Case Report

A human case of *Streptococcus suis* infection caused by an unencapsulated strain

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Introduction: *Streptococcus suis*, an emerging zoonotic pathogen, causes invasive infections in persons who are in close contact with infected pigs or contaminated pork-derived products. Although serotype 2 is the most prevalent type in *S. suis* infections in humans, to the best of our knowledge no human case caused by an unencapsulated strain has been reported previously.

Case presentation: A 53-year-old male alcohol misuser with liver cirrhosis was admitted with sepsis to a hospital in Sukhothai Province, Thailand. He had consumed a homemade raw pork product 3 days prior to the onset of illness. An isolate from blood culture was confirmed as *S. suis* by species-specific PCR and 16S rRNA gene sequencing, and as untypeable by a coagglutination test. The absence of a capsule around the bacterial cells was also confirmed by transmission electron microscopy. The isolate was also confirmed as sequence type 28 by sequence typing, and analysis of the capsule locus detected disruption of the *cpsE–cpsK* region, which comprises an approximately 2.5 kb fragment that contains phosphatase and kinase genes.

Conclusion: We have identified the first human *S. suis* infection caused by an unencapsulated strain in a patient with liver cirrhosis. This unencapsulated strain was attributable to a mutation of the *cps* gene. Clinicians should be aware of the emergence of *S. suis* infection caused by unencapsulated strains, especially in patients with liver cirrhosis.

Keywords: *cps* gene; electron microscopy; sepsis; serotype 2; *Streptococcus suis*; unencapsulated strain.

Introduction

*Streptococcus suis* is an important zoonotic pathogen in swine and humans, and causes sepsis and meningitis. Thirty-five serotypes have been identified based on their CPS, but most clinical isolates from human cases are serotype 2 strains (Gottschalk *et al*., 2010; Hill *et al*., 2005). However, human cases of serotypes 1, 4, 5, 14, 16 and 24 have also been reported in Southeast Asian countries (Kerdsin *et al*., 2009, 2011a, b; Nghia *et al*., 2008). A high incidence rate of *S. suis* infection has been reported in the general population of northern Thailand (Takeuchi *et al*., 2012).

Capsular polysaccharide (CPS) is considered a determinant of serotypes, but it is also an important virulence factor, and has anti-phagocytic effects against monocytes, neutrophils and dendritic cells (Benga *et al*., 2008; Fittipaldi *et al*., 2012; Smith *et al*., 1999a). To date, and to the best of our knowledge, no human case caused by unencapsulated *S. suis* has been reported. We describe here a case of sepsis caused by unencapsulated *S. suis* in a patient with liver cirrhosis. The capsule locus of the isolate

Abbreviations: CPS, capsular polysaccharide

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was analysed and the pathogenesis of this case caused by an unencapsulated strain is discussed.

Case report

In August 2011, a male alcohol misuser with liver cirrhosis, aged 53 years, was admitted to Srisangworn Hospital in Sukhothai Province, Thailand. On admission, he had headache, myalgia and fever with chills. He had no symptoms suggestive of a focal infection and no hearing loss. Three days prior to the onset of illness, he consumed a homemade raw pork product, called ‘Loo’. On the day of admission, his body temperature was 38.7 °C and his blood pressure was 106/55 mmHg. No neurological signs such as altered consciousness or nuchal rigidity were found. A haemogram suggested bacterial infection, based on an elevated white cell count (22.4 × 10^3 cells μl^{-1}) with 89.5 % neutrophils (20 × 10^3 cells μl^{-1}). Biochemical tests detected an elevated creatinine concentration (1.9 mg dl^{-1}) in the blood, with a blood urea nitrogen level of 16 mg dl^{-1}, aspartate aminotransferase of 32 international units (IU) l^{-1} and alanine aminotransferase of 33 IU l^{-1}. Immediately after the patient was diagnosed with sepsis, he was treated empirically with ceftriaxone (1.0 g at 6 h intervals). The patient was discharged on the fifth day of hospitalization.

Blood culture of this patient was positive, and three colonies were isolated for further characterization. The biochemical tests for three colonies demonstrated an identical result showing Voges–Proskauer (−), arginine (+), aesculin (+), 6.5 % NaCl (−), trehalose (+), mannitol (−), sorbitol (−) and bile aesculin (−) tests, and this result suggested S. suis (Hommmez et al., 1986). This isolate was sent to the National Institute of Health in Thailand. A multiplex PCR to identify S. suis and 15 serotypes (including serotypes 2 and 1/2) was done for this isolate (Kerdsin et al., 2012). The isolate was positive for S. suis species-specific tests but negative for all tested serotypes. Sequencing of the 16S rRNA gene confirmed that the strain belonged to the species S. suis (99 % nucleotide identity compared with GenBank accession nos NR036918, AF009487 and AM946016) (Brousseau et al., 2001). The isolate was untypeable by a coagglutination test using serotype-specific rabbit polyclonal antisera for all 35 described serotypes (Gottschalk et al., 1993).

Absorption to n-hexadecane was measured to evaluate the cell-surface hydrophobicity, which is an indirect indicator of the presence of a surface capsule (Bonifait et al., 2010). The isolate had a high hydrophobicity (89 %), which suggested the absence of CPS. Further analysis of the clinical isolate by transmission electron microscopy using ferritin to stabilize the capsule confirmed the almost complete absence of capsular material around the bacterial cells (Fig. 1a), unlike strain P1/7 of S. suis serotype 2, used as positive control (Lakkitjaroen et al., 2011), which was clearly surrounded by a thick capsule (Fig. 1b).

