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Short communication

Genomic epidemiological investigation of a *Streptococcus suis* outbreak in Guangxi, China, 2016

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ABSTRACT

In June 2016, a *Streptococcus suis* outbreak occurred in Guangxi, China. We determined the genetic characteristics of six clinically isolated strains by serotyping, PCR, and whole-genome sequencing, performing genome epidemiology analysis on these and 961 public available *S. suis* genomes. We also classified the first sequence type ST665 human case. Sporadic and outbreak cases were distinguished by whole-genome sequencing and phylogenomics. This approach could help to prevent and control *S. suis* epidemics in Guangxi and the wider region.

1. Introduction

Streptococcus suis, an important emerging zoonotic pathogen, can cause serious diseases in humans including acute meningitis, septicemia, and arthritis(Goyette-Desjardins et al., 2014). In recent decades, human *S. suis* infections have gained attention because of their high prevalence in China and Southeast Asia(Du et al., 2017). In China, two large *S. suis* infection outbreaks in 1998 and 2005, which were caused by a sequence type 7(ST7) clone, resulted in > 200 human infection cases with a fatality rate of nearly 20%(Du et al., 2017). In Vietnam, *S. suis* is the main cause of acute bacterial meningitis in adults(Mai et al., 2008) and has also been frequently detected in patients with encephalitis-meningitis syndrome in Guangxi Province, located on the Chinese–Vietnamese border(Dong et al., 2014). However, the phylogenetic position of the Guangxi isolates in the whole species tree and the evolutionary relationships among these strains are unknown. Thus, we collected six *S. suis* isolates from clinical patients during a *S. suis* outbreak, which resulted in two deaths in Guangxi in June 2016. We performed whole-genome sequencing of the six strains and rebuilt their phylogeny with 961 publicly available *S. suis* genomes to gain insights into their phylogenetic diversity and transmission patterns.

2. Results

2.1. Clinical and epidemiological features of the patients

Six patients, coded as C2227–C2232, were reported to be infected with *S. suis* in two Guangxi Province cities from June 2 to 13, 2016 (Fig. 1). All patients experienced high fever, vomiting and headache, and were treated with ceftriaxone and penicillin (Table 1). Patient C2227 was diagnosed with toxic shock-like syndrome and died two days after treatment. Patient C2231 was diagnosed with septicemia, swelling and pain in the right knee, leading to difficulty walking. The remaining four patients were diagnosed with meningitis and recovered from disease. According to our epidemiological investigation, patient C2227 had fed three pigs before the onset of the disease; patients C2228, C2229, C2230 all slaughtered pigs;, patient C2231 dealt with raw pork and ate pig tongue and bone one day before the disease onset. C2232 had no record of direct contact with pigs but > 200 pigs were fed in her village. All patients had trauma to their fingers or knees, indicating a route of infection. We suspect that their diseases were likely caused by exposure to diseased pigs. People who were in close contact with the six patients diagnosed with *S. suis* infections showed no clinical symptoms, hence in this outbreak, there was no obvious

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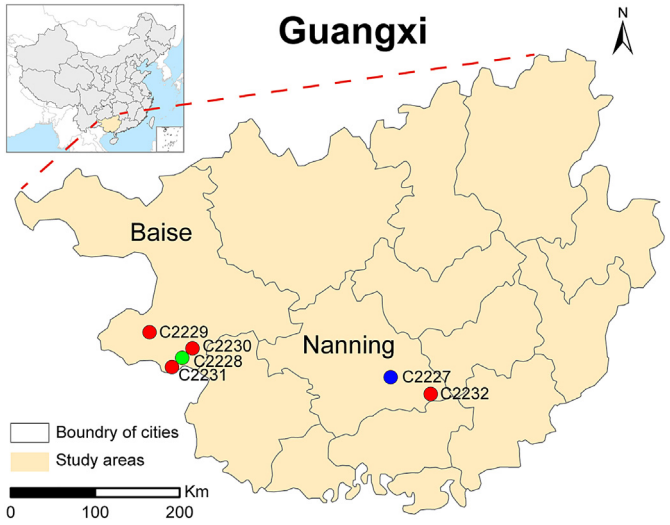

Fig. 1. Geographical distribution of the six strains. Isolates C2228 to 31 were from Jingxi, Guangxi. C2227 and C2232 were isolated from Hengxian, Guangxi. Genetically, Genetically, strain C2227 and C2228 belonged to distinct lineages compared with the other four strains, and therefore are indicated in blue and green, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Clinical characteristics of the six patients infected with *Streptococcus suis*.

