Short Communication

Characteristics of \textit{Streptococcus suis} Isolated from Patients in Japan

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SUMMARY: Seven cases of \textit{Streptococcus suis} infection in Japan during 1994 and 2006 were summarized. All cases had porcine exposure and five of them had hand skin injury during the exposure. Five cases presented symptoms of meningitis, three presented symptoms of sepsis, and one resulted in sudden death. All of the isolated \textit{S. suis} belonged to Lancefield’s group D and to serotype 2. They were susceptible to penicillin G, ampicillin, cefotaxime, and ciprofloxacin. However, six of them were resistant to both erythromycin and clindamycin, and four were also resistant to minocycline. Multilocus sequence typing of six isolates showed that they belonged to sequence type (ST) 1, and their pulsed-field gel electrophoresis (PFGE) patterns were similar. The remaining isolate was ST28 and its PFGE pattern was distinct from those of the others.

\textit{Streptococcus suis} is an important swine pathogen that has been associated with cases of meningitis, endocarditis, arthritis, and other infections in pigs (1). Thirty-five capsular serotypes based on capsular polysaccharides have been described for the bacterium, and the serotype 2 isolates are most frequently associated with these diseases (2). The bacterium is also recognized as a zoonotic agent, with the first human case of \textit{S. suis} infection having been reported in 1968 (3). Most patients have a contact history with infected pigs or swine products. Clinical presentations in humans include acute bacterial arthritis, sepsis, meningitis, and so on (4,5). Vestibular dysfunction and/or permanent hearing loss are described for the bacterium, and the serotype 2 isolates are most frequently associated with these diseases (2). The bacterium is also recognized as a zoonotic agent, with the first human case of \textit{S. suis} infection having been reported in 1968 (3). Most patients have a contact history with infected pigs or swine products. Clinical presentations in humans include acute bacterial arthritis, sepsis, meningitis, and so on (4,5). Vestibular dysfunction and/or permanent hearing loss are commonly noted sequelae (4,6). \textit{S. suis} infections in humans have sporadically occurred without obvious seasonal change, and almost all of them are caused by serotype 2 isolates (7). In China, two outbreaks caused by \textit{S. suis} serotype 2 occurred in summer (8,9). The reasons why \textit{S. suis} causes such outbreaks are still unknown. In Japan, several sporadic cases in humans have been reported (10-14). In this study, we summarize the clinical features of these infection cases in Japan, and perform a bacteriological analysis of the isolates.

All nucleic acid manipulations were accomplished according to the standard molecular biology techniques (15). Polymerase chain reaction (PCR) amplifications were carried out with Ex Taq polymerase (Takara, Otsu, Japan) by the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, Calif., USA). The DNA sequencing was performed with Genetic Analyzer 310 and Genetic Analyzer 3130 (Applied Biosystems).

Gram staining was performed by the Bartholomew & Mittwer method. The Lancefield’s group was determined by using Streptex (Remel Inc., Lenexa, Kans., USA), API 20 Strept Kit (bioMérieux, Marcy l’Etoile, France) was used for biochemical tests. The capsular types were determined by the Quellung method with Suis Type Antisera (Statens Serum Institut, Copenhagen, Denmark). Determination and homology searches of the 16S rDNA sequence were performed according to the instructions in MicroSeq (Applied Biosystems).

The antimicrobial susceptibility of the isolates to eight antibiotics was analyzed by the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) (16,17) by using dry plates (Eiken Chemistry, Tokyo, Japan). The eight antibiotics were penicillin G, ampicillin, cefotaxime, ciprofloxacin, minocycline, erythromycin, clindamycin, and telithromycin. Antimicrobial susceptibility breakpoints were defined according to CLSI criteria, while those of ciprofloxacin were the same as levofloxacin, and those of telithromycin were from \textit{Streptococcus pneumoniae} (17). To clarify the molecular mechanism of the drug resistance, the presence of the \textit{ermB} and \textit{mefA/E} genes, which responded to macrolide- and lincosamide-antibiotics resistance, was investigated by PCR as described previously (18). In addition to these two genes, we also investigated the presence of point mutation(s) in \textit{rplK}, \textit{ermB}, and \textit{ermA}, encoding 23S rRNA, L4, and L22 ribosomal proteins, respectively. Primers were designed according to the sequences of \textit{S. pneumoniae} (19).

Molecular typing of the isolates was done by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). PFGE was performed according to a method described previously (20). Restriction enzymes, \textit{Smal} (Takara) and \textit{ApaI} (Takara), were used for digestion of the genomic DNAs. Electrophoresis was carried out at 6 V/cm for 17 h,
with the pulse time ranging from 1.2 to 30 s. MLST was performed and primers were designed as described by King et al. (21). We sequenced both strands of the amplicons and trimmed the sequences of the seven genes (aroA, cpmB60, dpr, gki, mutS, recA, and thrA). The allelic numbers and sequence types of the isolates were determined by comparing their sequences with those in the database provided by King and Whatmore (http://susis.mlst.net/).

