Veterinary Microbiology 222 (2018) 109–113

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Emergence of a *vanG*-carrying and multidrug resistant ICE in zoonotic pathogen *Streptococccus suis*

Jinhu Huang[a], Li Chen[a], Daiwei Li[b,c], Mengli Wang[a], Fanshu Du[a], Yi Gao[a], Zuowei Wu[c,*], Liping Wang[a,*]

[a] College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China
[b] Department of Pharmacy, The Second Hospital of Dalian Medical University, Dalian 116027, China
[c] Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA 50011, USA

ARTICLE INFO

Keywords:
Vancomycin resistance
vanG-tpye locus
Streptococcus suis
ICEs

ABSTRACT

Vancomycin resistance occurs frequently in *Enterococcus* species, but has not yet been reported in *Streptococcus suis*, a previously neglected, newly emergent zoonotic pathogen. In this study, we tested the vancomycin susceptibility of 256 human and swine *S. suis* isolates from 2005 to 2016 and analyzed the mechanism of vancomycin resistance. We found that one isolate BSB6 was resistant to vancomycin with the MIC value of 4 mg/L and to another eleven kinds of tested antimicrobial agents. Whole genome sequencing showed that chromosomal gene mutations, and acquired genes in ICE*Ssu*BSB6 accounted for the resistance phenotypes. ICE*Ssu*BSB6 was ~83-kb in size and encoded two resistance gene regions, ARGR1 and ARGR2. ARGR1 harbored six resistance genes, namely *erm*(B), *aadE-apt-sat4-aphA3* cluster and *tet*(O/W/32/O), and showed highes similarity with corresponding sequences of *S. suis* ICE*Ssu*32457 and *Enterococcus faecalis* plasmid pEF418. ARGR2 encoded a *vanG*-type resistance operon. The resistance region showed highest similarity to that of *E. faecalis* BM4518 *vanG1*, but the regulatory region was more similar to that of *S. agalactiae* GBS-NM *vanG*2. Vancomycin resistance in isolate BSB6 was inducible. The study is the first report of *vanG*-type resistance in zoonotic pathogen *S. suis* and highlights importance of its surveillance.

1. Introduction

Streptococcus suis is a previously neglected, newly emergent zoonotic pathogen (Goyette-Desjardins et al., 2014; Yu et al., 2006). Globally, *S. suis* serotype 2 is the most prevalent serotype associated with disease in both humans and pigs, followed by serotype 14, 4, 5, 16, 21 and 24 in humans and 9, 3, 1/2 and 7 in pigs (Goyette-Desjardins et al., 2014). The outbreaks, such as in China, have caused great economic losses in the swine industry and posed severe threats to public health (Goyette-Desjardins et al., 2014; Yu et al., 2006). Recently, an uncommon serotype 24 has been involved in human infections in Thailand, inducing sepsis (Kerdsin et al., 2011) or fatal meningitis (Kerdsin et al., 2016), but has not been reported in other countries.

In the genus *Streptococcus*, mechanisms of resistance to vancomycin have been firstly documented to *vanA*- or/and *vanB*-type resistances in *Streptococcus bovis* (Mevius et al., 1998; Poyart et al., 1997). Thereafter, a *vanB* operon, which located on a 94-kb integrative and conjugative element (ICE*Sluvan*), was identified in *Streptococcus lutetiensis* (Bjorkeng et al., 2013). More recently, a *vanG*-type cluster was found on a mosaic ICE in *Streptococcus agalactiae* (Park et al., 2014; Srinivasan et al., 2014).

The *vanG* operon conferred low-level resistance to vancomycin by synthesizing peptidoglycan precursors with C-terminal D-Ala-D-Ser residues instead of the original D-Ala-D-Ala residues, thus reducing affinity to vancomycin. This operon consisted of a regulatory region encoding three genes (*vanURS*) and a resistance region encoding five genes (*vanYWG(XY)T*) (Depardieu et al., 2003). To date, the *vanG*-type resistance genes have been found in only a few species, including *Enterococcus*, *Clostridium*, *Ruminococcus* and *S. agalactiae* (Ammam et al., 2012; Berthet et al., 2015; Depardieu et al., 2003; Domingo et al., 2007; Srinivasan et al., 2014).