Characteristic	C2227	C2228	C2229	C2230	C2231	C2232
Sex	Male	Male	Male	Male	Female	Female
Age	53 y	71 y	64 y	53 y	65 y	56 y
Temperature, °C	39.5	38.8	38.3	38.4	NA	38.8
WBC, 10^9/L	6.6	12.44	26.43	16.94	NA	21.3
Pulse rate, beats/min	126	66	102	86	NA	NA
Respiratory rate, breaths/min	36	22	25	23	NA	NA
Blood pressure, mmHg	77/55	150/90	159/76	110/61	NA	NA
Outcome	Died	Survived	Survived	Survived	Survived	Survived

*NA: not available. WBC, white blood cell.

evidence of human to human transmission. 2.2 Sequence analysis and antibiotic resistance of the six strains.

S. suis was isolated from blood cultures, PCR-identified and named according to the patient code. We tested the antimicrobial susceptibility of the six isolates, and the minimum inhibitory concentration values of ampicillin, ceftriaxone, tetracyclines, erythromycin, ciprofloxacin and gentamycin were measured by *E*-test (Table 2). Surprisingly, the C2228 isolate had acquired resistance to erythromycin and we also found the ErmB gene in the C2228 genome by Blast. All six isolates were confirmed as serotype 2 via the serotype-specific PCR assay developed by Yukihiro Akeda(Kerdsin et al., 2014), consistent with the fact that

Table 2
MIC value (μg/mL) for six antibiotics against the six strains.

Strain	AMP	CEF	TET	ERY	CIP	GEN
C2227	0.016	0.125	32	0.125	0.75	1.5
C2228	< 0.016	0.125	128	> 256	3	4
C2229	0.023	0.094	32	0.125	2	3
C2230	0.023	0.064	64	0.125	1	2
C2231	0.032	0.125	32	0.19	1.5	4
C2232	0.032	0.094	32	16	1.5	2

AMP, ampicillin; CEF, ceftriaxone; TET, tetracyclines; ERY, erythromycin; CIP, ciprofloxacin; GEN- gentamycin.

human *S. suis* cases are mainly associated with serotypes 2 and 14(Zhou et al., 2017). Ten virulence genes (SalK, SalR, Fhb, IgA1, EF, MRP, Sly, SsnA, EndA and ScpA) linked with immune evasion or systemic infections(Zhou et al., 2017) were present in all six assembled genomes, except for salK/R, which was absent from the C2230 isolate. All of the outbreak strains from the human cases harbored these virulence factors, suggesting that all six strains are pathogenic. The sequences of seven housekeeping genes (aroA, cpn, dpr, gki, muts, recA and thrA) were extracted separately from the assembled genomes of the six isolates, and the allele sequences were uploaded to the Multilocus Sequence Typing website (http://www.mlst.net/) to confirm their sequence types (STs) based on MLST methodology(King et al., 2002). We also identified the sequence type of 961 strains published on the NCBI website, (https://www.ncbi.nlm.nih.gov/genome/genomes/199) using MLST software(Page et al., 2017), and defined the clonal complex 1–4 according to eBURST (http://eburst.mlst.net/v3/mlst_datasets/). While all six isolates in this study were located within clonal complex 1 (CC1) (Fig. 2A), five of them belonged to ST1 and one, C2228, belonged to ST665. C2228 was isolated from a 71-year-old male who was diagnosed with septic meningitis and had fever, nausea and vomiting. ST665 and ST1 differ in the allele profile of the gki gene, expressing gki189 and gki1, respectively. The only documented ST665 strain until now is the one isolated from the tonsils of a healthy pig (http://ssuis.mlst.net/sql/fulldetails.asp?id=1471). Our findings therefore confirm that ST665 can also infect humans. ST1 is the most common ST responsible for human *S. suis* cases and is also predominately isolated from pigs. Of the 967 recorded strains, 604 belong to ST1, including the present five strains isolated in Guangxi. However, the STs identified in this study are different from the STs that led to previously documented *S. suis* outbreaks in China (Ye et al., 2006), suggesting no epidemic association among them.

2.2. Phylogenetic analysis of the six isolates

To investigate the phylogenetic relationships between the six strains, we performed whole-genome sequencing (WGS) and phylogenomic analysis combined with 961 published genomes. WGS was performed on the Illumina HiSeq4000 platform, producing 2× 150 bp paired-end reads. We assembled the trimmed reads using SOAPdenovo (Li et al., 2010). The six assembled genomes were 2.0–2.1 Mb long with 41% GC content and were deposited in the GenBank database (accession no. PQGM00000000-PQGR00000000). A neighbor-joining (NJ) tree of 967 isolates was constructed by using whole genome wide SNPs identified as previously described(Cui et al., 2013), based on the software TreeBeST(Vilella et al., 2009) and visualized by using iTOL (Letunic and Bork, 2016). We found that the six isolates were located in the same clonal clade, which contained 565 genomes including the representative high virulence strains 05ZYH33, SC84, 98HAH33, BM407 and P1/7 (Fig. 2A). This result is consistent with the conclusion that high virulence strains always appear in one clade, as reported previously(Chen et al., 2013). To further investigate the relationships between the six newly isolated strains, we constructed a NJ tree based on the SNPs within the 565 genomes in the clonal clade containing the six Guangxi strains (Fig. 2B). We found that the six strains were divided into three sub-clades. C2229 to C2232 were closely related to each other, whereas C2227 and C2228 were located in two distinct subclades. Interesting, the six strains did not cluster together with the common Chinese clade containing the known epidemic strains 98HAH33, 05ZYH33 and SC84. The Guangxi strains appeared to be more closely related to the strains isolated from Vietnam than from elsewhere, suggesting a geographically-clustered *S. suis* population present in the region covering Vietnam and Guangxi province.

