



# Draft Genome Sequences of Three Porcine *Streptococcus suis* Isolates Which Differ in Their Susceptibility to Penicillin

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**ABSTRACT** The draft genome sequences of three *Streptococcus suis* isolates, IMT40343, IMT40201, and IMT40738, are presented here. These isolates were obtained from bronchoalveolar lavage fluid of healthy and diseased weaners from different German piglet-producing farms and differed in their susceptibility to penicillin.

*Streptococcus suis* strains are usually commensals colonizing the respiratory tract of pigs (1). However, they can cause meningitis, pneumonia, septicemia, arthritis, endocarditis, or abortion in pigs (1). Moreover, *S. suis* has been reported as a zoonotic pathogen, primarily in humans with occupational contact with pigs (2–4). To treat *S. suis* infections in pigs and humans,  $\beta$ -lactams are recommended. Unfortunately, reduced susceptibility of isolates against  $\beta$ -lactams, especially penicillins, has been reported during the last three decades (5–8).

The *S. suis* isolates IMT40343, IMT40201, and IMT40738 were obtained from bronchoalveolar lavage fluid of weaners originating from three different German piglet-producing farms. IMT40343 was isolated from a healthy weaner, whereas IMT40738 was from a weaner with respiratory tract disease. IMT40201 originated from a weaner that was cured of a respiratory tract infection and was isolated immediately after treatment with doxycycline. Aliquots of 100  $\mu$ l of the bronchoalveolar lavage fluid were plated onto Columbia sheep blood agar and incubated for 20 to 24 h at 37°C under aerobic and microaerophilic conditions. Colonies suspected to be *S. suis* were confirmed by a species-specific PCR and matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS) as described earlier (9). MIC determination, according to CLSI standards, identified IMT40343 as penicillin susceptible (MIC,  $\leq 0.015$  mg/liter), IMT40201 as penicillin intermediate (0.5 mg/liter), and IMT40738 as penicillin resistant (2 mg/liter) (10). The whole-genome sequences (WGSs) of IMT40343, IMT40201, and IMT40738 were determined and analyzed for point mutations in the genes for penicillin-binding proteins (PBPs) that might account for elevated penicillin MICs. The three *S. suis* isolates were grown for 20 to 24 h at 37°C in brain heart infusion broth under microaerophilic conditions prior to DNA extraction with the QIAamp DNA mini kit (Qiagen, Hilden, Germany). The libraries were prepared using a Nextera XT library preparation kit (Illumina, Inc., USA) according to the manufacturer's recommendations. The 2  $\times$  300-bp paired-end sequencing in 30-fold multiplexes was performed on the MiSeq (Illumina, Inc.) platform. Genome sequences were *de novo* assembled using the Mimicking Intelligent Read Assembler (MIRA) v4.0 (Biomatters Ltd., New

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**TABLE 1** Characteristics of the draft genome sequences of the three *S. suis* isolates

Strain (IMT no.)	NCBI reference sequence no.	BioSample no.	Genome size (bp)	No. of contigs	$N_{50}$ length (bp)
IMT40201	NZ_RRZQ00000000	SAMN10440285	2,344,245	66	179,911
IMT40738	NZ_RRZO00000000	SAMN10440287	2,458,255	192	34,794
IMT40343	NZ_RRZP00000000	SAMN10440286	2,254,559	42	155,466

Zealand) with default settings as a plugin within Geneious v10.1.3 (Biomatters Ltd.) (Table 1). The coverage of the sequences was approximately 50-fold.

The total genome size of IMT40343 was 2,254,559 bp, and that of IMT40201 was 2,344,245 bp, while IMT40738 had a size of 2,458,255 bp. The G+C contents ranged from 41.0 to 41.5%. Gene annotation was performed via the Rapid Annotations using Subsystems Technology (RAST) server (11). For IMT40343 and IMT40201, RAST identified 2,316 and 2,338 coding DNA sequences, with 10 and 13 rRNAs and 48 and 51 tRNAs, respectively. For IMT40738, RAST annotated 2,536 coding sequences with 8 rRNAs and 54 tRNAs. The Geneious BLAST tool identified class A (PBP2x and PBP2b) and class B (PBP1a, PBP2a, and PBP1b) PBPs with identity thresholds between 94 and 100% (12). Analyses of the PBPs revealed an accumulation of amino acid exchanges in PBP2x and PBP2b of IMT40738, followed by that in IMT40201. However, no amino acid alterations within the conserved motifs were detected in all three sequences. Based on the detection of the respective loci in the draft genome sequences, isolates IMT40343 and IMT40201 were classified as serotypes 8 and 21, respectively. However, IMT40738 was not serotypeable, as it harbored a novel *cps* locus (13–15). IMT40343 was assigned to sequence type 308 (ST308) using the multilocus sequence typing (MLST) service of the Center for Genomic Epidemiology (16). In contrast, IMT40201 and IMT40738 were assigned to new STs 1097 and 1098, respectively, by the pubMLST service. No acquired antimicrobial resistance genes were found in IMT40343 using ResFinder, while in IMT40201, the resistance genes *mef(A)*, *msr(D)*, and *tet(O)* were found. In IMT40738, the resistance genes *erm(B)* and *tet(O)/W/32/O* were detected, in addition to *mef(A)* and *msr(D)* (17).