The acquisition and dissemination of antibiotic resistance genes in *S. suis* and other streptococci is strongly associated with mobile genetic elements, mainly integrative and conjugative elements (ICEs) and prophages (Huang et al., 2016b; Palmieri et al., 2011). Although multidrug resistance has emerged to various antimicrobials in *S. suis*, particularly to agents of last resort such as oxazolidinones (Huang et al., 2017), vancomycin resistance has not been reported yet. In this study,

* Corresponding authors.
E-mail addresses: wuzw@iastate.edu (Z. Wu), wlp71@163.com (L. Wang).

https://doi.org/10.1016/j.vetmic.2018.07.008
Received 24 October 2017; Received in revised form 3 February 2018; Accepted 11 July 2018

Table 1
Antibiotic resistance profile of *S. suis* isolate BSB6 and the resistance mechanisms.

Antibiotics	MICs (mg/L)	Phenotype	Resistance mechanisms
Penicillin	16	R	57 substitutions in PBP2X compared with P1/7
Ampicillin	16	R	57 substitutions in PBP2X compared with P1/7
Ceftiofur	0.5	S	–
Ciprofloxacin	64	R	Mutations in GyrA (Ser81 to Lys) and in ParC (Ser79 to Tyr)
Enrofloxacin	32	R	Mutations in GyrA (Ser81 to Lys) and in ParC (Ser79 to Tyr)
Erythromycin	> 256	R	*erm*(B), located on ICE*Ssu*BSB6
Tilmicosin	> 256	R	*erm*(B), located on ICE*Ssu*BSB6
Clindamycin	> 256	R	*erm*(B), located on ICE*Ssu*BSB6
Tetracycline	128	R	*tet*(O/W/32/O), located on ICE*Ssu*BSB6
Gentamicin	16	Not HLGR[a]	–
Spectinomycin	> 512	HLSR[a]	*sat4, apt, aadE*, located on ICE*Ssu*BSB6
Kanamycin	> 256	R	*aphA3*, located on ICE*Ssu*BSB6
Florfenicol	4	S	–
Linezolid	1	S	–
Vancomycin	4	R	*vanG* operon, located on ICE*Ssu*BSB6
Teicoplanin	0.0625	S	–

[a] HLGR means high level gentamycin resistance and HLSR stands for high level spectinomycin resistance.

we tested vancomycin susceptibility of *S. suis* isolates in China from 2005 to 2016 and reported the emergence of vancomycin resistance in *S. suis* serotype 24, which was mediated by a *vanG*-carrying and multidrug resistant ICE.

2. Materials and methods

2.1. Bacterial strains

256 *S. suis* isolates were collected from humans and pigs in 2005–2016. Isolates were grown on Todd-Hewitt broth or agar plates supplemented with 5% calf serum at 37 °C unless otherwise indicated. All the isolates were identified by PCR and sequencing using primers targeting the *gdh* gene as previously described (Okwumabua et al., 2003).

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by broth microdilution method following CLSI standards (CLSI, 2016). The antimicrobial agents in the study were summarized in Table 1.

2.3. Vancomycin resistance mechanism and WGS analysis

Isolates with vancomycin minimum inhibitory concentration (MIC) value ≥2 mg/L were selected to screen the presence of vancomycin resistance genes *vanA*, *vanB*, *vanD*, *vanE*, and *vanG* as previously described (Domingo et al., 2005).

Genomic DNA of the *vanG*-carrying *S. suis* isolate BSB6 was purified and submitted for WGS on the Illumina Hiseq2000 platform (Novogene Bioinformatics Technology, China). Draft genome was assembled with SOAP *denovo* version 2.04 by default parameters (Li et al., 2008). Assembled contigs were ordered by Mauve v2.4.0 (Darling et al., 2004). Gaps between the contigs of *vanG* element were closed by PCR and sanger sequencing. ORFs were predicted using ORF Finder (http://ncbi.nlm.nih.gov/gorf/gorf.html) and annotated by BLAST searching NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Genes and mutations associated with antibiotic resistance were identified using ARG-ANNOT (Gupta et al., 2014). Muti-locus sequence types of the isolate was determined using the scheme introduced by King et al (King et al., 2002).

