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Case report

A *Streptococcus suis* serotype 2 caused streptococcal toxic shock syndrome(STSS) in a patient☆

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Abstract

Streptococcus suis (*S. suis*) is a Gram-positive, facultatively anaerobic coccus that has been implicated as the cause of a wide range of clinical disease syndromes in swine and other domestic animals. *S. suis* has also been implicated in disease in humans, especially among abattoir workers, swine and pork handlers. Here we report a case of streptococcal toxic shock syndrome(STSS) caused by *S. suis* in a 59-year-old man. Despite of intensive treatment, the patient died of shock with multiple organ failure 14 h after admission. One bacterial isolate obtained from blood culture was identified to the species level by biochemical tests and serological tests as *S. suis* serotype 2. Identification was confirmed by PCR amplification of genes encoding 16sRNA of *S. suis* and the capsule of *S. suis s*erotype 2(cps *2J*). Genes encoding virulence factors were also detected. An investigation to identify the source of *S. suis* revealed that several days before admission the affected man had been handling sick pigs or their meat. Transmission may occur through breaks in the skin of feet with tinea due to that no measures for personal protection was taken. This case should highten awareness of the potential for occupational exposure and human infection with *S. suis.*

Key words: *Streptococcus suis*; streptococcal toxic shock syndrome; PCR

INTRODUCTION

Streptococcus suis(*S. suis*) is a worldwide causative agent of many different swine diseases such as meningitis, endocarditis, septicemia, and arthritis[1,2]. Of the 35 official serotypes described to date for *S. suis*, serotype 2 is the most virulent and frequently isolated from diseased animals[2]. This serotype is also recognized as a zoonotic agent since it has been identified as a cause of meningitis, septicemia, and endocarditis in humans, and particularly those occupationally exposed to pigs or pig products[3-6]. Outbreaks of *S. suis* serotype 2 in humans were reported in Jiangsu and Sichuan province of China in 1998 and 2005, respectively; hundreds of people were infected, and some people died in streptococcal toxic shock syndrome(STSS) and meningitis[7, 8]. Diagnosis of *S. suis* infection is based on characteristic macroscopical lesions, bacterial cultures, and biochemical and serological characterization[9]. Early diagnosis and effective antibiotic therapy are key points for increasing the survival rate. In this report we describe a patient with STSS from a primary infection by *S. suis.*

CASE REPORT

Patient

A 59-year-old man presented to a general hospital with malaise, decreased consciousness over the last 6 h, fever and rapidly developing hyperpyrexia, hypotension, and a decline of pulse pressure. Vital signs on admission were: temperature, 39.8℃; pulse, 140 times/min;

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and blood pressure,90/40 mmHg. Subcutaneous haemorrhage was also observed(***Fig. 1***). Venipuncture blood collected in EDTA tubes was used for blood examination. Laboratory investigation revealed a leukocyte count of 16 700/mm^3 with 96% polymorphonuclear cells, a platelet count of 57 000/ mm^3, prothrombin time 24.8 s, and activated partial thromboplastin time more than 120 s. Although systemic treatment, including anti-shock, anti-infection, and supportive management, was taken, the patient died of shock complicated with disseminated intravascular coagulation at 3 o'clock the following morning. His medical history indicated a severe tinea of the feet(***Fig. 2***). During pig slaughtering he had worn slippers not protective boots on his feet.

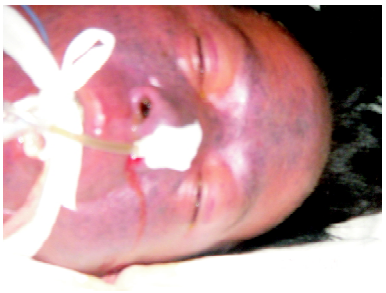


Fig. 1 Clinical signs of subcutaneous haemorrhage found in the face of the patient infected with *S suis*.

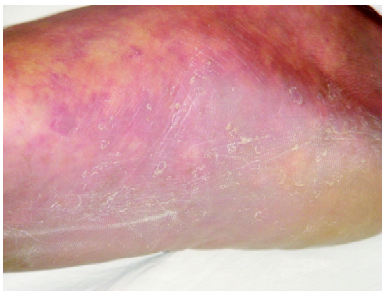


Fig. 2 Tinea pedis image shown in the foot of the patient infected with *S suis*

Bacterial isolation

To isolate the possible bacterium pathogen, blood sample was directly inoculated on the nutritional agar medium with 8%(vol/vol) of defibrinated sheep blood and incubated at 37℃, 5% CO_2.

