



Molecular Characterization of a Novel Integrative Conjugative Element ICEHpa1 in *Haemophilus parasuis*

Hua-Run Sun[†], Xiao-Die Cui[†], Xiao-Kang Liu[†], Shuai-Hua Li, Kai-Fang Yi, Yu-Shan Pan, Hua Wu, Li Yuan, Gong-Zheng Hu* and Dan-Dan He*

OPEN ACCESS

Department of Pharmacology and Toxicology, College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, China

Edited by:

Axel Cloeckaert,
Institut National de la Recherche
Agronomique (INRA), France

Reviewed by:

Kristina Kadlec,
Independent Researcher, Wunstorf,
Germany
Patrick Boerlin,
University of Guelph, Canada
Ruth M. Hall,
The University of Sydney, Australia
Séamus Fanning,
University College Dublin, Ireland

*Correspondence:

Gong-Zheng Hu
yaollab@163.com
Dan-Dan He
9951278142@qq.com

[†] These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Antimicrobials, Resistance
and Chemotherapy,
a section of the journal
Frontiers in Microbiology

Received: 24 April 2020

Accepted: 17 July 2020

Published: 13 August 2020

Citation:

Sun H-R, Cui X-D, Liu X-K,
Li S-H, Yi K-F, Pan Y-S, Wu H,
Yuan L, Hu G-Z and He D-D (2020)
Molecular Characterization of a Novel
Integrative Conjugative Element
ICEHpa1 in *Haemophilus parasuis*.
Front. Microbiol. 11:1884.
doi: 10.3389/fmicb.2020.01884

ICEHpa1 was identified in the genome of a serovar 8 *Haemophilus parasuis* ST288 isolate YHP170504 from a case of swine lower respiratory tract infection. The aim of the present study was to characterize the integrative conjugative element ICEHpa1 and its multiresistance region. Susceptibility testing was determined by broth microdilution and the complete ICEHpa1 was identified by WGS analysis. The full sequence of ICEHpa1 was analyzed with bioinformatic tools. The presence of ICEHpa1, its circular intermediate and integration site were confirmed by PCR and sequence analysis. Transfer of ICEHpa1 was confirmed by conjugation. ICEHpa1 has a size of 68,922 bp with 37.42% GC content and harbors 81 genes responsible for replication and stabilization, transfer, integration, and accessory functions, as well as seven different resistance genes [*bla*_{Rob-3}, *tet*(B), *aphA1*, *strA*, *strB*, *aac*(6)^I-*le-aph*(2')-*Ia*, and *sul2*]. Conjugation experiments showed that ICEHpa1 could be transferred to *H. parasuis* V43 with frequencies of 6.1×10^{-6} . This is the first time a multidrug-resistance ICE has been reported in *H. parasuis*. Seven different resistance genes were located on a novel integrative conjugative element ICEHpa1, which suggests that the ICEHpa1 is capable of acquiring foreign genes and serving as a carrier for various resistance genes.

Keywords: multiresistance, *H. parasuis*, ICE, conjugation, transposon

INTRODUCTION

The gram-negative bacterium *Haemophilus parasuis* is the causative agent of Glasser's disease characterized by polyarthritis, fibrinous polyserositis, and meningitis in swine (Oliveira and Pijoan, 2004). The *H. parasuis* infection may cause great economic losses to the global pig industry (Oliveira et al., 2001).