We next characterized the cps locus of this isolate by PCR and DNA sequencing of all PCR products, as described previously (Lakkitjaroen et al., 2011), and confirmed the homology of the cpsA–cpsD and cpsL–cpsT sequences in this strain to serotype 2 and 1/2 cps sequences in GenBank (99 % nucleotide identity against GenBank accession nos BR001000, CP003736, CP000837 and JF273645). However, the cpsE–cpsK region (~7.4 kb) could not be amplified using the primer pairs in PCR5–12 (Lakkitjaroen et al., 2011), which explains the lack of positive reactions when multiplex PCR was used for the serotyping of this strain (Kerdsin et al., 2012). Interestingly, we detected an amplified product from the cpsE–cpsK region of ~3.4 kb using the primer pair cps2-F5 and cps2-R12 (Lakkitjaroen et al., 2011). Sequencing and analysis of this fragment (3458 bp) detected three sequence components: (i) a 565 bp partial sequence of the galactosyl transferase gene (cpsE); (ii) 2523 bp of a serine/threonine protein kinase gene and a partial protein phosphatase gene, which were identical to genes in S. suis serotype 2 strains in GenBank, including strain P1/7; and (iii) 370 bp of a partial glycosyl transferase gene (cpsK). Compared with a previous report (Lakkitjaroen et al., 2011), this mutation is a new type of structural alteration in the cps loci of unencapsulated S. suis isolates (Fig. 2).

Discussion

In this study, we identified the first human case, to the best of our knowledge, of S. suis infection caused by an unencapsulated strain, which was associated with a mutation that disrupted an ~2.5 kb fragment in the cpsE–cpsK region. A recent study reported that the cpsE–cpsK region is characteristic of both serotypes 2 and 1/2, and that the genes of the cps locus in serotypes 2 and 1/2 are almost identical (Okura et al., 2013; Smith et al., 1999b). Furthermore, the cpsE–cpsK region appears to be replaced by genes originally found in other genomc loci of S. suis. Therefore, it is impossible to determine the genetic backbone of this strain with a disruption of the cpsE–cpsK region.

The patient suffered from sepsis caused by this strain of S. suis; he was an alcohol misuser with liver cirrhosis and...
he had consumed a raw pork product 3 days prior to the onset of illness. In a previous study, we also reported similar patient conditions that led to the isolation of atypical \textit{S. suis} serotypes 5 and 24 for the first time from humans (Kerdsin \textit{et al}., 2011b). Sepsis or spontaneous bacterial peritonitis has been identified as possibly occurring via bacterial translocation of \textit{S. suis} after the consumption of raw pork products by patients with liver cirrhosis in Southeast Asia. As we have already identified 700 patients with invasive infection whose culture results were positive for \textit{S. suis} between 2006 and 2011 in Thailand (A. Kerdsin, Y. Akeda & K. Oishi, unpublished data), the isolation rate of the unencapsulated strain is still low in this country (0.14 %).

We cannot completely dismiss the possibility of a loss of capsule production during culture. The isolate in this case was subcultured only four times before it was analysed. The CPS of \textit{S. suis} is considered to be highly stable during \textit{in vitro} culture. In fact, several passages in the presence of hyperimmune serum against CPS are needed to induce an unencapsulated strain (Gottschalk \textit{et al}., 1992). In addition, the reference strain of serotype 2 (S735), isolated in 1963, has been cultured for more than 50 years without losing its capsular expression (de Moore, 1963).

A recent study of cps2J-positive \textit{S. suis} isolates from pigs in Japan reported that 34 \% of 256 isolates from pigs with endocarditis were also unencapsulated, whereas all 32 isolates from pigs with meningitis were encapsulated (Lakkitjaroen \textit{et al}., 2011). The adherence to porcine platelets of the unencapsulated strain isolated from pigs with endocarditis was also significantly greater than that of the encapsulated serotype 2 strain P1/7 (Lakkitjaroen \textit{et al}., 2011). These results indicate that the loss of capsule production may be advantageous for \textit{S. suis} during the development of endocarditis. A previous study also reported a high level of epithelial cell adhesion and invasion by unencapsulated strains (Benga \textit{et al}., 2004). Loss of the capsule may expose the adhesins on the bacterial surface (Fittipaldi \textit{et al}., 2012), thereby increasing bacterial adhesion to platelets and epithelial cells.

Previous studies have also reported that an unencapsulated strain was associated with increased biofilm formation (Bonifait \textit{et al}., 2010; Tanabe \textit{et al}., 2010). The formation of biofilms may allow bacteria to become persistent colonizers and resist clearance by the host immune system and antibiotics. In addition, unencapsulated \textit{S. suis} induced a higher inflammatory response in macrophages resulting in an increased secretion of cytokine such as TNF-α, IL-1β, IL-6 and IL-8 (Segura \textit{et al}., 2006; Tanabe \textit{et al}., 2010). Collectively, the loss of capsule may exaggerate the immunological response induced by cell-wall components, leading to uncontrolled inflammatory reactions that may promote the development of a severe disease outcome. Moreover, alcohol abuse and cirrhosis may have contributed to the impaired neutrophil function in this patient (Shawcross \textit{et al}., 2008; Tritto \textit{et al}., 2011). These observations may explain why the unencapsulated strain was able to survive in the blood circulation.

In conclusion, we have detected the first human case of \textit{S. suis} infection caused by an unencapsulated strain in Thailand. Although the isolation rate for this type of strain is still very low, clinicians should be aware of the emergence of \textit{S. suis} infections caused by unencapsulated as well as uncommon serotype strains, especially in patients with liver cirrhosis (Kerdsin \textit{et al}., 2011b). Finally, the use of PCR targeted directly at \textit{cps} genes for serotyping from clinical isolates could miss this type of strain, as well as strains belonging to other serotypes. Thus, PCR that identifies \textit{S. suis} species would be more effective (Gottschalk \textit{et al}., 2010; Kerdsin \textit{et al}., 2011b).

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