2.4. Inducible vancomycin resistance assays

Strain BSB6 was further tested for inducible vancomycin resistance phenotype by preincubation of 1/10 × MIC (0.4 mg/L) vancomycin for 1 h in Todd-Hewitt broth plus 0.2% yeast extract (THY) prior to dilution back to OD_{595} = 0.05 in the same medium containing 1/2 × MIC (2 mg/L) vancomycin as described previously (Srinivasan et al., 2014). The reference strain P1/7 was served as negative control.

RT–PCR was used to investigate transcription of *vanG*in *S. suis* strain BSB6. Transcription of *vanG* were compared between the absence of and incubation with 1/10 × MIC (0.4 mg/L) or 1/2 × MIC (2 mg/L) of vancomycin for an hour in the culture medium. In brief, total RNA was extracted from 1 mL of broth culture (OD_{595} = 1) using the RNAiso Plus (Takara Bio, China) following the manufacturer's instructions. RNA was used as the template for cDNA synthesis using the PrimeScrip RT reagent Kit with gDNA Eraser (Takara Bio, China). The cDNA products of each sample were amplified using primers *vanG*-F (5′-AGCAAATGAG GTGGATTTGG-3′) and *vanG*-R (5′-CTGCGATTTGACTCTTGCTG-3′). The resulting PCR products were visualized with GoldView after separation by agarose gel electrophoresis.

2.5. Mating experiments

The transferability of *vanG* element was examined by filter mating experiment as described previously (Davies et al., 2009). In mating experiments, *E. faecalis* JH2-2 (Jacob and Hobbs, 1974) and *S. suis* P1/7RF, a rifampicin- and fusidic acid-resistant derivative strain (Huang et al., 2016b) were used as recipients and the *vanG*-carrying strain BSB6 was utilized as donor. Selection of BSB6 transconjugants was performed on Todd-Hewitt agar containing 0.2% yeast extract containing 2 mg/L vancomycin, 25 mg/L rifampicin and 100 mg/L fusidic acid.

2.6. Nucleotide sequence accession number

The complete sequence of ICE*Ssu*BSB6 has been deposited in GenBank (Accession no. MF616023).

3. Results and discussion

A total of 256 *S. suis* isolates were subjected to vancomycin susceptibility test in the surveillance of vancomycin resistant *S. suis* in China between 2005–2016 (Fig. S1A). Their vancomycin MIC values ranged from 0.125 to 4 mg/L (Fig. S1B). Six isolates showed a vancomycin MIC of 2 mg/L. But only strain BSB6 isolated from a diseased pig in 2016 had the highest MIC of 4 mg/L and was positive in *vanG* PCR detection. To understand the genetic context of *vanG* in isolate BSB6, we determined its genome by Illumina sequencing. Sequence analysis showed that BSB6 belonged to serotype 24 according to the genes in the capsular polysaccharide synthesis (CPS) locus. Multi-locus sequence typing showed that the isolate belonged to a novel sequence type ST-852. Although serotype 24 of *S. suis* has been reported in human infection cases (Kerdsin et al., 2011, 2016), the virulence mechanisms remain largely unknown. BLAST search showed that BSB6 did not possess the most prevalent virulence markers (*epf*, *mrp*, and *sly* genes) of serotype 2 (Goyette-Desjardins et al., 2014), which is consistent with a previous study (Kerdsin et al., 2016). The virulence factors of strain BSB6 remains to be further characterized.

Excepting resistance to vacomycin, BSB6 was also resistant to penicillin, ampicillin, ciprofloxacin, enrofloxacin, erythromycin, tilmicosin, clindamycin, tetracycline, spectinomycin, gentamycin and kanamycin (Table 1). The mechanisms of resistance to these antibiotics were further investigated by analyzing its genome sequence (Table 1).