

Molecular characterization of *Streptococcus pyogenes* isolated from children with pharyngitis

Abstract

Streptococcus pyogenes is a significant issue all around the world. The *S. pyogenes* infection “pharyngitis” is not much more studied previously. To examine the prevalence of streptococcal pharyngitis in children gives a better idea about its occurrence and pattern of development. We also determine the role of sAg and M protein in the spread of infection and surveillance in the human body in the presence of strong immune system. This study help to find the measure for the control of streptococcal pharyngitis like the production of vaccine using M protein should be a better option for safe and efficient eradication of the disease from society.

Keywords: streptococcus pharyngitis, sAg, m protein, immune system, vaccine

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Introduction

Streptococcus pyogenes is important gram-positive pathogens that cause various diseases in human ranging from mild to lethal.¹ Streptococcal pharyngitis is most significant disease, and its peak incidence occurs in children, these infections impose the economic burden on the society.² The symptoms of streptococcal pharyngitis include a sore throat, pain in swallowing, fever, swollen, and tender lymph nodes in the neck and fatigue.³ *S. pyogenes* oftenly results from direct contact with another person with streptococcal pharyngitis.⁴ Asymptomatic carriers can play a role in transmission, particularly during outbreaks. *S. pyogenes* contact large number of virulence factor that's contributing to its pathogenicity, important virulence factor,⁵ encoded by emm gene are super antigen protein, and M protein is an important virulence factor of *S. pyogenes*.⁶ superantigens are a potent immune stimulatory activity that contributes to the pathogenesis of *Streptococcus pyogenes*. The gene distribution of sAgs has used as an epidemiologic tool to explore genomic heterogeneity and the possible correlation between super antigen gene content and clinical manifestation.⁷

sAg gene distribution and emm typing analysis used in comparing different isolates of *S. pyogenes*,⁸ along with these techniques pulsed-field gel electrophoresis (PFGE) analysis are also used widely used in comparing different isolates of same bacterial species.⁹ Highly variability occurs in emm gene among *S. pyogenes* strain which is important surveillance tools. For example in Taiwan, emm1, emm4, and emm12 strains were the leading causes of noninvasive disease; few emm 1 strain had speC and speH: few emm12 had speJ and smcZ,⁹ in Spain, emm 1 *S. pyogenes* associated with pharyngitis possessed speA, speG and speJ, but not speC, speH, speL, and ssa.¹⁰ The M protein is virulence factors associated with colonization and resistance to phagocytosis.¹¹ This protein is possibly one of the best-studied molecules among the known streptococcal virulence determinants.

The M protein has usually been targeted for serotyping of GAS strains because of its significance as a virulence determinant,¹² Though; sequencing of the emm gene is now becoming the standard

method. Now, more than 170 emm types and 750 sub types of *S. pyogenes* are identified and distribution of emm types significantly varies among the different countries and regions.¹³ *S. pyogenes* is a significant gram-positive bacterial pathogen that causes various human diseases. Streptococcal pharyngitis is the most common disease it causes, and its peak incidence occurs in school aged children. These infections impose significant loss of health and wealth on society.² *S. pyogenes* harbors a large quantity of virulence factor that contributes to its complex pathogenicity.¹¹ Recently initial characterized of *S. pyogenes* isolated from Chinese children with pharyngitis through the minimal inhibitory concentration test Identification of constitutive or inducible phenotype (Figure 1). The presence of macrolide-resistant genes (*mefAermB* and *ermA*) in the present study, further analyzed these strains by emm genotyping, PFGE, and analyze of sAg gene profiles.¹¹ Among the *S. pyogenes* strains causing pharyngitis in Chinese children, 13 different emm types were identified: emm 1, and emm 12 types were more prevalent and were found to be specific for 84.9% of all the strains. More ever, 2 predominant PFGE clusters that is cluster A (53 strains,28.6%) and group 1 (81 strains,43.8%) belonged to emm1 and emm 1 and emm 12, respectively. Thus, the result from our collection seem to point out that emm 1and emm 12 strains are major causative agents of scarlet fever and pharyngitis in china during 2007.¹⁴