More and more attention has been drawn to the antimicrobial resistance in bacteria from food-producing animals. In *H. parasuis*, the resistant genes are usually located on small plasmids, in which *mob* genes (*mobA*, *mobB*, *mobC*, *mobA-like*, *mobC-like*, and *mobA-L*) and IS*AplI* are usually identified flanking the resistant genes (Lancashire et al., 2005; Chen et al., 2010; Yang et al., 2013; Li et al., 2015; Moleres et al., 2015). However, no other mobile genetic elements [transposons, integrons, and integrative and conjugative elements (ICEs)] have been found to be associated with the resistant genes in *H. parasuis*. ICEs are self-transmissible mobile elements that are widespread

among different bacteria (Burrus et al., 2002; Burrus and Waldor, 2004; Lei et al., 2016). ICEs are composed of a set of core genes that are responsible for replication, maintenance, conjugation, recombination, and regulation, with other accessory modules, such as antimicrobial resistance genes (Robinson et al., 2013; Johnson and Grossman, 2015). In addition, ICEs usually have a single insertion site, which is often in the 5' or 3' end of a tRNA or other highly conserved genes such as the gene *prfC*, in the chromosome of their host (Mulvey et al., 2001; Johnson and Grossman, 2015). ICEs, as vehicles for active DNA exchange among different bacteria, contain some specific genes or sites needed for processing their DNA for transfer. Most of these genes are not expressed when the ICE is integrated in the chromosome; however, expression of the genes needed for excision, integration, and conjugation is induced under certain conditions, and the ICE may excise from the host chromosome to form a dsDNA circular intermediate. Some ICE-encoded proteins assemble into a mating pore that is responsible for transferring the ICE. The new host may recognize the origin of transfer (*oriT*), process the ICE dsDNA to generate a linear ssDNA-protein (T-DNA) through the ICE-encoded relaxase, and pump the T-DNA into the recipient. Then the ICE was recombined into the new host chromosome through an ICE-encoded integrase (Toleman and Walsh, 2011; Johnson and Grossman, 2015; Wright et al., 2015).

Quite a few ICEs have been identified in *Pasteurellaceae*, such as *Haemophilus influenzae*, *Pasteurella multocida*, *Mannheimia haemolytica*, and *Actinobacillus pleuropneumoniae* (Juhás et al., 2007; Brenner et al., 2012; Eidam et al., 2015; Bossé et al., 2016; Li Y. et al., 2018). However, no complete multidrug-resistance ICE in *H. parasuis* has been described in detail to date. In this study, we identified ICEHpa1, a novel ICE carrying multiple resistance genes, in the chromosome of a serovar 8 *H. parasuis* ST288 isolate YHP170504, in a feedlot from Henan, China, in 2017.

MATERIALS AND METHODS

Bacterial Strains and Susceptibility Testing

The strain YHP170504 was obtained from a case of swine lower respiratory tract infection in a feedlot from Henan, China, in 2017. Owing to the unavailability of an approved method for *H. parasuis*, MICs of *H. parasuis* isolates were determined using broth microdilution method following CLSI standard (Clinical and Laboratory Standards Institute [CLSI], 2018) for *A. pleuropneumoniae*. The antimicrobial agents tested were oxytetracycline, doxycycline, ampicillin, amoxicillin, ceftiofur, cefquinome, enrofloxacin, streptomycin, gentamicin, tilmicosin, tylosin, florfenicol, sulfamethoxazole/trimethoprim (19/1), lincomycin, and colistin. *A. pleuropneumoniae* ATCC27090 and *Escherichia coli* ATCC 25922 were used as control strains.

WGS and Analysis

Total genomic DNA of strain YHP170504 was extracted using the TIANamp Bacteria DNA Kit (TIANGEN, Beijing, China) and subjected to WGS using Illumina Nextseq 500 and the Oxford Nanopore Technologies (ONT) MinION platforms. Sequencing

reads including short-read and long-read data were assembled using Unicycler 0.4.4 with the hybrid strategy (Wick et al., 2017; Li R. et al., 2018). The complete sequence of ICEHpa1 was initially annotated using the RAST server¹ and corrected manually. Comparison analysis was conducted using the genome comparison visualizer Easyfig.

