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Note

The change of macrolide resistance rates in group A *Streptococcus* isolates from children between 2002 and 2013 in Asahikawa city

Hiroshi Sakata*

Department of Pediatrics, Asahikawa Kosei Hospital, 1-24, Asahikawa, Hokkaido 078-8211, Japan

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ABSTRACT

This study targeted patients in the Department of Pediatrics, Asahikawa Kosei Hospital, between January 2002 and December 2013. In patients suspected of having hemolytic streptococcal infection, Group A *Streptococcus* (GAS) strains isolated from a throat swab were examined for antimicrobial susceptibility testing. The MICs were measured by the broth microdilution method. The annual number of GAS strains examined for antimicrobial susceptibility testing ranged from 28 to 65 strains, for a total of 574 strains. Some of the isolates obtained from 2006 to 2009 and from 2011 to 2013 were analyzed to determine their *emm* types. An erythromycin (EM) resistant strain was not detected until 2004, but one EM-resistant strain appeared in 2005. Subsequently, EM-resistant strains rapidly increased, and 48 of 65 strains (73.8%) examined in 2009 were resistant. In 2010, the number of EM-resistant strains decreased to 12 of 36 strains (33.3%). However, it gradually increased afterwards, and 37 of 60 strains (61.7%) were resistant in 2013. Out of 574 strains examined, 184 exhibited EM-resistance, and the overall resistance rate was 31.9%. Partitioning the 124 strains examined between 2006 and 2008 according to *emm* types, only *emm*28 strains, which exhibited a high resistance rate, and *emm*12 strains demonstrated resistance. For the 142 strains examined between 2011 and 2013, the resistance rate of *emm*28 strains was similarly high; the resistance of *emm*12 strains significantly increased, and *emm*1 strains exhibited a high resistance rate. The number of *emm* types associated with the resistant strains increased.

Group A *Streptococcus* (GAS) causes various infections ranging from common diseases in the pediatric field such as pharyngitis, tonsillitis, impetigo, and scarlet fever in the pediatric field, to serious diseases such as toxic shock syndrome and fulminant skin and soft tissue infection. Furthermore, they occasionally result in non-infective complications such as rheumatic fever. Even after the acute symptoms are relieved, the continued prophylactic administration of antimicrobial agents is necessary.

For the treatment of infections with GAS, guidelines in the United States and elsewhere recommend penicillins as the first choice and macrolide derivatives for patients with penicillin allergies [1]. However, in Japan, resistance to macrolides has been increasing for GAS, and they are now considered inadequate for the initial treatment of hemolytic streptococcal infection [2]. Thus, we investigated longitudinal fluctuations in macrolide resistance rates of GAS in Asahikawa Kosei Hospital and evaluated the association between resistance and *emm* type.

This study targeted patients in the Department of Pediatrics, Asahikawa Kosei Hospital, between January 2002 and December 2013. In patients suspected of having hemolytic streptococcal infection, GAS isolated from a throat swab was examined for antimicrobial susceptibility testing. Though several pediatricians work in the hospital, this study presents the results obtained for the strains isolated from patients examined by me only. The MICs were measured by the broth microdilution method [3] using with MicroScan WalkAway (Siemens healthcare Diagnostics Inc), a fully automated bacterial identification system. Panels for test were used Pos Combo41J to 2009, MicroFAST 3J from 2010 to 2011, and MicroFAST 5J from 2011. In particular, susceptibilities to penicillin G (PCG), erythromycin (EM), and clindamycin (CLDM), were examined. Measurements were made the criteria for drug resistance were set at Penicillin G $\geq$ 0.25 μg/ml, erythromycin $\geq$1 μg/ml, clindamycin $\geq$1 μg/ml, and levofloxacin $\geq$8 μg/ml. The macrolide-resistant strains were defined as those strains exhibiting resistance to EM. Some of the isolates obtained from 2006 to 2009 and

* Tel.: +81 166 33 7171; fax: +81 166 33 6075.
E-mail address: sakhiro@mac.com.

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from 2011 to 2013 were analyzed to determine their *emm* types. The Laboratory of Molecular Epidemiology for Infectious Agents, Kitasato Institute of Life Sciences, was requested to perform determination of the *emm* typing according to the Center for Disease Control (CDC) guideline (http://www.cdc.gov/streplab/M-ProteinGene-typing.html). Statistical analysis was performed with StatMate V (ATMS Co., Ltd.). A p-value of less than 0.05 was considered to indicate a statistically significant difference.

The annual number of GAS strains examined for antimicrobial susceptibility testing ranged from 28 to 65 strains, for a total of 574 strains. Throughout both targeted periods, none of the strains were resistant to PCG. Fig. 1 shows the annual fluctuations in EM-resistance rates. An EM-resistant strain was not detected from 2002 to 2004, but one EM-resistant strain appeared in 2005. Subsequently, EM-resistant strains rapidly increased, and 48 of 65 strains (73.8%) examined in 2009 were resistant. In 2010, the number of EM-resistant strains decreased to 12 of 36 strains (33.3%). However, it gradually increased afterwards, and 37 of 60 strains (61.7%) were resistant in 2013. Out of 574 strains examined, 184 exhibited EM-resistance, and the overall EM-resistance rate was 31.9%.

