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ScienceDirect
Diagnostic Microbiology and Infectious Disease 58 (2007) 289–295
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Molecular epidemiology of group A *streptococcus* causing scarlet fever in northern Taiwan, 2001–2002

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Received 9 November 2006; accepted 19 January 2007

Abstract

In this study, 830 *Streptococcus pyogenes* isolates collected between 2001 and 2002 from patients with scarlet fever in northern Taiwan were analyzed by M protein gene (*emm*) sequence typing, pulsed-field gel electrophoresis (PFGE), and antimicrobial susceptibility testing. A total of 21 *emm* types and 56 PFGE patterns were identified. The most frequent *emm* types were *emm*1 (29.2%), *emm*4 (24.1%), *emm*12 (19.0%), *emm*6 (15.8%), stIL103 (5.7%), and *emm*22 (1.9%). Antimicrobial resistance profiles were determined, and resistance to erythromycin (24.6%), clindamycin (2.0%), and chloramphenicol (1.3%) was detected. Five major *emm* types (*emm*4, *emm*12, *emm*1, *emm*22, and *emm*6) accounted for 95.6% of the erythromycin-resistant isolates. The decreased prevalence of erythromycin-resistant *emm*12 strains coincided with the overall decrease in erythromycin resistance from 32.1% in 2001 to 21.1% in 2002 in Taiwan. Five major clones (*emm*4/2000, *emm*12/0000, *emm*4/2010, *emm*1/1000, and *emm*22/8100) represented 72.1% of the erythromycin-resistant isolates. The survey of group A *Streptococcus emm* types, genetic diversity, and antibiotic resistance has direct relevance to current antimicrobial use policies and potential vaccine development strategies.

Keywords: GAS; *Streptococcus pyogenes*; Erythromycin resistant; Molecular epidemiology; Vaccine

1. Introduction

Streptococcus pyogenes (group A *Streptococcus* [GAS]) causes a wide range of human diseases and clinical manifestations including necrotizing fasciitis, toxic shock, pharyngitis, impetigo, acute rheumatic fever, and acute glomerulonephritis (Cleary et al., 1992; Cunningham, 2000). Scarlet fever is one of the most common infections caused by GAS in schoolchildren in Taiwan and is a notifiable disease, with 105 to 1033 confirmed cases per year during 1994 to 2004. Besides, temporal and geographic clustering of cases with this potentially severe GAS manifestation has been described (Chiou et al., 2004; Yan et al., 2003).

Multivalent vaccines targeted against common M types are the focus of intense study because, in many regions, relatively few M types account for most of the GAS causing both invasive and noninvasive infections (O'Brien et al., 2002; Shulman et al., 2004). *emm* sequence typing is an essential tool for determining the M serotype distribution among large sets of isolates (O'Brien et al., 2002; Li et al., 2003). Some *emm* types are linked to certain manifestations more frequently than others (Johnson et al., 1992; Musser et al., 1995; Bisno, 1991; Kiska et al., 1997). For example, type *emm*28 is more associated with postpartum infections than other types (Chuang et al., 2002).

S. pyogenes is still susceptible to penicillin but has shown increasing resistance to macrolides in many countries recently (Malhotra-Kumar et al., 2005; Szczypa et al., 2004). An erythromycin-resistant clone was reported to cause an outbreak of pharyngitis cases among schoolchildren (Martin et al., 2002). In Taiwan, the prevalent rate of erythromycin-resistant isolates had been approximately 40% to 70% (Hsueh et al., 2002; Hsueh et al., 2003), which was much higher than most of other countries. Taiwan has implemented a policy for decreased use of antibiotics for treatment of various infections, particularly the uncomplicated upper respiratory tract infection since 2001, which has

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doi:10.1016/j.diagmicrobio.2007.01.013

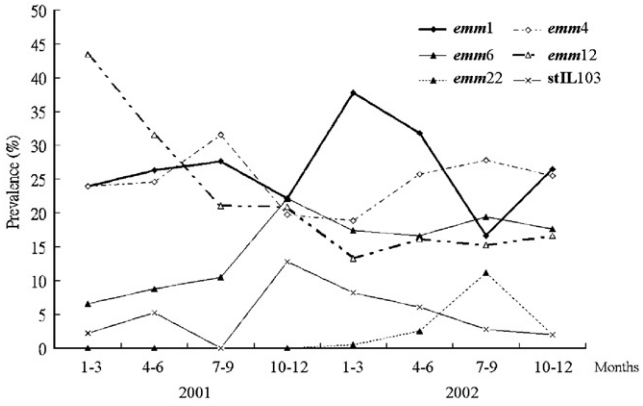

Fig. 1. The trimonthly distribution of major emm types as a percentage of the total number studied during 2001 to 2002.

significantly reduced the incidence of erythromycin resistance in GAS. Continuous monitoring of antimicrobial resistance trends is vital for continued development of sound antibiotic treatment policies. Long-term assessment of the M type distribution and genetic diversity of GAS in Taiwan may prove valuable for developing effective vaccines.