Confirmation of the Circular Extrachromosomal Form of the ICE and Conjugal Transfer of ICEHpa1

Chromosomal insertion sites were confirmed by PCR in YHP170504 and transconjugants. For the 5' junction, primers F1 and R1 were designed to amplify a 515-bp fragment from upstream of the ICEHpa1 insertion to a sequence within the 5' end of ICEHpa1. For the 3' insertion, primers F2 and R2 were designed to amplify a 458 bp fragment from within the 3' end of ICEHpa1 insertion to a sequence of the downstream of the ICEHpa1 insertion. To confirm the extrachromosomal circular form of the ICE, outward-facing primers ICE-out-F and ICE-out-R were used.

To investigate self-transfer ability of the ICEHpa1, the conjugation assay was performed using the *H. parasuis* strain YHP170504 as the donor and *H. parasuis* V43 (rifampicin resistance) as the recipient. The serovar 4 *H. parasuis* ST170 isolate V43 was from the strain collection of our laboratory, and the rifampicin-resistant mutant of this strain was generated by selection on Tryptic Soy Agar (TSA) plates supplemented with 10% fetal bovine serum, 10 mg/L nicotinamide adenine dinucleotide (NAD), and increasing rifampicin concentration. For the conjugation assay, overnight cultures of donor and recipient strains grown in Tryptic Soy Broth supplemented with 10% fetal bovine serum and 10 mg/L NAD were mixed (1:5) and incubated for 4 h at 37°C. Bacterial cultures were spread on TSA plates supplemented with 10% fetal bovine serum, 10 mg/L NAD, oxytetracycline (8 mg/L), and rifampicin (100 mg/L), incubated at 37°C for 24 h. Then the transconjugants were screened on the plates. The conjugation frequency was calculated as the number of transconjugants per donor. All the transconjugants were confirmed with PCR using the primers *virB4*-F and *virB4*-R, susceptibility testing, and MLST. All the primers used are listed in Table 1.

Serotyping and Multilocus Sequence Typing (MLST)

The strain YHP170504, V43, and the transconjugant V43::ICEHpa1 were typed by serotyping and MLST. Serovars of the strains were determined using the primers Howell previously described (Howell et al., 2015). Seven housekeeping genes (*atpD*, *infB*, *mdh*, *rpoB*, *6pgd*, *g3pd*, and *frdB*) were amplified and sequenced as described previously (Mullins et al., 2013), after registration of sequences at <https://pubmlst.org/hparasuis/> for assignment of allele numbers and STs; data were analyzed using software available on the website.

¹<http://rast.nmpdr.org>

TABLE 1 | PCR primers used in this study.

Target	Primer	Sequence 5'–3'	Amplicon size (bp)	Annealing temperature (°C)
5' junction of ICEHpa1	F1	ATGTGGTGAATATTTAACTA	515	53
	R1	GGTGCAGAATCTTCAATATG		
3' junction of ICEHpa1	F2	TCTAGACTTTACAAGAAAAC	458	50
	R2	AGCTACATTGACTATAACGC		
Circular form and insertion sites of the ICEHpa1	ICE-out-F	CGAGTGAAAAATTCATACAA	712	47
	ICE-out-R	TGGTTTCCCTATTCTAGCC		
virB4	virB4-F	CAATACAAGCCATCGCTATC	393	55
	virB4-R	TTTGTCTTCGAATAGACCAC		
Circular form of the Tn6742	Tn-out-F	GGCTATTTTACCCACGCACT	1036	55
	Tn-out-R	TGAGTACTTCTACCCGACAT		

Evolutionary Analyses of the Integrase

The integrase, an important core gene of ICE, is needed for both integration and excision. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016) and the analysis involved 11 integrases complete sequences of eight ICEs from *Pasteurellaceae*. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log-likelihood value (Kumar et al., 2016).

Nucleotide Sequence Accession Number

The complete sequences of the chromosome and the ICEHpa1 in *H. parasuis* YHP170504 have been submitted to GenBank with the following accession numbers: CP054198 and MN844034.

