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Molecular typing of Chinese *Streptococcus pyogenes* isolates

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ABSTRACT

Streptococcus pyogenes causes human infections ranging from mild pharyngitis and impetigo to serious diseases including necrotizing fasciitis and streptococcal toxic shock syndrome. The objective of this study was to compare molecular *emm* typing and pulsed field gel electrophoresis (PFGE) with multiple-locus variable-number tandem-repeat analysis (MLVA) for genotyping of Chinese *S. pyogenes* isolates. Molecular *emm* typing and PFGE were performed using standard protocols. Seven variable number tandem repeat (VNTR) loci reported in a previous study were used to genotype 169 *S. pyogenes* geographically-diverse isolates from China isolated from a variety of disease syndromes. Multiple-locus variable-number tandem-repeat analysis provided greater discrimination between isolates when compared to *emm* typing and PFGE. Removal of a single VNTR locus (*Spy2*) reduced the sensitivity by only 0.7%, which suggests that *Spy2* was not informative for the isolates screened. The results presented support the use of MLVA as a powerful epidemiological tool for genotyping *S. pyogenes* clinical isolates.

1. Introduction

Streptococcus pyogenes, (group A *Streptococcus*; GAS), is an important human pathogen that causes a wide range of skin, mucosal surface and invasive infections, including pharyngitis, impetigo, scarlet fever and necrotizing fasciitis [1]. Common approaches toward molecular typing of GAS includes *emm*-gene typing [2–5], pulsed field gel electrophoresis (PFGE) typing [6–8], and multi-locus sequence typing (MLST) [9–11]. Each of these methods has shown various advantages and disadvantages in the study of the molecular epidemiology and evolution of GAS. Recent studies have demonstrated that multiple-locus variable-number tandem-repeat analysis (MLVA) is an additional powerful tool used to identify and genotype *S. pyogenes* strains. MLVA uses the naturally occurring variation in the number of tandem repeated nucleotide sequences found in the genome of *S. pyogenes* strains [12]. In China, *emm* typing and PFGE have been widely used to investigate molecular characteristics of GAS isolates from different geographical regions, diseases and outbreaks [13–20]; however, no reports describe the use or utility of MLVA for GAS genotyping. To determine the utility of this typing method for GAS isolates from China, the MLVA-7 scheme [12] was used to type a collection of 169 GAS isolates from 7 different regions throughout China.

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2. Materials and methods

2.1. GAS isolates and DNA preparation

One hundred and sixty-nine GAS clinical isolates were obtained from Beijing (n = 40), Shandong (n = 54), Guizhou (n = 55), Shenzhen (n = 9), Guangzhou (n = 2), Shanghai (n = 7), and Chongqing (n = 2) over the period 1993 to 2011. The strains were collected from patients presenting with a range of streptococcal pathologies, including acute glomerulonephritis (AGN), scarlet fever, tonsillopharyngitis, and non-symptomatic carriers from hospitals and pathology labs networked with the Chinese Centre for Disease Control and Prevention. Two ATCC strains, MGAS2096 and MGAS9429, were included in the strain set. All isolates showed beta-hemolysis on Trypticase soy agar containing 5% sheep blood, and were identified negative in the catalase test, susceptible to 0.04 U of bacitracin, and Lancefield grouped as GAS using the streptococcal grouping kit (Oxoid). Strains were stored at −70 °C in brain heart infusion broth containing 15% glycerol before laboratory use. Bacteria were suspended in 100 μl of TE buffer and treated with lysozyme (10 mg/ml) (Sigma) and mutanolysin (500 U/ml) (Sigma). DNA was extracted using a Qiagen Mini kit according to the manufacturer's protocol.

2.2. Molecular emm gene typing

All isolates identified as GAS were tested for *emm* gene type. The sequence of the first 240 bases of *emm* gene was submitted to the National Centers for Disease Control Biotechnology Core Facility Computing Laboratory. The *emm* type and subtype were determined using parameters described by the United States Centers for Disease Control and Prevention *emm* sequence database (http://www.cdc.gov/ncidod/biotech/strep/M-ProteinGene_typing.htm).

2.3. Multilocus variable number tandem repeat analysis

MLVA typing using the MLVA-7 scheme was performed as described previously [12], except that the primers were fluorescently labeled and PCR products were sized on an automated DNA sequencer to accurately determine the number of tandem repeats. The number of repeats in each of the seven VNTR loci was calculated by subtracting the size of the flanking regions from the size of the total PCR product.

