J Infect Chemother xxx (2017) 1–6

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

Molecular characterization and antibiotic resistance of *Streptococcus dysgalactiae* subspecies *equisimilis* isolated from patients with streptococcal toxic shock syndrome☆

T. Ikebe [a, *], R. Okuno [b], M. Sasaki [c], Y. Kanda [c], H. Otsuka [d], R. Kawahara [e], H. Ohya [f], M. Suzuki [f], K. Uchida [g], H. Nihonmatsu [h], M. Ohnishi [a], The Working Group for Beta-Hemolytic Streptococci in Japan[1]

[a] Department of Bacteriology I, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan
[b] Department of Microbiology, Tokyo Metropolitan Institute of Public Health, 3-24-1 Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan
[c] Laboratory of Microbiology, Oita Prefectural Institute of Health and Environment, 2-8 Takae-Nishi, Oita 870-1117, Japan
[d] Department of Public Health Sciences, Yamaguchi Prefectural Institute of Public Health and Environment, 2-5-67 Aoi, Yamaguchi 753-0821, Japan
[e] Division of Bacteriology, Osaka Institute of Public Health, 1-3-69 Nakamichi, Higashinari-ku, Osaka 537-0025, Japan
[f] Division of Microbiology, Kanagawa Prefectural Institute of Public Health, 1-3-1 Shimomachiya, Chigasaki, Kanagawa 253-0087, Japan
[g] Department of Bacteriology, Toyama Institute of Health, 17-1 Naka-Taikouyama, Imizu, Toyama 939-0363, Japan
[h] Department of Microbiology, Fukushima Institute of Public Health, 16-6 Mitouchi, Katakida, Fukushima 960-8560, Japan

ARTICLE INFO

Article history:
Received 30 July 2017
Received in revised form
27 September 2017
Accepted 28 September 2017
Available online xxx

Keywords:
Streptococcus dysgalactiae subspecies *equisimilis*
Streptococcal toxic shock syndrome
emm genotype
Antibiotic resistance

ABSTRACT

Streptococcal toxic shock syndrome (STSS) is a severe invasive infection characterized by the sudden onset of shock, multiorgan failure, and high mortality. Although STSS is mainly caused by *Streptococcus pyogenes*, group G streptococcus identified as *S. dysgalactiae* subsp. *equisimilis* (SDSE) causing STSS has also been reported; however, no study has analyzed >100 isolates of SDSE causing STSS. Therefore, we characterized the *emm* genotype of 173 SDSE isolates obtained from STSS patients in Japan during 2014–2016 and performed antimicrobial susceptibility testing using the broth microdilution method and *emm* gene typing. The predominant *emm* genotype was found to be *stG6792*, followed by *stG485*, *stG245*, *stG10*, *stG6*, and *stG2078*. These six genotypes constituted more than 75% of the STSS isolates. The proportion of each *emm* genotype in STSS isolates correlated with that in invasive isolates previously reported. We found that 16.2% of the isolates showed clindamycin resistance. The proportion of clindamycin-resistant SDSE isolates was significantly higher than that of *S. pyogenes* isolates. Thus, while treating STSS caused by SDSE, it is necessary to consider the possibility of clindamycin resistance and to ensure judicious use of the drug.

1. Introduction

Group G streptococci (GGS) are common members of the normal flora of the human skin, pharynx, and gastrointestinal tract [1]. GGS cause pharyngitis, skin and soft tissue infections, septic arthritis, bacteremia, and endocarditis. Since the late 1980s, streptococcal toxic shock syndrome (STSS) caused by *Streptococcus pyogenes* (group A streptococci) has become a severe problem in many countries. The characteristic symptoms progress very rapidly and are fulminant from the onset. Patients can develop necrotizing fasciitis, acute kidney failure, adult respiratory distress syndrome, disseminated intravascular coagulopathy, and multiorgan failure within a few hours, leading to shock and death. GGS identified as *S. dysgalactiae* subsp. *equisimilis* (SDSE) have also been reported to cause STSS [2,3]. The first case of STSS caused by SDSE was reported in 1995 in Japan [4]. Our group and another group found that there have been dozens of cases of SDSE causing STSS in Japan to date [4,5].

☆ All authors meet the ICMJE authorship criteria.
* Corresponding author.
E-mail address: tdikebe@nih.go.jp (T. Ikebe).
[1] Members are listed in the Acknowledgements.

https://doi.org/10.1016/j.jiac.2017.09.013
1341-321X/

In SDSE, many virulence factors have been implicated in the pathogenesis of streptococcal infections, including the M protein encoded by the *emm* gene. On the basis of *emm* genes that show polymorphisms similar to those in *S. pyogenes* [6], gene sequence analysis has been applied to *emm* typing for epidemiologic study of SDSE. According to the Centers for Disease Control and Prevention (CDC; http://www2a.cdc.gov/ncidod/biotech/strepblast.asp), >30 *emm* types have been recognized among SDSE.