RESULTS AND DISCUSSION

The strain YHP170504 was serotyped as serovar 8, belonging to ST288. Also, it exhibited high MICs of oxytetracycline (64 mg/L), doxycycline (8 mg/L), ampicillin (64 mg/L), amoxicillin (64 mg/L), gentamicin (256 mg/L), streptomycin (128 mg/L), sulfamethoxazole/trimethoprim (513/27 mg/L), and enrofloxacin (8 mg/L).

The chromosome of *H. parasuis* YHP170504 is 2,520,015 bp long with a GC content of 39.64%. Sequence analysis showed that the genome harbors seven resistance genes including the β -lactamase-encoding gene *bla*_{Rob-3}, tetracycline resistance gene *tet*(B), the aminoglycoside resistance genes (*aphA1*, *strA*, and *strB*), aminoglycoside and fluoroquinolone resistance gene [*aac*(6')-*Ie-aph*(2')-*Ia*], and sulfonamide resistance gene *sul2*. WGS analysis showed that all seven resistance genes were located on a novel integrative conjugative element, designated as ICEHpa1 (Figure 1A) according to the nomenclature of ICEs².

ICEHpa1 (68,922 bp, corresponding to bases 1,511,383–1,580,304 in GenBank accession number CP054198 or bases 5938–74,859 in GenBank accession number MN844034), a novel ICE with a lower GC content (37.42%), differing from the GC content (39.64%) of entire genome of *H. parasuis* YHP170504,

was integrated into the tRNA^{Leu}, a common insertion site for ICEs in other species of the family *Pasteurellaceae* (Juhás et al., 2007; Brenner et al., 2012; Eidam et al., 2015; Bossé et al., 2016). WGS analysis showed that the ICEHpa1 was flanked by two tRNA^{Leu} copies (a truncated copy and an intact copy, Figure 1A). The presence of an extrachromosomal circular form of ICEHpa1 was confirmed in YHP170504 using primers ICE-out-F and ICE-out-R. Sequencing of the closed circular form of ICEHpa1 showed that the junction (*attP* sequence) sequence was formed by 56 bp imperfect direct repeats (Figure 1B).

PCR assays and susceptibility testing confirmed the presence and activity of the ICEHpa1-associated resistance genes in the *H. parasuis* V43. The transconjugant *H. parasuis* V43::ICEHpa1 showed, in comparison with *H. parasuis* V43, increased MICs of oxytetracycline (from < 0.5 to 32 mg/L), doxycycline (from < 0.5 to 8 mg/L), ampicillin (from 1 to 64 mg/L), amoxicillin (from 1 to 64 mg/L), gentamicin (from < 0.5 to 128 mg/L), streptomycin (from 2 to 128 mg/L), sulfamethoxazole/trimethoprim (from 1.9/0.1 to 513/27 mg/L), and enrofloxacin (from < 0.5 to 4 mg/L). Also, the transconjugant *H. parasuis* V43::ICEHpa1 showed the same serovar and ST as *H. parasuis* V43. Although only *traD*, *traG*, *virB4*, *pilT*, and *pilL* genes encoding components of the type IV secretion system were identified, many other conjugal transfer proteins and membrane proteins were present in ICEHpa1, which may also be involved in the conjugal transfer and responsible for the host specificity of the ICE. The ICEHpa1 could be transferred to *H. parasuis* V43 at a low frequency of conjugation, with 6.1×10^{-6} transconjugants per donor. Our results revealed that ICEHpa1 has the self-transmissible capacity to facilitate the dissemination of the resistance genes.