Biochemical identification

Isolates were identified biochemically by routine procedures. All suspected *S. suis* colonies were tested with the APIE® 20 Strep system according to the manufacturer's directions(bioMérieux, Marcy-I'Etoile, France).

Serology identification

Cells scrapped off the plate were characterized by using *S. suis* antisera specific against individual serotypes(Statens Serum Institut, Copenhagen, Denmark) and confirmed by the coagglutination test as reported[10].

Molecular identification

General genomic DNA preparation from whole blood was done with the QIAamp DNA Blood Maxi kit (Qiagen, Inc., Valencia, CA) according to the instructions of the manufacturer. General PCR was performed to detect genes encoding for 16S rRNA of *S. suis* and for cps *2J.* genes encoding proteins associated with virulence including muramidase-released protein(MRP)[11], extracellular factor(EF)[11], suilysin(SLY)[12], dipeptidyl peptidase Ⅳ(DPP Ⅳ)[13] , and fibronectin-binding protein(FBPS)[14] were also detected. Genomic DNA from the 98HAH33 strain of *S. suis* 2, was used as a positive control template. The DNA template was replaced by double-distilled water for the negative control. Seven pairs of primers specific for the target genes were designed in this study(***Table 1***). The PCR assay was performed in PTC-225 thermocycler(MJ Research, INC., Watertown, MA). The PCR products were separated in a 1.2% agarose gel electrophoresis consisting of 5% gold view(SBS Genetech, Shanghai, China) in 0.5 × TBE buffer(90 mmol/L Tris, 90 mmol/L borate, 2.5 mmol/L EDTA(pH 8.0)) for 30 min at a constant voltage of 80 V, and then were visualized by Gel DocTM XR system(Bio-Rad, Milan, Italy). The 100 bp DNA Ladder (Huamei Biotech, Luoyang, China) and DL2000 DNA Marker(TAKARA, BIOTECHNOLOGY(DALIAN) CO., LTD, Dalian, China) were used as molecular size standards.

Table 1 **Nucleotide sequences of the PCR primers**

Gene	Primer sequences(5'-3')	Tm(℃)	Size(bp)
16sRNA	F: CAGTATTTACCGCATGGTAGATAT	60	319
	R: GTAAGATACCGTCAAGTGAGAA		
cps *2J*	F: GTTGAGTCCTTATACACCTGTT	60	460
	R: CAGAAAATTCATATTGTCCACC		
ef	F: GCTACGACGGCCTCAGAAATC	60	626
	R: TGGATCAACCACTGGTGTTAC		
mrp	F: GGTATACCTTGCTGGTACCGTTC	60	532
	R: AGTCTCTACAGCTGTAGCTGG		
sly	F: GCTTGACTTACGGGCCACAAG	50	1277
	R: CCACCATTCCCAAGCTAATCC		
ddp	F: CCTCCGCAATAAAGCAGC	54	156
	R: TGTGAGCCGTGGGTAAAG		
fbps	F: AAGGTTTGGGTCGGGATA	51	127
	R: AGAGCAGCATAGGATTTGT		

RESULTS

Based on the clinical features and epidemiological investigation, we suspected that the patient had bacte-

rial toxic shock syndrome(TSS), and we sought to identify the pathogenic bacteria. After one day cultivation, we observed enriched growth of isolates from blood samples of the affected patient. Colonies were small(1~2 mm diameter), greyish, and slightly mucoid. Strains produced variable types of haemolysis on sheep blood agar plates, but the majority produced narrow zones of haemolysis that became more complete and wider with prolonged incubation(***Fig. 3***). All of the typical features of *S. suis* were observed: positive biochemical reactions with lactose, trehalose, raffinose, starch, and glucogen and negative biochemical reactions with ribose, L-arabinose, mannitol, sorbitol, and inulin. Strains were determined as serotype 2 by using diagnostic antiserum against *S. suis* and confirmed by the coagglutination method. The identity of the isolate as *S. suis* serotype 2 was further verified by positive PCR for the genes coding for the 16s rRNA of *S. suis* and for cps 2*J*. PCR also showed the isolate to be positive for the virulence genes coding for MRP, EF, SLY, DPP IV, and FBPS(***Fig. 4***).

