Spread of Streptococcus suis Sequence Type 7, China

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Streptococcus suis sequence type (ST) 7 has been spreading throughout China. To determine events associated with its emergence, we tested 114 isolates. In all 106 ST7 strains responsible for human outbreaks and sporadic infections, the tetracycline-resistance gene, tetM, was detected on the conjugative transposon Tn916. Horizontal transmission of tetM is suspected.

A large outbreak of Streptococcus suis serotype 2 infection emerged in the summer of 2005 in Sichuan Province, People’s Republic of China, and resulted in 215 cases and 38 deaths among humans (1). Sporadic infections were identified in 4 other provinces. A smaller, previously overlooked, outbreak occurred in Jiangsu Province in 1998; 25 cases and 14 deaths were reported (1,2). The causative agent of the Sichuan and Jiangsu outbreaks was identified as a clone of S. suis sequence type (ST) 7 (3). ST7 was first identified in 1996 in a patient with meningitis in Hong Kong and later caused the 1998 outbreak in Jiangsu; it spread further to cause the largest outbreak in Sichuan in 2005 (3,4). The spread of S. suis ST7 across China underscores the need to better understand the genetic and ecologic events associated with its emergence as an important pathogen in humans.

The Study

Using the MICroSTREP Plus system (Dade Behring, Deerfield, IL, USA), we tested 114 ST7 isolates from China and found that all isolates were resistant to tetracycline and susceptible to 12 of 13 antimicrobial drugs. Of these 114, 6 were isolated in 2006, 84 were from human patients and 8 from diseased pigs in the 2005 Sichuan outbreak, 7 were from sporadic human cases and 3 from diseased pigs in other provinces in 2005, and 4 were from human patients and 2 from diseased pigs in the 1998 Jiangsu outbreak (Table 1). The isolates were susceptible to penicillin, ampicillin, cefotaxin, cefixime, ceftriaxone, ceferone, levofloxacin, chloramphenicol, erythromycin, azithromycin, clindamycin, and vancomycin. In contrast, 7 of 12 S. suis serotype 2 strains from other countries and 18 of 34 serotype reference strains were resistant to tetracycline; 3 tetracycline-resistant strains were also resistant to erythromycin, azithromycin, and clindamycin.

Multilocus sequence typing analysis showed that of the 114 isolates from China, 106 were typed as ST7: 98 from the Sichuan and Jiangsu outbreaks, 5 from sporadic infections in other provinces in 2005 and 2006, and 3 from diseased pigs from other provinces in 2005. Of the other 8 isolates from sporadic cases in 2005 and 2006, 7 were ST1 and 1 was untypeable. Of the 12 serotype 2 strains from other countries, 8 were ST1 and 4 were ST25. Of the 34 serotype reference strains, serotype 2 strain R735 was ST1, 10 serotypes were untypeable, and 22 STs were identified as ST6 (serotypes 17 and 19), ST35, ST35-55, ST68-73, ST75-82, ST87, or ST91-2. Serotype 17 and 19 strains were identified as ST76 (Table 1) (5,6).

PCR was used to screen all isolates for tetracycline resistance genes; primers specific for tetABCDEGKLMQS were used (7,8). Of the 114 tetracycline-resistant isolates from China, 111 (all 106 ST7 strains and 5 of 7 ST1 strains) harbored the tetM gene. The tetO gene was carried by 1 ST1 and 1 sequence-untypeable strain. All 7 tetracycline-resistant serotype 2 strains from other countries and 16 of 18 tetracycline-resistant strains in the 34 reference serotypes carried the tetO gene (Table 1) (5,6). The only other tetM-positive strain was from serotype 13, an ST71 isolated from a diseased pig in Denmark (Table 1). The PCR results were confirmed by sequencing the PCR-synthesized fragments.