2. Materials and methods

2.1. Strains

Isolates of *S. pyogenes* (GAS) were collected from patients with specific symptoms (strawberry tongue, skin rash, and sore throat) of scarlet fever in northern Taiwan from 2001 to 2002. Isolates recovered from throat swabs were submitted to Centers for Disease Control (CDC) of Taiwan with reporting forms that contained detailed patient information. A total of 830 isolates collected from 818 patients were confirmed to be GAS using a series of tests including β-hemolysis, Gram stain, pyrrolidonyl aminopeptidase (Rosco Diatabs, Taastrup, Denmark), bacitracin susceptibility (BD Diagnostics Taxo A Discs, Franklin Lakes, NJ), and coagglutination (Boule Phadebact Strep A Test, Huddinge, Sweden) (Kurzynski and Van Holten, 1981). From 12 patients, more than one isolate was obtained from another episode of infection.

2.2. Antimicrobial susceptibility tests

Minimum inhibitory concentration for all 830 isolates of *S. pyogenes* were determined by the agar dilution method and interpreted according to the guidelines recommended by the Clinical and Laboratory Standards Institute. The isolates were cultured overnight on trypticase soy agar plates supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, MD) in 5% CO_2 at 37 °C overnight. The freshly grown bacteria were suspended in sterile normal saline and adjusted to a density of 0.5 McFarland standard. A density of 104 CFU/spot was inoculated onto the appropriate plate with various concentrations of antimicrobial agents with a Steers replicator. The concentration ranges of antibiotics were as follows: penicillin, 0.015 to 2.0 μg/mL; cefotaxime, 0.015 to 2.0 μg/mL; erythromycin, 0.015 to 4.0 μg/mL; clindamycin, 0.015 to 4.0 μg/mL; and vancomycin, 0.03 to 4.0 μg/mL.

2.3. Pulsed-field gel electrophoresis analysis

Strains were inoculated on blood agar plates (BBL) incubated in 5% CO_2 at 37 °C for 16 to 24 h. Pulsed-field gel electrophoresis (PFGE) using *Sma*I endonuclease was conducted as previously described (Lin et al., 2006; Chen et al., 2006).

2.4. Analysis of banding patterns

The digital images of PFGE pattern were analyzed with BioNumerics software (Applied Maths, Courtrai, Belgium). The dice similarity coefficient was used with optimization and position tolerance settings of 1.0% and 1.5%, respectively. Pulsed-field gel electrophoresis–based clusters were defined as isolates with ≥80% genetic relatedness on the dendrogram. Dendrograms derived from the PFGE patterns were constructed by use of the unweighted pair group method with arithmetic averages (UPGMA) algorithm, based on the Dice similarity coefficients.

2.5. emm typing

The protocol was accomplished using a method developed by Whatmore et al. (1994) and as modified at the CDC Web site (http://www.cdc.gov/ncidod/biotech/strep/protocols.htm). *emm* types and subtypes were assigned from the *emm* database at the CDC Web site (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm).

3. Results

A total of 830 GAS isolates collected from scarlet fever patients in northern Taiwan during 2001 to 2002 were analyzed from which 21 *emm* sequence types were identified. Similar to recent studies in the United States (Li et al., 2003; Shulman et al., 2004), for each common *emm* type, a predominant *emm* subtype was also found. All major erythromycin-resistant sets corresponding to specific *emm* types were associated with the predominant *emm* subtype.

The most frequent *emm* types were *emm*1 (29.2%), followed by *emm*4 (24.1%), *emm*12 (19.0%), *emm*6 (15.8%), stIL103 (5.7%), and *emm*22 (1.9%). These 6 major *emm* types accounted for 95.7% of GAS isolates. Other minor *emm* types included *emm*101, *emm*106, *emm*11, *emm*113, *emm*2, *emm*33, *emm*49, *emm*58, *emm*77, *emm*92, st11014, st463, st5240, stIL62, and sts104. It is noteworthy that when performing polymerase chain reaction-based assays of GAS isolates recovered in the United States of types *emm*1, *emm*4, *emm*6, and *emm*12, these isolates usually carry one or both of the 2 phage-encoded exotoxin genes *speA* or *speC*, whereas most of *emm*22 isolates carry neither of these genes, which have been associated with scarlet fever. The trimonthly distribution of major *emm* types during 2001 to 2002 was shown in Fig. 1. The