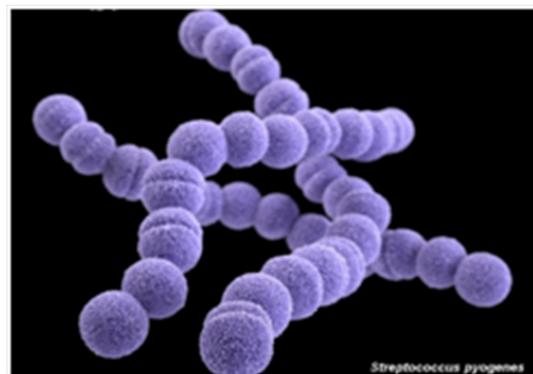


Figure 1 *Streptococcus Pyogenes* (3d model).

Despite the presence of numerous SAg gene profiles within nemm type, each emm type was characterized by 1 or 2 predominant SAg gene profile. For example, SAg profile 3 and 4 are predominant among emm 12 strains, while SAg profiles 1 and 2 are predominant among emm 1 types.¹⁵ The distribution of *S. pyogenes* mm types and SAg genes profiles varied in different geographic areas. For instance, in Taiwan emm 1, emm 4, and emm 12 strains were the leading cause of noninvasive disease, few emm 1 strains had spec ad spec H, few emm 12 strains ha spe J and smeZ (Figure 2).¹⁶

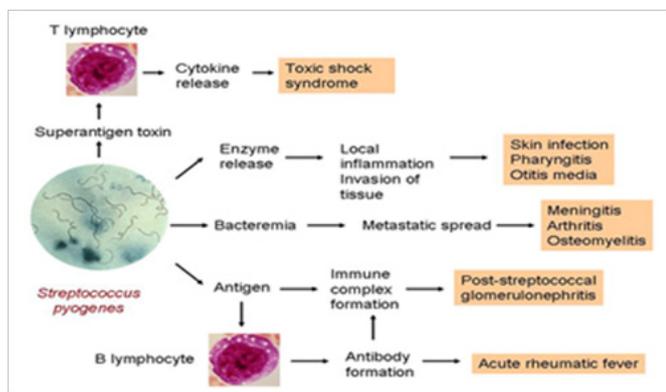


Figure 2 Virulence cycle of *streptococcus pyogenes*.

Bacterial identification

Throat swab is taken from all outpatient children and inoculated into sheep's blood agar plate. After 24hr. of inoculation at 37°C, the preliminary identification of the isolate as *S. pyogenes* based on beta hemolysis on sheep's blood agar, colony morphological characteristics, and the presence of group an antigen. Which is confirmed using the Diagnostic streptococcal grouping Kit.¹⁶

Emm genotyping

The *S. pyogenes* chromosomal DNA isolated using a DNA extracting kit.¹⁷ For identification of types and subtype specific region of extracting DNA is amplified using specific primer mix (Forward and Reverse) and sequenced using emm typing protocol and compared with available reference emm sequences.¹⁸

PFGE analysis

Pulse field gel electrophoresis gives better resolution compare the conventional method, so run the DNA on PGFE to obtain the better result using large DNA fragment (about more than 600kb).¹⁹ After isolation of DNA and amplification of specific DNA fragment, it is run on PFGE to obtain specific information about the strain of *s. pyogenes*.

Detection of SAg genes

PCR was used to determine the presence of 11 Sag genes in *s. pyogenes* strain. PCR is performed under the conditions, denaturation for 1min at 95°C, 29 cycles containing denaturation for 30 s at 95°C, annealing for 30s at primer dependent temperature, and extension for 1 min at 72°C for 5min.¹⁶

Conclusion

The study, help us to identify the strains of *streptococcus pyogenes* which cause severe infection in children's and the role of the microbes in the development of pharyngitis in children. Molecular analysis of *S. pyogenes* strains associated with children's pharyngitis by applying the 3 typing method. Isolation

of emmgene, genotyping and comparison with previously studied isolates. Exploring genomic heterogeneity and the possible correlation between super antigen gene content and clinical manifestation. To educate the community about the hazardous effects of *streptococcus pyogenes* to prevent infection as well as to reduce the economic burden on the society.

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None.

Conflict of interest

The author declares no conflict of interest.

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