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Original Article

Isolation of *Streptococcus pyogenes* from children with pharyngitis and *emm* type analysis

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Abstract

Background: The group A streptococcus (GAS) M protein, encoded by the *emm* gene, acts as a major virulence factor. *Emm*-typing is the GAS gold standard molecular typing and is based on the DNA sequence of the nucleotides of the *emm* gene. The aim of the present study was to isolate GAS from patients and to detect the *emm* types of the isolates using *emm* typing.
Methods: A total of 1000 throat samples were collected from patients with pharyngitis referred to Aboozar Children's Hospital in Ahvaz, Iran. We performed antimicrobial susceptibility testing on all isolates using the Kirby–Bauer disk diffusion method. Additionally, amplification of the *emm* gene was performed using polymerase chain reaction using the standard primers and described protocol.
Results: From all throat samples screened, 25 isolates (2.5%) were identified as GAS. Antibiotic susceptibility testing revealed that all the GAS isolates were susceptible to penicillin and erythromycin, but 44% showed resistance to vancomycin. Based on polymerase chain reaction for the *emm* gene, the obtained *emm* types were: *emm-3*, observed in 20 isolates (80%); *emm-1* observed in four isolates (16%); and *emm-75* observed in one isolate (4%).
Conclusion: The result of the present study showed that penicillin and erythromycin are still the most effective antibiotics against the organism. The *emm* typing revealed that *emm* type-3 was detected in most of the isolates from patients with purulent pharyngitis. On the basis of the findings of this study, we may conclude that *emm* typing provides new insights on the genetic diversity of the M proteins, and is of demonstrable value for molecular studies of GAS.

Keywords: antibiotic resistance; *emm* typing; *Streptococcus pyogenes*

1. Introduction

Group A Streptococcus (GAS) or *Streptococcus pyogenes* is a gram-positive cocci with humans as its specific host. It is capable of causing a large variety of infections ranging from simple benign infections like sore throats and impetigo to fatal diseases like streptococcal toxic shock syndrome and necrotizing fasciitis, acute rheumatic fever, and acute glomerulonephritis.[1,2] The mortality rate of severe GAS infections remains high, both in developed and developing countries.[3,4] Additionally, there are reports of outbreaks of invasive GAS infections in the community and in hospitals.[5,6]

Due to an elevated global prevalence of GAS disease, epidemiological surveillance is necessary to detect changes in disease distribution in various populations. Typing of a collection of GAS isolates is important as part of the epidemiological surveillance for the disease. There are several

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typing methods available for screening GAS isolates. Among them, typing based on the M protein, a cell-surface protein that is the major virulence and immunological determinant of GAS, has been the most widely used method.[7] The M protein which is encoded by the *emm* gene possesses a hypervariable region of the amino-terminal with 40–50 amino acid residues.[8] A GAS typing system based on sequencing of this N-terminal hypervariable region of the M protein (*emm*) gene is known as *emm* typing and is the "gold standard" method used to characterize GAS isolates.[9] This method has been used for identification of different *emm* types. The surface proteins are not only a suitable substrate for typing and studying the molecular epidemiology of GAS isolates, but also represents choice candidates for the development of an effective vaccine against GAS-related serious diseases due to their critical role in host–bacteria relationships.[10,11] Currently, more than 170 *emm* types and 750 *emm* subtypes of GAS are known.[12] The distribution of *emm* types reportedly varies among different countries and regions.[13]

Due to the lack of comprehensive information about different types of M protein among GAS isolates in Ahvaz city, the current study was proposed to isolate GAS from the patients' throat suffering from pharyngitis and typing their M proteins. This undertaking is the first molecular epidemiologic analysis of GAS strains associated with children's pharyngitis in south western Iran.

2. Methods

2.1. Sampling

Our study reviewed a total of 1000 throat samples obtained from children with pharyngitis ranging in age from 2 years to 14 years, who were referred to Aboozar Children's Hospital in Ahvaz, southwestern Iran, from November 2012 to June 2013. The preliminary proposal of the work was reviewed and approved by the hospital's Institutional Review and Ethics Board, and the necessary permission to collect the requisite samples and initiate the work was obtained.

Standard patient demographics and clinical data were recorded, including age, sex, and disease onset, and patient symptoms were recorded. All patients who presented with fever and sore throat were entered into the study, and those with prior antibiotic therapy or patients with other respiratory tract symptoms such as rhinorrhea or nasal congestion were excluded from the study by the available pediatrics infectious diseases specialist at the time of admission.

2.2. Phenotypic identification of GAS

A single throat swab was taken from each patient and immediately placed in a thioglycolate broth and transferred to the microbiology laboratory, where the broth was incubated at 37°C for 24 hours, with subsequent subculture on a sheep blood agar plate (HiMedia, Mombai, India) the next day. The identities of the colonies were confirmed based on morphological and growth characteristics, including gram staining, beta-hemolysis on blood agar medium, bacitracin susceptibility, pyrrolidonyl arylamidase test, and resistance to trimethoprim-sulfamethoxazole.[14]

2.3. Antimicrobial susceptibility testing

For confirmed GAS isolates, antimicrobial susceptibility testing was done using the standard disk-diffusion method on Müeller–Hinton agar with 5% sheep blood, incubated overnight at 37°C in air enriched with 5% CO_2 according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.[15] The commercial antibiotic discs (MAST Co., London, UK) were as follows: penicillin G, ampicillin, ceftriaxone, vancomycin, azithromycin, chloramphenicol, clindamycin, and erythromycin. The interpretation criteria of the susceptibility testing were in accordance to the CLSI recommendations.

2.4. Polymerase chain reaction amplification for the detection of the emm gene

For DNA extraction from the isolates, a commercial extraction and purification kit (Roche, Berlin, Germany) was used according to the manufacturer's instructions. The extracted DNA purity was measured with a photobiometer (Eppendorf, Hamburg, Germany) in 260/280 nm UV long waves. In order to amplify the *emm* gene, the set of primers of *emm1* (5′-TATTCGCTTAGAAAATTAA-3′) and *emm2* (5′-GCAAGTTCTTCAGCTTGTTT-3′) were used,[16] which amplifies a 914 bp fragment of the target gene. Polymerase chain reaction (PCR) amplification was performed in a final volume of 25 μL containing 1 × PCR buffer, 1.5mM $MgCl_2$, 200μM deoxynucleotide, 0.4mM of each primer, 1.5 U *Taq* polymerase, and 1 μL of template DNA. All the reagents were purchased from Qiagen, Hilden, Germany. Amplification was performed on a thermocycler nexus gradient (Eppendorf) and the cycling program consisted of initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 46°C for 45 seconds, extension at 72°C for 59 seconds, and a final extension at 72°C for 7 minutes. A control positive for *S. pyogenes* ATCC 8668 and a control negative for *S. pyogenes* ATCC 8668 were included in each PCR run. The products were run on 1.5% agarose gel (w/vol.) containing 0.5 μg/mL ethidium bromide (Qiagen). Results were recorded using the gel documentation system (Protein Simple, San Jose, CA, USA). A 100-bp DNA ladder was used as a size marker (Roche). The PCR products were sent for sequence analysis (Bioneer Co., Daejeon, South Korea). The *emm* sequences were blasted against the *emm* database at the BLAST program, National Center for Biotechnology (www.ncbi.nlm.nih.gov/BLAST/) to determine the *emm* sequence type.

The data were analyzed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). In the univariate analysis, continuous and categorical data were analyzed using Student *t* test and the Mantel–Haenszel test, respectively.