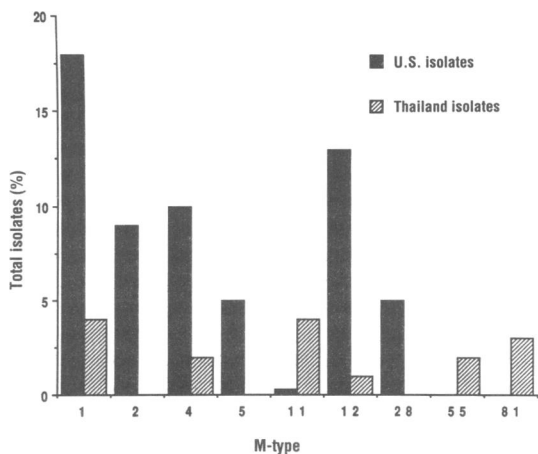
pared with 50% of the Thai isolates). However, there was a marked difference in typability, both by determining the T-agglutination pattern and by M/OF typing.

While 93% of the U.S. isolates could be typed by T-agglutination pattern, only 61% of the Thai isolates could be identified by this method ( $P < 0.001$ ). The difference in typability was even more striking when the isolates from the two groups were characterized by M protein and/or OF typing: 80% of the 866 U.S. isolates, compared with only 15.4% of the 123 Thai isolates ( $P < 0.001$ ).

The 123 Thai strains were isolated in 1989. Although the 866 U.S. strains were isolated in 1988–90, the percentage typable by either M/OF or T typing was not different if one compares the spectrum of all 866 U.S. strains or only those 462 strains isolated in 1989.

Fig. 2 compares the distribution of the six most common M types of the U.S. pharyngeal isolates (80% M typable) with the M type distribution of the Thai strains (15% M typable). Although admittedly the numbers are relatively small, of those isolates that could be identified by either M typing or OF-inhibition typing, the distribution of typable strains was very different for the two geographical areas. Nineteen of the Thai strains were M/OF typable with the 57 available M and/or OF-typing sera. These 19 strains included six serotypes (M types 1 (4% of total isolates), 4 (2%), 11 (4%), 12 (1%), 55 (2%),

Fig. 2. Comparison of the distribution of M serotypes of upper respiratory tract group A streptococcal isolates from the USA and Thailand.



and 81 (3%). Of the 696 (80%) strains from the USA that were M/O/F typable, the relative proportions of the same six serotypes were very different. M types 1, 4 and 12 were found in both the U.S. and Thailand, but constituted a much larger percentage of the total U.S. isolates (18%, 10%, and 13% respectively). M types 11, 55 and 81 either were only found among the isolates from Thailand or were very uncommon among the U.S. isolates. Of interest is the observation that M type 5, a serotype very frequently associated with rheumatic fever in the USA and other western hemisphere countries, was relatively common among the U.S. isolates, but not among the upper respiratory tract isolates from Thailand.

It was also noted that 48% (413/866) of the T-typable strains from the USA were T-1, T-12, or T-3. In contrast, these three T-agglutination patterns accounted for less than 10% of the corresponding Thai isolates (data not shown).

## Discussion

Although caution must be exercised in drawing conclusions based on the relatively limited number of isolates examined, these results clearly indicate that there are significant differences in the prevalent serotypes of group A streptococci isolated from the two countries. Of primary importance is the fact that, whereas we were able to characterize 93% of U.S. pharyngeal isolates by T-agglutination pattern and 80% by M and/or OF typing, using the identical

panels of typing sera we could identify only about 60% of the Thai strains by T-agglutination pattern and only approximately 15% by M/O/F type. While such a difference in typability of strains from other countries has been briefly alluded to by Colman (10), to our knowledge the extent of this difference has not previously been documented.

There are several explanations for these contrasting findings. Sampling error must be considered as one distinct possibility. The sample size is limited, as it must be. It is practically impossible to sample a statistically significant percentage of all group A streptococci in either country. It is also possible that a large percentage of the untypable Thai strains actually lack M protein, thus making its detection impossible. The strains reported here have not been tested for M-protein content by measuring their ability to grow in normal human blood. However, it would be unusual, in our experience, for such a very large percentage of strains collected from patients in a normal population to lack M protein. Variation in percentage typability is not uncommonly reported among streptococcal reference laboratories. However, the fact that 93% of the U.S. strains could be typed by T-agglutination pattern and 80% by M/O/F typing, while a statistically significantly much smaller percentage of the Thai strains were typable in the same experienced laboratory, strongly supports the most likely and credible explanation—i.e., that many of the strains in that Asian country are currently of different serotype from those in the USA. These non-typable strains may well represent as yet unrecognized or undefined serotypes of group A streptococci.