**Data availability.** The WGSs of the isolates IMT40343, IMT40201, and IMT40738 have been deposited with BioProject number [PRJNA505967](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA505967) at DDBJ/ENA/GenBank under the accession numbers [RRZP00000000](https://www.ncbi.nlm.nih.gov/nuccore/RRZP00000000), [RRZQ00000000](https://www.ncbi.nlm.nih.gov/nuccore/RRZQ00000000), and [RRZO00000000](https://www.ncbi.nlm.nih.gov/nuccore/RRZO00000000), respectively. The raw data are available as SRA data with the numbers [SAMN10440285](https://www.ncbi.nlm.nih.gov/sra/SAMN10440285) (IMT40201), [SAMN10440286](https://www.ncbi.nlm.nih.gov/sra/SAMN10440286) (IMT40343), and [SAMN10440287](https://www.ncbi.nlm.nih.gov/sra/SAMN10440287) (IMT40738).

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## REFERENCES

- Staats JJ, Feder I, Okwumabua O, Chengappa MM. 1997. *Streptococcus suis*: past and present. *Vet Res Commun* 21:381–407. <https://doi.org/10.1023/A:1005870317757>.
- Gottschalk M, Segura M, Xu J. 2007. *Streptococcus suis* infections in humans: the Chinese experience and the situation in North America. *Anim Health Res Rev* 8:29–45. <https://doi.org/10.1017/S1466252307001247>.
- Arends JP, Zanen HC. 1988. Meningitis caused by *Streptococcus suis* in humans. *Rev Infect Dis* 10:131–137. <https://doi.org/10.1093/clinids/10.1.131>.
- Wertheim HF, Nghia HD, Taylor W, Schultz C. 2009. *Streptococcus suis*: an emerging human pathogen. *Clin Infect Dis* 48:617–625. <https://doi.org/10.1086/596763>.
- Gottschalk M, Turgeon P, Higgins R, Beaudoin M, Bourgault AM. 1991. Susceptibility of *Streptococcus suis* to penicillin. *J Vet Diagn Invest* 3:170–172. <https://doi.org/10.1177/104063879100300214>.
- Turgeon PL, Higgins R, Gottschalk M, Beaudoin M. 1994. Antimicrobial susceptibility of *Streptococcus suis* isolates. *Br Vet J* 150:263–269. [https://doi.org/10.1016/S0007-1935\(05\)80006-5](https://doi.org/10.1016/S0007-1935(05)80006-5).
- Zhang C, Ning Y, Zhang Z, Song L, Qiu H, Gao H. 2008. *In vitro* antimicrobial susceptibility of *Streptococcus suis* strains isolated from clinically healthy sows in China. *Vet Microbiol* 131:386–392. <https://doi.org/10.1016/j.vetmic.2008.04.005>.
- Hernandez-Garcia J, Wang J, Restif O, Holmes MA, Mather AE, Weinert LA, Wileman TM, Thomson JR, Langford PR, Wren BW, Rycroft A, Maskell DJ, Tucker AW, BRADP1T Consortium. 2017. Patterns of antimicrobial resistance in *Streptococcus suis* isolates from pigs with or without streptococcal disease in England between 2009 and 2014. *Vet Microbiol* 207:117–124. <https://doi.org/10.1016/j.vetmic.2017.06.002>.
- Niemann L, Müller P, Brauns J, Nathaus R, Schäkel F, Kipschull K, Höltig D, Wendt M, Schwarz S, Kadlec K. 2018. Antimicrobial susceptibility and

- genetic relatedness of respiratory tract pathogens in weaner pigs over a 12-month period. *Vet Microbiol* 219:165–170. <https://doi.org/10.1016/j.vetmic.2018.03.030>.
10. CLSI. 2018. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 4th ed, CLSI supplement VET08. CLSI, Wayne, PA.
  11. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
  12. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. 2008. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev* 32:234–258. <https://doi.org/10.1111/j.1574-6976.2008.00105.x>.
  13. Zheng H, Ji S, Liu Z, Lan R, Huang Y, Bai X, Gottschalk M, Xu J. 2015. Eight novel capsular polysaccharide synthesis gene loci identified in nontypeable *Streptococcus suis* isolates. *Appl Environ Microbiol* 81:4111–4119. <https://doi.org/10.1128/AEM.00315-15>.
  14. Pan Z, Ma J, Dong W, Song W, Wang K, Lu C, Yao H. 2015. Novel variant serotype of *Streptococcus suis* isolated from piglets with meningitis. *Appl Environ Microbiol* 81:976–985. <https://doi.org/10.1128/AEM.02962-14>.
  15. Qiu X, Bai X, Lan R, Zheng H, Xu J. 2016. Novel capsular polysaccharide loci and new diagnostic tools for high-throughput capsular gene typing in *Streptococcus suis*. *Appl Environ Microbiol* 82:7102–7112. <https://doi.org/10.1128/AEM.02102-16>.
  16. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Ponten T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol* 50:1355–1361. <https://doi.org/10.1128/JCM.06094-11>.
  17. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <https://doi.org/10.1093/jac/dks261>.