2.4. MLVA data analysis

All MLVA data were analyzed using BioNumerics version 5.1 software (Applied Maths, Belgium). Clustering analysis was based on the categorical coefficient and unweighted pair group method using arithmetic averages (UPGMA) method.

2.5. PFGE analysis

To compare MLVA and PFGE, 33 strains isolated from a scarlet fever outbreak in Qingdao were selected from the 169 strains and were characterized by PFGE. All 33 strains were initially identified as *emm12* type. Genomic DNA was digested using SmaI (New England Biolabs) and analyzed by using a CHEFDR II system (Bio-Rad) for 19 h (initial forward time, 4 s; final forward time, 40 s). Gels were stained with GelRed and analyzed using Bionumerics software (Applied Maths, Kortrijk, Belgium). The unweighted pair group method with arithmetic averages and the DICE coefficient (1.0% optimization, 1.0% position tolerance) were used to construct a dendrogram.

3. Results

Of the 169 strains tested, 12 *emm* types and 74 MLVA types were identified (Fig. 1). The most common *emm* types were *emm1*, *emm12*, *emm60*, *emm63* and *emm95*. MLVA0023 was the most prevalent MLVA type, which was consistent with the high proportion of *emm12* GAS. Of the 33 scarlet fever outbreak strains, six *emm* subtypes, namely, *emm12.0, emm12.1, emm12.4, emm12.7, emm12.19*, and *emm12.32*, were identified.

To further assess the discriminatory power of MLVA, the 33 *emm12* scarlet fever isolates were analyzed by PFGE and compared with the MLVA results. Seven PFGE patterns were associated with eleven different MLVA types (Fig. 2). The predominant PFGE group SPYS16C00011 was shown to encompass five different MLVA types (MLVA0005, MLVA0023, MLVA0025, MLVA0030, MLVA0031), which demonstrates that MLVA was able to identify additional diversity within PFGE types. This is also consistent with the results reported by Obszańska et al. [12].

In this study, not all loci of the MLVA-7 scheme were equally effective; 112 isolates were PCR-negative at the *Spy2* locus. Removal of the *Spy2* locus in the analysis only resulted in a slight decrease in the discrimination of strains (0.944 compared to 0.951 when *Spy2* was included), which suggests that *Spy2* did not significantly contribute to the discriminatory power of GAS MLVA typing of isolates from China. Among the tested Chinese GAS isolates, the most diverse *emm* types were as follows: *emm1* (9 profiles among 17 strains), *emm60* (18 profiles among 29 strains), and *emm63* (7 profiles among 9 strains).

The two reference strains MGAS2096 and MGAS9429 were clustered into two different clades. MGAS2096 was isolated in Trinidad from a patient with acute glomerulonephritis in 1960. MLVA typing shows MGAS2096 is genetically identical to CH-7, a strain isolated in Beijing from a patient with impetigo in 1993. These strains belong to a single clade, MLVA0024. MGAS9429 was isolated in Texas from a patient with pharyngitis in 2001. MLVA typing shows it is genetically similar to several strains isolated from patients of scarlet fever outbreak in China in 2011. These findings suggest that MGAS2096 and CH-7 may have quite different genetic features in the *emm*12 GAS population, while MGAS9429 may be genetically closer to strains involved in the recently reported scarlet fever outbreak in China.

4. Discussion

In this study, MLVA was used to analyze strain diversity among 169 *S. pyogenes* isolates from China. A total of 74 MLVA genotypes were identified among the 12 initially defined *emm* genotypes, which therefore supports the use of MLVA over conventional *emm* typing as a discriminatory technique. Although there was a high degree of congruence between MLVA and *emm* types, minor differences were also observed. For example, in the MLVA clustered *emm12* strains, one isolate, *emm86*, was unexpectedly detected, and in the *emm1* clade clustered using MLVA, a single *emm6* isolate was included. It is possible that these strains contain interesting genome characteristics that warrant further analysis.

GAS *emm12* strains are usually considered clonal [21,22]; however, multiple MLVA patterns were detected in this study. In the MLVA profiling results of the *emm12* GAS strains, we found two independent clusters (designated as HSC1 and HSC2) that include a high proportion of *emm12* strains isolated before 2011, when a scarlet fever outbreak occurred in China. Most of these strains were isolated from patients with pharyngitis. Clustered in the branch with the predominant clone involved in the scarlet fever outbreak, were three historic strains CH-38, CH-26 and CH-41 (MLVA0031, MLVA0023 and MLVA0026 respectively). These findings are