To further characterize the tetM genes, the open reading frame (ORF) was completely sequenced by using 16 selected strains: 9 isolates from humans and 1 from a pig from the Sichuan outbreak; 3 from sporadic infections in Guangxi, Jiangsu, and Guangdong; 1 from a diseased pig in Jiangxi Province in 2005; and 2 from the Jiangsu outbreak in 1998 (Table 1). Sequence alignments showed 2 groups (GenBank accession nos. EF101931, EF016118). The first group comprised 15 of the 16 isolates typed as ST7 (3). The second group had only 1 isolate, GX1, typed as ST1 (3). The sequences of tetM gene for ST7 (strain SC84) and ST1 (GX1) were 1,920 and 1,917 bp, respectively, with 90 nt variations between the 2 sequences leading to 32 aa changes. Comparison of the 53 tetM sequences with those from public databases showed that the tetM of S. suis SC84 was most related to Enterococcus faecium isolate 9830470-4 plasmid pYA470-4 (DQ223243) (7). The tetM sequence

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of *S. suis* ST1 strain GX1 was most closely related to *S. pneumoniae* Tn916-like/Tn2009 (AY466395) and to *Gardnerella vaginalis* (U58986) (Figure 1) (9).

Because the gene tetM is reported to be associated with transposon Tn916, we designed 24 pairs of primers targeting its 24 ORFs based on published Tn916 sequences (Table 2). The complete sequence of Tn916 from SC84 was obtained by sequencing the PCR-synthesized fragments. Between Tn916 of SC84 and plasmid pYA470-4 of *E. faecium*, we observed 133 nt variations, 91 of which were in the tetM gene, 3 in the integrase gene, 1 in excisionase gene, and 38 in 10 additional ORFs. PCR showed that 111 isolates from China and 1 from Denmark have intact Tn916 (Table 1).

The most recognized virulence genes of *S. suis*, including *mrp, sly, and ef*, were detected by PCR in all 114 Chinese isolates tested in this study. Of the 12 serotype 2 strains from other countries, 6 of 8 ST1 strains were posi-

| Table 1. Source, serotype, sequence type, and tetracycline-resistant genes in *Streptococcus suis* strains* |
|---|---|---|---|---|---|---|
| No. strains | Source (no.) | Place of isolation (no.) | Year of isolation (no.) | Serotype (no.) | ST (no.) | tet gene (no. positive) | Tn916 (no.) | Virulence genes (no.) |
| 98 outbreak-associated ST7 strains in China | 84 Human patients | Sichuan, China | 2005 | 2 | ST7 | tetM (84) | Intact (84) | + (84) + (84) + (84) |
| 8 Diseased pigs | Sichuan, China | 2005 | 2 | ST7 | tetM (8) | Intact (8) | + (8) + (8) + (8) |
| 4 Human patients | Jiangsu, China | 1998 | 4 | ST7 | tetM (4) | Intact (4) | + (4) + (4) + (4) |
| 2 Diseased pigs | Jiangsu, China | 1998 | 2 | ST7 | tetM (2) | Intact (2) | + (2) + (2) + (2) |
| 8 ST7 strains isolated from sporadic cases in China | 5 Human patients | 6 provinces, China | 2005 (2) | 2 | ST7 | tetM (5) | Intact (5) | + (5) + (5) + (5) |
| 3 Diseased pigs | Jiangxi, China | 2006 (3) | 2 | ST7 | tetM (3) | Intact (3) | + (3) + (3) + (3) |
| 7 ST1 and 1 untypeable strain isolated from sporadic cases in China | 5 Human patients | Guizhou, Guangxi (4) | 2005 | 2 (4) | ST1 | tetO (4) | Intact (4) | + (4) + (5) + (5) |
| 3 Human patients | 3 provinces | 2006 | 2 | ST1 (2) UT | tetO (2) tetM | Intact (1) | + (3) + (3) + (3) |
| 12 serotype 2 strains from other countries | 5 Human patients | Netherlands (2), France (3) | NA | 2 | ST1 | tetO (3) | Intact (3) | + (5) + (5) + (5) |
| 3 Diseased pigs | Netherlands, France, England | NA | 2 | ST1 | tetO (3) | Intact (3) | + (5) + (5) + (5) |
| 4 Human patients (2), healthy pigs, diseased pigs | Canada (3), England | NA | 2 | ST25 | tetO (4) | Intact (4) | + (4) – – – |
| 34 serotype reference strains | 1 Human patients | Netherlands | NA | 14 | ST6 | tetO (3) | Intact (3) | – – + – |
| 1 Diseased pig | Denmark | NA | 13 | ST71 | tetM | Intact (1) | – – + – |
| 25 Diseased pigs | Denmark (11), Denmark (5), Netherlands (9) | NA | 1/2, 2–12, 15–16, 22–30, 32, 34 | ST1, ST35, ST53–55, ST68, ST69, ST72–73, ST75, ST77–78, ST80–82, ST87, ST91–92, UT (7) | tetO (11) | Intact (1) | + (2) + (2) + (2) + | + (1) |
| 2 Diseased calves | Canada, United States | NA | 20, 31 | ST70 (1), UT (1) | tetO (1) | Intact (1) | – – – – |
| 1 Diseased lamb | Canada | NA | 33 | UT | tetO (1) | Intact (1) | – – – – |
| 4 Healthy pigs | Canada | NA | 17–19, 21 | ST76 (2), ST79, UT | tetO (4) | Intact (4) | – – + (2) – |