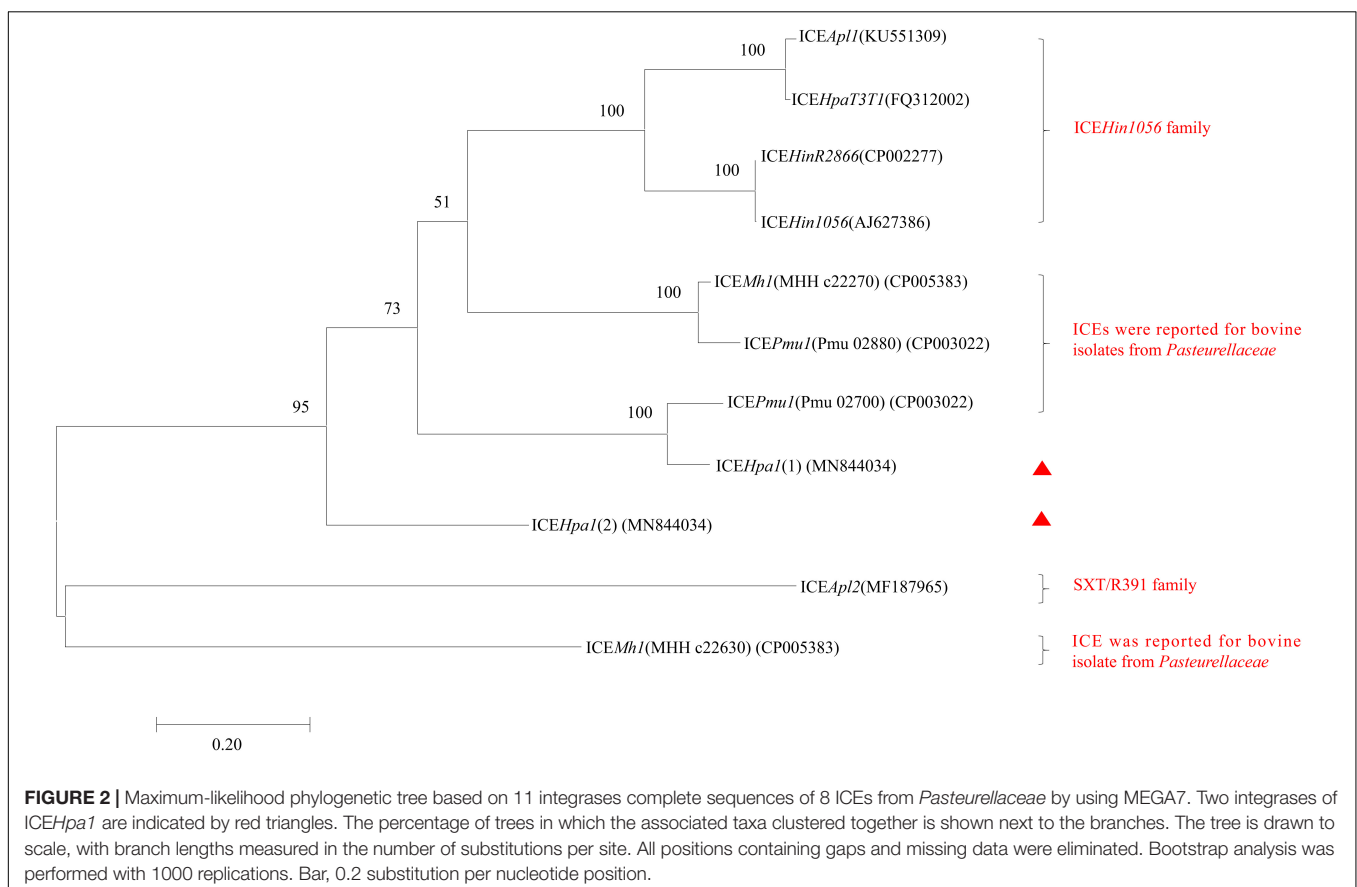
Sequence analysis indicated that the insertion point of ICEHpa1 in the transconjugant was located in a tRNA^{Leu}. Also, the tRNA^{Leu} locus, in which the ICEHpa1 was inserted in the *H. parasuis* V43, showed the same sequence as the one in the *H. parasuis* YHP170504 (Figure 1B). The tRNA^{Leu} into which ICEHpa1 was inserted in *H. parasuis* YHP170504 was flanked on one side by a peptide transporter and on the other side by a hypothetical protein. A comparison between the genome of *H. parasuis* YHP170504 and another similar genome of *Glaesserella parasuis* (*H. parasuis*) D74 showed that the genome