Other groups reported the *emm* genotype of SDSE isolated from invasive infections [7–10]. In France [7] and USA [8], *stG6* was most prevalent, and in Taiwan [9], *stG485* was most prevalent. In Japan, *stG6792* was most prevalent in invasive SDSE isolates [10]. There are regional differences in the *emm* genotype of invasive isolates. However, these studies included small numbers of STSS cases (0–19 cases), and the SDSE in these studies was mainly isolated from patients with cellulitis, bacteremia without focus, arthritis, or abscess [7–10]. To date, no study had analyzed >100 isolates of SDSE causing STSS, and it was not known which *emm* genotype was prevalent in STSS isolates. In the current study, we investigated the *emm* genotypes of STSS isolates in Japan and performed antimicrobial susceptibility tests.

2. Materials and methods

2.1. Ethical approval

This study complies with the guidelines of the Declaration of Helsinki. This study protocol was approved by the Institutional Individual Ethics Committees for the use of human subjects (the National Institute of Infectious Diseases Ethics Review Board for Human Subjects).

2.2. Bacterial isolates

Isolates of SDSE causing STSS were obtained from pathogen collections at the National Institute of Infectious Diseases and prefectural Public Health Institutes (PHIs) in Japan. A total of seven reference center branch offices are located in the PHIs of Fukushima, Tokyo, Kanagawa, Toyama, Osaka, Yamaguchi, and Oita. Data on streptococcal infections and clinical isolates are sent to PHIs from hospitals. The diagnostic criteria of SDSE-induced STSS were based on the definite cases described by the Working Group on Severe Streptococcal Infections (1993) [11]. A total of 173 SDSE isolates were obtained from sterile body sites of patients with STSS during 2014–2016 and cultured. Isolates were obtained as part of standard patient care at hospitals.

2.3. emm typing

emm gene sequencing was performed as described by Beall et al. [6] with modifications as described at https://www.cdc.gov/streplab/index.html. As per the CDC protocol, the primers emm-1 (5′-TATT(C/G)GCTTAGAAAATTAA-3′) and emm-2 (5′-GCAAGTTCTT CAGCTTGTTT-3′) were used for amplifying the N-terminal region of the *emm* gene. The purified PCR products were sequenced using the emmseq2 primer (5′-TAA TCG CTT AGA AAA TTA AAA ACA GG-3′). The first 160 bases of the 5′-end of the *emm* gene were compared with those in the CDC *emm* sequence database (https://www2a.cdc.gov/ncidod/biotech/strepblast.asp).

2.4. Antimicrobial susceptibility tests

The antimicrobial susceptibility of the isolates to penicillin G, ampicillin, cefotaxime, erythromycin, clindamycin, and linezolid was examined using the broth microdilution method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [12]. The breakpoints for the resistance of each drug were set according to the CLSI recommendations [12]. Screening for inducible clindamycin resistance was performed using the broth microdilution method, as recommended by the CLSI [12].

2.5. Detection of erythromycin-resistance genes

The *ermA*, *ermB*, *mefA*, and *mefE* genes, which are responsible for erythromycin resistance, were detected using PCR performed using published primer sequences [13–15].

2.6. Statistical analyses

Data were compared using $\chi 2$ test. Differences were considered significant when p was <0.05.

3. Results

3.1. Age distribution of patients with STSS due to SDSE

During 2014–2016, isolates were collected from 173 patients with STSS due to SDSE (Table 1). Of these patients, 52.0% were male individuals (male patients, 90; female patients, 83). The age range of the patients was 14–101 years (Fig. 1); their average age (75.0 years) was greater than that reported earlier for patients with STSS due to *S. pyogenes* (average age, 60.8 years; $p < 0.01$) [16]. The average age of the female patients (80.9 years) was significantly higher than that of the male patients (69.5 years; $p < 0.01$). The median age of patients with STSS due to SDSE was 79 years (male patients, 72.5; female patients, 80.9). The mortality rate was 31.2% (male patients, 27.8%; female patients, 34.9%) and was significantly lower than that of STSS due to *S. pyogenes* (40.9%; $p < 0.05$) [16].

3.2. emm typing of STSS isolates

A total of 21 *emm* genotypes were identified; the prevalence of each genotype is presented in Table 1. The most prevalent genotype

Table 1
Frequency (%) and the number (n) of STSS isolates of each *emm* genotype during 2014–2016.

emm type	STSS (n)	STSS (%)	Invasive (%)[a]
stC36	2	1.2	3.6
stC46	1	0.6	
stC5345	3	1.7	
stC74a	6	3.5	2.6
stC839	1	0.6	
stG10	14	8.1	6.2
stG11	1	0.6	
stG166b	4	2.3	2.6
stG2078	8	4.6	3.8
stG245	18	10.4	10.7
stG4222	2	1.2	1.4
stG480	2	1.2	2.3
stG485	23	13.3	13.3
stG4974	4	2.3	1.4
stG5420	1	0.6	2.2
stG6	8	4.6	5.5
stG643	3	1.7	
stG652	5	2.9	6.2
stG653	5	2.9	3.9
stG6792	60	34.7	27.1
stGLP1	1	0.6	
Untypeable	1	0.6	
total	173	100.0	

[a] Genotype distributions of isolates from patients with invasive infections as per data reported by Wajima et al. [10].

Please cite this article in press as: Ikebe T, et al., Molecular characterization and antibiotic resistance of *Streptococcus dysgalactiae* subspecies *equisimilis* isolated from patients with streptococcal toxic shock syndrome, J Infect Chemother (2017), https://doi.org/10.1016/j.jiac.2017.09.013