*ST, sequence type; tet, tetracycline; UT, untypeable by multilocus sequence typing; NA, not available.
tive for all 3 virulence genes. However, none of the 4 ST25 strains tested positive (Table 1). Of the 34 serotype reference strains, serotype 2 strain R735 was positive for \textit{mrp} and \textit{sly}. The \textit{sly} gene was detected in 10 reference strains that were typed serotype 1/2 as untypeable, serotype 2 as ST1, serotype 4 as ST54, 5 as ST53, 7 as untypeable, 13 as ST71, 14 as ST6, 16 as ST73, 17 as ST76, and 18 as ST79 (Table 1).

**Table 2. Primers used to detect \textit{tet} genes and conjugative transposon \textit{Tn916} in \textit{Streptococcus suis}**

| Gene | Primers (5’→3’) | Product size, bp | Annealing temperature, °C |
|------|----------------|-----------------|--------------------------|
| \textit{tetA} | GCTACATCTGATGGACGAG; CATAGATCCCGGTGAAGGAGG | 210 | 55 |
| \textit{tetB} | TTTGTTAGGGGCAAGTTTTTGG; GTAATGGGCCAATAACCCG | 659 | 55 |
| \textit{tetC} | CTTGAGACCCCTCAACCCG; ATGGTCGTCATCTACCTGCC | 418 | 55 |
| \textit{tetD} | AAACCATCGCGATCATGCC; GACGGGACACCTAATCCATAC | 787 | 55 |
| \textit{tetE} | AAACACATCTCCATACG; AAAATGCGCAACCCGACG | 278 | 55 |
| \textit{tetG} | GTCTCGGTGTTATCTGCTCT; AGCAACAGAATCGGGAACAC | 468 | 55 |
| \textit{tetK} | TCGATAGAAGGACACAGA; CAGCAGATCTCAATCTCT | 169 | 55 |
| \textit{tetL} | TCGTATTGGTGTGCAATACC; GTATCCCGCTAATGACCC | 267 | 55 |
| \textit{tetM} | GTCGCTGGTGTTATCTGCTCT; AGCAACAGAATCGGGAACAC | 468 | 55 |
| \textit{tetO} | ACGTACATCCTGATGGACGAG; CATAGATCCCGGTGAAGGAGG | 210 | 55 |
| \textit{tetQ} | TTATCTTCCCTCCGCTGTTCTG; ATGGTCGTCATCTAATCC | 904 | 55 |
| \textit{tetS} | CATAGACAAAGCCTGTTGAC; ATGTCTTGGGAAGCCGACAG | 667 | 55 |
| ORF 24 | ATGAGGGTCATTTTTTTTAA; TTTGTTGGGCTAATGATGTT | 120 | 52 |
| ORF 23 | AATTGTGATTCACACAG; GTCGTCGCGCCTGAGCTG | 315 | 52 |
| ORF 22 | ATGTGAGTAGAAAG; ATGGTCGTCATCTAATCC | 387 | 52 |
| ORF 21 | TTATCTTCCCTCCGCTGTTCTG; ATGGTCGTCATCTAATCC | 904 | 55 |
| ORF 20 | ATGTCTTGGATTGATGAA; TTTATCTTCTTGGTTTATCA | 990 | 52 |
| ORF 19 | ATGACATGGTCTATTTTTTT; TTAATGTTGGGAACGCACG | 667 | 55 |
| ORF 18 | CATAGACAAAGCCTGTTGAC; ATGTCTTGGGAAGCCGACAG | 667 | 55 |
| ORF 17 | ATGAGGGTCATTTTTTTTAA; TTTGTTGGGCTAATGATGTT | 120 | 52 |
| ORF 16 | ATGAGGGTCATTTTTTTTAA; TTAATGTTGGGAACGCACG | 2,448 | 52 |
| ORF 15 | ATGAGGGTCATTTTTTTTAA; TTAATGTTGGGAACGCACG | 2,448 | 52 |