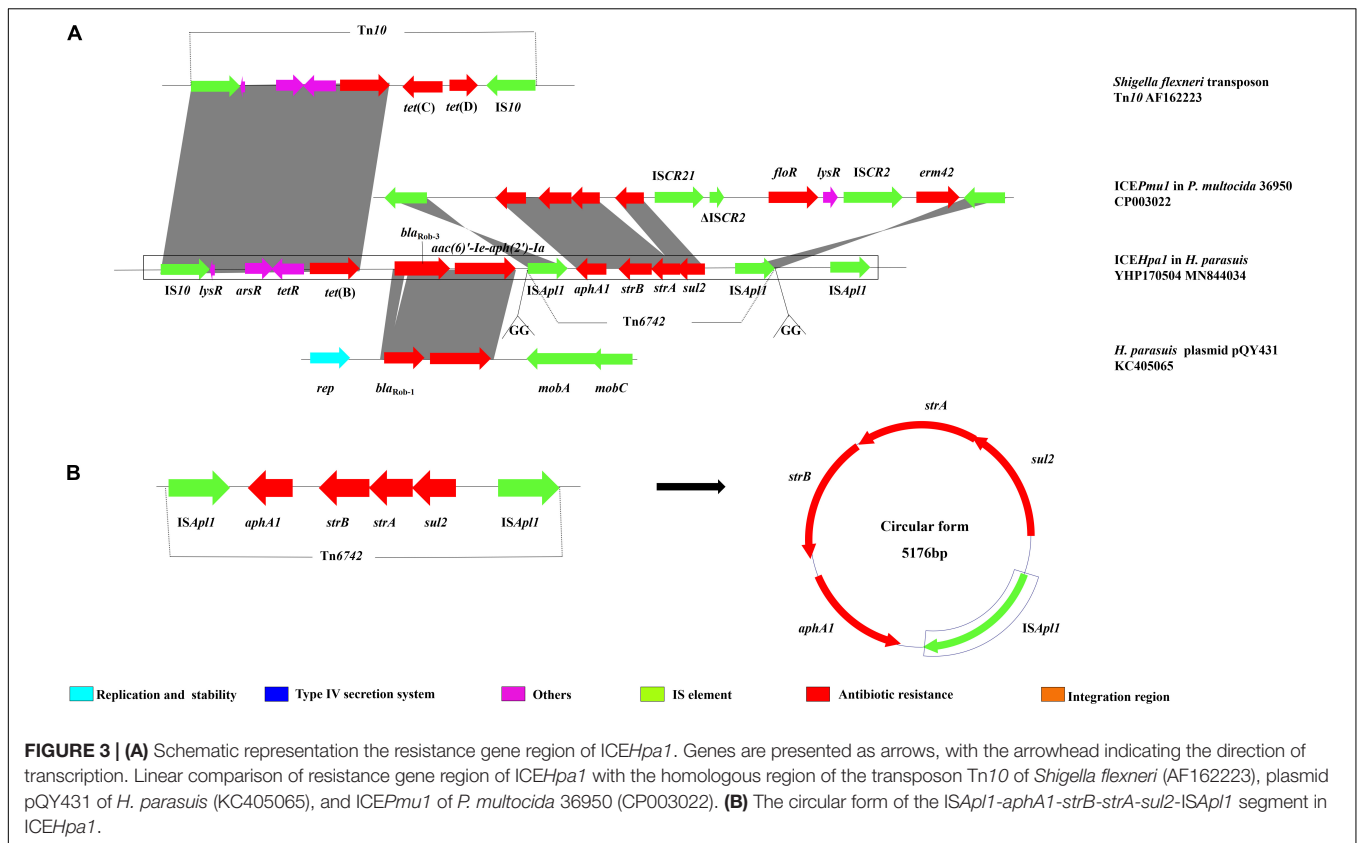
²<https://db-mml.sjtu.edu.cn/ICEberg/>

(**Figure 1A**). Similar to ICEPmu1 in *P. multocida* (Brenner et al., 2012), two integrases were found in ICEHpa1. Identity of 47.35% (93% coverage) was seen when the amino acid sequences of these two integrases were aligned. Comparative sequence analysis revealed that these two integrases are tyrosine recombinases of the Xer family, which are responsible for the integration by site-specific recombination. The integrase 1 in ICEHpa1, belonging to tyrosine recombinase XerD, shared 93.98% (98% coverage) amino acid identity to integrase (Pmu_02700) of ICEPmu1 (CP003022). The integrase 2 in ICEHpa1, belonging to tyrosine recombinase XerC, shared 100% (100% coverage) amino acid identity to integrase of *Glaesserella parasuis* strain F9 (KEZ23006.1). However, both of them differed from the integrases reported in ICEs from *Pasteurellaceae* according to the maximum-likelihood tree obtained by using MEGA 7 software (**Figure 2**). Other experiments are necessary to show which of them or if both are responsible for the integration of ICEHpa1.

The multiresistance region (**Figure 3A**) contains three segments harboring seven different resistance genes. The first segment harboring *tet(B)* was characterized by a truncated transposon Tn10, which shows 99% identity with the corresponding region of the Tn10 transposon. The second segment carries two resistance genes, *bla_{Rob-3}* and *aac(6)'-Ie-aph(2')-Ia*. Notably, although this region showed 99% identity with the corresponding region of the *H. parasuis* pQY431 complete sequence, its *bla_{Rob}* gene significantly differed from its

counterpart in *H. parasuis* pQY431. Compared with the Bla_{Rob} protein encoded by *bla_{Rob-1}* from the *H. parasuis* pQY431, two alanine residues [leucine (L) and threonine (T)] were added between positions 16 and 17 in the Bla_{Rob} from ICEHpa1. The Bla_{Rob} from ICEHpa1 shared 100% amino acid identity with the Bla_{Rob-3} from *Moraxella pluranimalium* CCUG 54913 (NG059331). Resistance to β -lactam