The *emm* type that 124 strains examined according to CDC database between 2006 and 2008 exhibited *emm*28 that indicated high EM-resistance rate and *emm12* that indicated low one, only two types (Figs. 2 and 3). According to the *emm* types of the 124 strains isolated between 2006 and 2008, *emm*28 exhibited a high EM-resistance rate of 82.3%, followed by *emm*12 with a rate of 14.6%. Among the 142 strains isolated between 2011 and 2013, although *emm*28 exhibited a similarly high EM-resistance rate of 77.8%, the EM-resistance rate was the highest, at 87.8%, for *emm*1 ($p < 0.001$). In addition, the EM-resistance rate of *emm*12 increased to 68.0% ($p < 0.001$). EM-resistance also developed in the *emm*4 and *emm*11 strains, though the difference did not reach statistical significance. The number of *emm* types associated with EM-resistant strains increased from 2 in 2006 to 5 in 2008.

CLDM-resistant strains were first detected in 2006 (Fig. 1). Unlike the EM-resistance rates, rates of CLDM resistance fluctuated from 3.1% to 15.0%. Overall, 37 of 574 strains (6.4%) were CLDM-resistant. CLDM-resistance was observed in 14 of 124 strains (11.3%) examined between 2006 and 2008 and 17 of 142 strains (12.0%) examined between 2011 and 2013, indicating no significant difference in resistance rate over time. Considering the *emm* types, the CLDM-resistant strains detected between 2006 and 2008 included 10 *emm*28 strains (58.8%) and 4 *emm*12 strains (9.8%), while those detected between 2011 and 2013 included 12 *emm*28 strains (66.7%), 4 *emm*12 strains (16.0%), and one *emm*1 strain (2.0%).

GAS acquires resistance to macrolides by two mechanisms [4]. In the first mechanism, GAS obtains the erythromycin resistance methylase (*erm*) genes, which confer drug resistance by dimethylation of adenine in domain V of the 23S rRNA to prevent macrolide derivatives from combining. While there are *erm* (A) and *erm* (B) genes, only the latter exhibits high resistance. In the second mechanism, GAS obtains the macrolide efflux, coding to a *mef* (A) gene, which promotes the excretion of macrolide derivatives from the bacterium. GAS carrying the *erm* (A) gene exhibit resistance to CLDM, and GAS carrying the *mef* (A) gene are often sensitive to CLDM.

We have investigated macrolide resistance rates in the Asahikawa region since the 1970s. Although the resistance rates were 60% or greater in 1970 [5], the prevalence of macrolide-resistant strains began declining in 1980s, and they nearly disappeared in the late 1980s and early 1990s [6]. The resistant strains were detected again in 2005, leading to the data presented in this study. The results of other studies conducted in Japan show a similar trend [7,8]; no resistant strains were detected in the early 1990s. According to nationwide results, resistant strains began to be detected in the late 1990s, which is earlier than was observed at our hospital. Wajima et al. [9] report that 332 strains isolated from samples obtained from pediatric patients with pharyngotonsillitis at 45 medical institutions throughout Japan between 2003 and 2006 included 49 macrolide-resistant strains (14.8%). In a similar investigation conducted in 2012, the number of macrolide-resistant strains was 211 out of 363 strains (58.1%), indicating a significant increase [2]. The EM-resistance rate in our hospital increased from 10.0% in 2006 to 50.0% in 2012 similar to previous report [2].

In recent studies in other countries, the macrolide-resistance rates were 6.1% in the United States [10] and 2.6% in Germany [11], which are each lower than the rate observed in Japan, whereas in China the rate was extremely high at nearly 100% [12]. Substantial differences were observed among countries.

Such regional and periodic differences in resistance rates are considered to be greatly attributable to antimicrobial agents [13]. Although their actual consumption is unknown, macrolide derivatives were used more than penicillin derivatives in Japan in the 1970s to compensate for penicillin allergy. However, many cephem derivatives were developed and sold in the 1980s, and

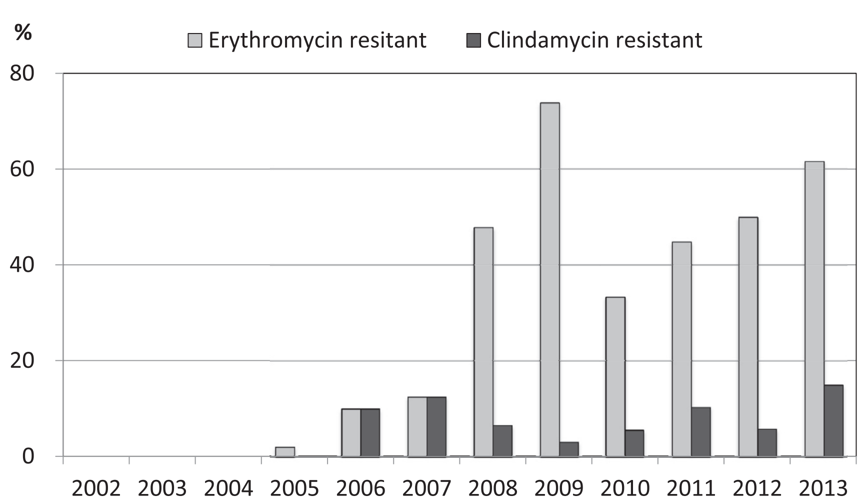

Fig. 1. Annual change of erythromycin or clindamycin resistant strains among group A *Streptococcus* isolates from 2002 to 2013.