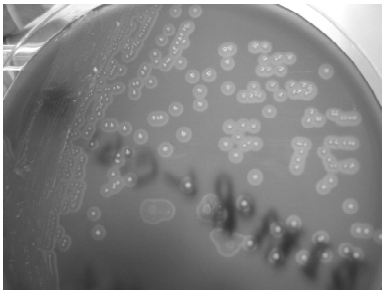
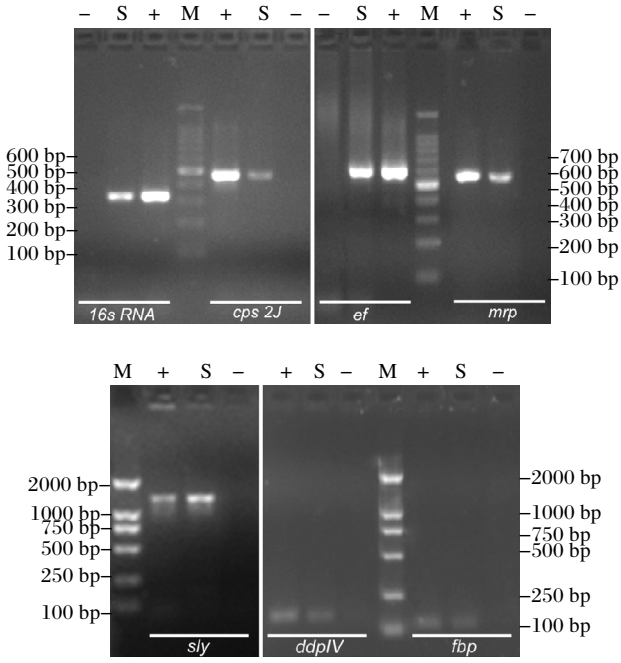


Fig. 3 Haemolytic colonies on agar after 48 hours of incubation at 37℃

DISCUSSION

S. suis is an increasingly important pathogen, causing meningitis, septicaemia, arthritis, and endocarditis in both pigs and human beings[3]. People in daily contact with pigs(such as butchers, abattoir workers, and pig farmers) are usually affected through the skin via a cut, infected wound or abrasion. In our case, the patient was exposed to pathogen in the contaminated water in the slaughterhouse when he killed his animal(sick or healthy carrier), because he had not protected himself by wearing boots. The portal of entry of the organism may be skin lesions in his feet caused by tinea pedis. As there is no *S. suis* vaccine for humans, suitable preventive measures and supervision of high risk population are critically important to decrease the transmission of *S. suis* to humans.

Usually infections due to *S. suis* have a favorable outcome with quick diagnosis and appropriate therapy.



M:marker; S:sample; "+":positive; "–":negative.

Fig. 4 PCR detection of the patient's blood sample with a set of unique primers specific for 16s rRNA, cps *2J*, ef, mrp, sly, ddpIV, and fbp of *S. suis*, respectively.

Delay in treatment will develop TSS and adversely affect the outcome. Despite of early diagnosis and prompt treatment when brought to medical attention, the patient died from STSS finally because of a personal delay in seeking treatment and not receiving the adequate therapy in the early stage. This case should remind us that increasing awareness of the disease within populations that are at high risk is also expected to help avoid human infections[15-16], early recognition of the disease and prompt seeking appropriate treatment is of crucial importance to increase survival.

In our case, *S. suis* was isolated from the blood culture, and the diagnosis of STSS was confirmed. The organism was typed as serotype 2. This pathogen was responsible for the majority of *S. suis* infections in humans in our country[16-17]. Five genes encoding known major virulence factors are likely to be involved in the pathogenesis of human infection: mrp, ef, sly, ddp IV, and fbps were present in the isolated strain, which was related to European strains(*mrp*+, *ef*+, *sly*+) that are considered to be more virulent than North American strains(*mrp*+, *ef*+, *sly*-)[18]. This may be the main underlying cause of the patient's rapid development to STSS and death. But further studies are urgently needed to elucidate the exact pathogenic mechanism as well as the pathophysiology of the disease.

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