antibiotics in *H. parasuis* is conferred by two potential mechanisms: mutations in the *ftsI* gene encoding PBP3 and/or production of β -lactamases. WGS analysis showed that no point mutation was found in the *ftsI* gene and no other *bla* gene was detected in *H. parasuis* YHP170504 except *bla_{Rob-3}*, which suggests that the *bla_{Rob-3}* gene conferred resistance to ampicillin and amoxicillin. To our knowledge, this is the first report of *bla_{Rob-3}* gene in *Pasteurellaceae* species. The third segment harboring a resistance module *aphA1-strB-strA-sul2*, which is flanked by two IS*Apl1* elements oriented in the same direction, was regarded as a putative small transposon designated Tn6742 (**Figure 3B**). To confirm the excision and cyclization of this structure, PCR was conducted using the primers, Tn-out-F and Tn-out-R. The result showed this structure can be looped out, which indicated IS*Apl1* might accelerate the dissemination of the module *aphA1-strB-strA-sul2*. IS*Apl1* has been reported to produce a 2 bp direct duplication GG at its integration site (Tegetmeyer et al., 2008). The 2 bp direct duplication GG was detected upstream of the left-hand copy and downstream of the right-hand copy





of ISAp11, which suggested that the transposon Tn6742 was reassembled into the host chromosome via ISAp11-mediated insertion rather than homologous recombination. However, the formation of a similar structure ISAp11-aphA1-strB-strA-sul2-ISCR21-ΔISCR2-floR-ISCR2-erm42-ISAp11 in ICEPmu1 from *P. multocida* was proved to insert the chromosome by producing a 2-bp direct duplication GT rather than GG (Brenner et al., 2012). In addition, all the four resistance genes *aphA1*, *strB*, *strA*, and *sul2* oriented in the same direction, which is opposite the direction of the two ISAp11. Compared with that, the four genes and two ISAp11, all oriented in the same direction, were identified in ICEPmu1 from *P. multocida* (Brenner et al., 2012). Additional experiments are necessary to show the role of the third ISAp11 in the transmission of these resistance genes.