The importance of these data is emphasized by the recent advances in microbiological laboratory technology which facilitate higher resolution analyses of the structure of group A streptococcal antigens (16, 17). Such molecular biological techniques not only have the possibility of enhancing the understanding of somatic and extracellular virulence factors of these organisms (18), but also have allowed significant progress in the molecular analyses of the M protein in preparation for the design of a safe and effective group A streptococcal vaccine for use in public health control programmes in different countries or regions of the world (19–21).

However, since group A streptococcal infections and their sequelae are still very prevalent and present problems in many countries of the world (12), until the spectrum of globally prevalent serotypes is more completely defined and the responsible organisms are characterized, current efforts to understand the pathogenesis of the disease process will be severely hampered. Even if the presence of a widely shared “conserved” epitope(s) on the M-protein molecule proves to be a major factor in successful

vaccine design (19), the production of a clinically useful and effective vaccine for public health programmes can never be initiated without knowledge of and subsequent testing of prevalent specific serotypes with confirmation of their molecular structure.

Finally, our results emphasize the need for continuous epidemiological surveillance of group A streptococci (9). More importantly, if the antigenic composition of a future group A streptococcal vaccine is based only on serotypes in isolates from a limited geographical region, there is a definite risk of omitting other important serotypes that are required to protect patients in other areas of the world where these infections and their sequelae are very common.

### Acknowledgements

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### Résumé

#### Comparaison entre les sérotypes de streptocoques du groupe A isolés dans les voies respiratoires supérieures aux Etats-Unis d'Amérique et en Thaïlande: conséquences

D'après des observations publiées en Amérique du Nord, en Europe et dans d'autres régions du monde depuis le milieu des années 80, la virulence des streptocoques bêta-hémolytiques du groupe A est à nouveau soulignée, de même que leur importance en tant qu'agents pathogènes chez l'homme. Une association dans le temps entre la prévalence de certains sérotypes du groupe A dans la population et les infections streptococciques graves, avec séquelles suppuratives et non suppuratives, a été évoquée. On avait déjà noté une association entre certains sérotypes de streptocoques du groupe A et des syndromes cliniques (par exemple néphrite aiguë, rhumatisme articulaire aigu), mais il importe maintenant d'étendre et d'actualiser ces observations, pour deux raisons: tout d'abord, pour aider à définir les facteurs de virulence et le rôle pathogène des souches de groupe A dans ces infections et leurs séquelles, et ensuite pour identifier les principaux sérotypes prévalents afin de les inclure dans un vaccin antistreptococcique groupe A qui serait utilisé dans des programmes de santé publique nationaux, régionaux ou même mondiaux.

La caractérisation d'environ un millier de streptocoques bêta-hémolytiques du groupe A dans des prélèvements de voies respiratoires supérieures aux Etats-Unis d'Amérique et en Thaïlande montre qu'alors que 80% des souches isolées aux Etats-Unis peuvent être typées selon la protéine M ou le facteur d'opacité, moins de 20% des isolements obtenus en Thaïlande peuvent être caractérisés au moyen des sérums anti-type A disponibles ( $P < 0,001$ ). On a également observé une différence statistiquement significative du pourcentage de souches pouvant être caractérisées selon le mode d'agglutination des antigènes T (93% aux Etats-Unis contre 61% en Thaïlande,  $P < 0,001$ ). Même parmi les souches identifiables, des différences sensibles dans la distribution des sérotypes retrouvés ont été notées entre les deux pays. Par exemple, les types M 1, 4 et 12 figuraient parmi les isolements des deux pays. En revanche, les types M 11, 55 et 81 étaient très rares voire absents parmi les souches américaines, alors qu'ils étaient parmi les plus courants en Thaïlande.

Comme les infections à streptocoques du groupe A et leurs séquelles ont encore une très forte prévalence dans de nombreux pays, où elles posent de graves problèmes, tant que l'on n'aura pas plus complètement défini le spectre des sérotypes répandus dans le monde entier et caractérisé les germes responsables, les efforts actuels en vue d'élucider la pathogénie de la maladie seront sérieusement compromis. Même si la présence d'un ou plusieurs épitopes "conservés" largement partagés sur la molécule de la protéine M s'avère en fin de compte un facteur primordial pour l'élaboration d'un vaccin, il ne sera pas possible d'entreprendre la production d'un vaccin efficace à l'intention des programmes de santé publique sans connaître quels sont les sérotypes du groupe A les plus couramment prévalents dans les régions géographiques concernées. D'après nos résultats, il semblerait que l'on risque d'omettre d'importants sérotypes, nécessaires pour protéger les populations, dans certaines régions où les infections qu'ils provoquent et leurs séquelles sont très courantes. Enfin, ces données soulignent à nouveau la nécessité pratique d'une surveillance épidémiologique continue des streptocoques du groupe A.

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