According to the geographical distribution of the six isolates (Fig. 1), we can infer that C2228, C2229, C2230 and C2231 are descended from a common source and are possibly more closely related to each other epidemiologically than to the C2227 and C2232 strains.

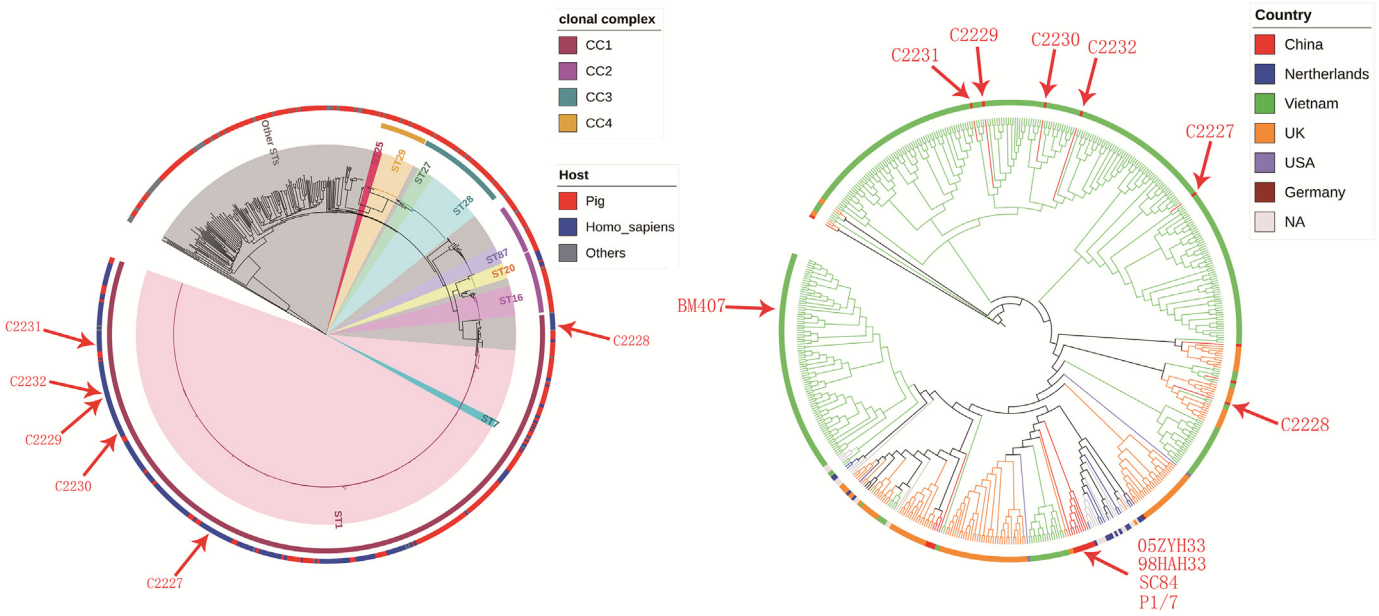

Fig. 2. NJ tree for the 967 *S. suis* isolates (A) and the 565 clonal clade isolates (B) We aligned each genome to the BM407 reference genome using the MUMmer package (Delcher et al., 2002). In total, 68,183 SNPs were extracted from all 967 genomes and 35,927 SNPs were extracted from the 565 genomes of the clonal clade to construct the NJ tree.

These phylogenetic analyses have provided us with additional insight into the relationships between the six strains. Strains C2227 and C2228 are located in different sub-clades from the other four strains and are genetically distinct, indicating that they are more likely to represent sporadic cases, rather than an outbreak. Although strain C2232 is geographically distinct from C2229–31 strains, the fact that both belong to the same sub-clade indicates that they might come from the same outbreak and that strain C2232 seems to have spread a relatively long distance from the border between Vietnam and China to inland China.

3. Conclusion

We have described the characteristics of *S. suis* isolates from two Guangxi Province counties in terms of the clinical cases and the bacterial genotypes and phylogenies. We have provided evidence that *S. suis* ST665 can infect humans and have distinguished the sporadic cases from the disease outbreak cases according to the phylogenetic relationships between the isolated strains. This study supports the notion that combining genomic and clinical epidemiological data with phylogenomics is a robust way to source-trace pathogens, with strong potential as a strategy for preventing and controlling epidemics involving *S. suis*.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2018.12.023.

Declarations of interest

None.

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