From 1994 to January 2006, seven cases of *S. suis* infections emerged in Japan. Six of them have been published as case reports (10-14). The remaining case occurred in January 2006. The patient was a 56-year-old previously healthy woman. She had come into contact with raw viscera from swine, and suffered from sepsis, disseminated intravascular coagulation (DIC), arthritis, and endophthalmitis. Blood culture on admission yielded *S. suis*. After treatment with effective antibiotics, she recovered and had no aftereffects.

Clinical characteristics of these cases are summarized in Table 1. The ages of the seven patients (4 males and 3 females) ranged from 47 to 58 and the average age was 53.7. All of them had history of exposure to swine materials and five of them had skin injury on their hands during the exposure. Five patients showed meningitis, which is the most prevalent (75.2-84.6%) symptom in the reported exposure. Five patients suffered from DIC, three from sepsis, disseminated intravascular coagulation (DIC), arthritis, and endophthalmitis. Blood culture on admission yielded *S. suis*. After treatment with effective antibiotics, she recovered and had no aftereffects.

### Table 1. Summary of clinical characteristics of patients with *Streptococcus suis* infection

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases reported (n = 7)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure history</td>
<td></td>
</tr>
<tr>
<td>Porcine exposure</td>
<td>7 (100.0)</td>
</tr>
<tr>
<td>Skin injury during exposure</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>Type of infection</td>
<td></td>
</tr>
<tr>
<td>Meningitis</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>Endophthalmitis</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>Epidural abscess</td>
<td>1 (14.3)</td>
</tr>
<tr>
<td>Complication</td>
<td></td>
</tr>
<tr>
<td>Deafness</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>Death</td>
<td>1 (14.3)</td>
</tr>
</tbody>
</table>

\(^a\): Data of six cases are adapted from references (10-14).

All of the seven *S. suis* isolates were susceptible to penicillin G (minimum inhibitory concentration [MIC], 0.015-0.06 μg/ml), ampicillin (MIC, 0.015-μg/ml), cefotaxime (MIC, 0.03-0.06 μg/ml), and ciprofloxacin (MIC, 0.5-1 μg/ml). The six isolates other than SUIS2 were resistant to erythromycin (MIC, ≥2 μg/ml) and clindamycin (MIC, ≥4 μg/ml); four (SUIS1, SUIS4, SUIS5, and SUIS6) of them were also resistant to minocycline (MIC, ≥8 μg/ml). All the six resistant isolates were positive for *ermB*, but negative for *mefA/E* in the PCR. SUIS2, which was isolated in 1994 (11), carried neither the *ermB* nor the *mefA/E* genes. Among the six resistant isolates, four (SUIS4, SUIS5, SUIS6, and SUIS7) showed reduced susceptibility to telithromycin (MIC, 1-2 μg/ml). It had been reported that telithromycin-resistant *S. pneumoniae* possessed *ermB*, and showed a combination of mutations in the *rrl*, *rplD*, and/or *rplV* genes (18,22). Therefore, the sequences of *rrl* (position 31-2891), *rplD* (position 1-625), and *rplV* (position 1-345) of the *S. suis* isolates were determined. No difference was observed between the telithromycin-susceptible and the telithromycin-reduced-susceptible isolates (data not shown). The molecular mechanism(s) for the reduced susceptibility to telithromycin of these isolates is still unclear.

PFGE analysis was performed and the *Smal*-digested profiles of *S. suis* are shown in Figure 1. The PFGE patterns of six isolates (SUIS1, SUIS2, SUIS3, SUIS4, SUIS5, and SUIS6) were similar to each other; in particular, those of SUIS1, SUIS4, SUIS5, and SUIS6 were indistinguishable, although they were isolated in different years and/or regions (10,13,14). However, the pattern of SUIS7 was clearly distinct from those of the others (Fig. 1). SUIS7 was isolated from the case occurring in January 2006, as described above. The same result was obtained when restriction enzyme *ApaI* was used for digestion of the genomic DNAs (data not shown). The MLST revealed that SUIS1, SUIS2, SUIS3, SUIS4, SUIS5, and SUIS6 belonged to sequence type (ST) 1, whereas SUIS7 belonged to ST28.

STs of six of the seven isolates in Japan were the same as

Sequences of the 16S rDNA of these isolates shared 99.4-100% homology with that of the *S. suis* type strain ATCC 43765, which also belongs to serotype 2.
ST1, which has been reported to be the most common ST of \textit{S. suis}, and were isolated in European nations, the United States, and Hong Kong (21). Based on these observations, the ST1 isolates are recognized as a highly successful clone that arose relatively recently and has rapidly spread worldwide (21).

In this study, we summarized the clinical characteristics of seven cases of \textit{S. suis} infection in Japan, determined the serotypes and antimicrobial susceptibilities, and compared PFGE patterns and STs of the isolates. Since all of the patients had come into contact with swine materials, enhanced protection during work will be the best defense against \textit{S. suis} infection. The observed mortality rate of these cases was high (14.3%), and was comparable to those reported previously (13.2 - 20.2%) (7). Therefore, early diagnosis and administration of appropriate antibiotics will be indispensable for effective treatment and reduction in mortality of \textit{S. suis} infections. Although there may be additional unreported cases of \textit{S. suis} infection in Japan, the number of cases over the past 12 years seems to be rather small. Surveillance of \textit{S. suis} infections should be emphasized in Japan.

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REFERENCES