| ORF 14 | ATGAGGGTCATTTTTTTTAA; TTAATGTTGGGAACGCACG | 2,448 | 52 |
| ORF 13 | ATGAGGGTCATTTTTTTTAA; TTAATGTTGGGAACGCACG | 2,448 | 52 |
| ORF 12-\textit{tetM} | ATGAGGGTCATTTTTTTTAA; TTAATGTTGGGAACGCACG | 2,448 | 52 |
| ORF 11 | ATGAGGGTCATTTTTTTTAA; TTAATGTTGGGAACGCACG | 2,448 | 52 |
| Xis-Tn | ATGAGGGTCATTTTTTTTAA; TTAATGTTGGGAACGCACG | 2,448 | 52 |
| \textit{Int}-\textit{Tn} | ATGAGGGTCATTTTTTTTAA; TTAATGTTGGGAACGCACG | 2,448 | 52 |

*\textit{tet}, tetracycline; ORF, open reading frame.*
To determine the significance of horizontal gene transfer of Tn916 with the tetM and virulence genes, we constructed a rooted phylogenetic tree by using the maximum-parsimony method. The sequence of S. pneumoniae R6 was chosen as the outgroup that is closely related to S. suis (10). The data suggest S. suis ST7 evolved originally from ST1 and ST48. The horizontal transfer of tested virulence genes and tetM occurred in various stages of the evolution of S. suis and played a major role in the emergence of ST1 and ST7 (Figure 2).

Conclusions

We report that S. suis ST7 was responsible for 2 large outbreaks and sporadic infections in several provinces of China and has recently acquired the tetracycline resistance gene, tetM, associated with the conjugative transposon Tn916 (3, 7). Horizontal transfer of Tn916 with the tetM gene occurred in at least 3 STs located at various stages in the constructed phylogenetic tree and played a central role in the evolution of the epidemic S. suis ST7 clone. All 3 virulence genes tested in this study were shown to be transferred horizontally (11–13).

Our data support the contention that Tn916 with tetM acts as an important selective factor that provides considerable advantages for the clone emergence and spread of S. suis ST7 (3). The widespread use of tetracycline in swine feed could provide the selective pressure for clone amplification and spreading, thus contributing to the outbreak of S. suis ST7 through Tn916 (14). The countrywide spread of S. suis ST7-tetM represents a model of selective pressure leading to the emergence of a bacterium as a virulent pathogen in humans. The case of S. suis ST7 is a sign that pathogens present in food animals can result in substantial public health problems if no action is taken to prevent the indiscriminate use of antimicrobial drugs in animal feed (15).

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