CONCLUSION

In summary, a novel ICE was identified from a serovar 8 *H. parasuis* ST288 isolate. To our knowledge, this is the first time a multidrug-resistance ICE has been reported in *H. parasuis*. A total of seven different resistance genes were located on the ICEHpa1, which suggests that the ICEHpa1 may act as a reservoir for various resistance genes. Therefore, more research and effective surveillance is needed to monitor the dissemination of multidrug-resistance ICEs.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

This study was carried out in accordance with the guidelines of Henan Agricultural University Animal Ethics Committee. The owners of the farm animals from which samples were taken gave permission for their animals to be used in this study.

AUTHOR CONTRIBUTIONS

G-ZH conceived and designed the experiments. H-RS, X-DC, X-KL, and K-FY produced the data. H-RS, Y-SP, D-DH, HW, LY, and G-ZH analyzed the data. H-RS and G-ZH wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was financed by the National Key Research and Development Program of China (2016YFD05101304).

REFERENCES

- Bossé, J. T., Li, Y., Roberto, F. C., Chaudhuri, R. R., Rogers, J., Holden, M. T. G., et al. (2016). ICEApl1, an integrative conjugative element related to ICEHin1056, identified in the pig pathogen *Actinobacillus pleuropneumoniae*. *Front. Microbiol.* 7:810. doi: 10.3389/fmicb.2016.00810
- Brenner, M. G., Kristina, K., Sweeney, M. T., Elzbieta, B., Heiko, L., Rolf, D., et al. (2012). ICEPmu1, an integrative conjugative element (ICE) of *Pasteurella multocida*: analysis of the regions that comprise 12 antimicrobial resistance genes. *Jo. Antimicrob. Chemother.* 67, 84–90. doi: 10.1093/jac/dkr406
- Burrus, V., Pavlovic, G., Decaris, B., and Guedon, G. (2002). Conjugative transposons: the tip of the iceberg. *Mol. Microbiol.* 46, 601–610. doi: 10.1046/j.1365-2958.2002.03191.x
- Burrus, V., and Waldor, M. K. (2004). Shaping bacterial genomes with integrative and conjugative elements. *Res. Microbiol.* 155, 376–386. doi: 10.1016/j.resmic.01.012
- Chen, L. P., Cai, X. W., Wang, X. R., Zhou, X. L., Wu, D. F., Xu, X. J., et al. (2010). Characterization of plasmid-mediated lincosamide resistance in a field isolate of *Haemophilus parasuis*. *J. Antimicrob. Chemother.* 65, 2256. doi: 10.1093/jac/dkq304
- Clinical and Laboratory Standards Institute [CLSI] (2018). “Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals,” in *CLSI supplement VET08*, 4th Edn (Wayne, PA: CLSI).
- Eidam, C., Poehlein, A., Leimbach, A., Michael, G. B., Kadlec, K., Liesegang, H., et al. (2015). Analysis and comparative genomics of ICEMh1, a novel integrative and conjugative element (ICE) of *Mannheimia haemolytica*. *Jo. Antimicrob. Chemother.* 70, 93–97. doi: 10.1093/jac/dku361
- Howell, K. J., Peters, S. E., Wang, J., Hernandez-Garcia, J., Weinert, L. A., Luan, S. L., et al. (2015). Development of a multiplex PCR assay for rapid molecular serotyping of *Haemophilus parasuis*. *J. Clin. Microbiol.* 53, 3812–3821. doi: 10.1128/jcm.01991-15
- Johnson, C. M., and Grossman, A. D. (2015). Integrative and Conjugative Elements (ICEs): what They Do and How They Work. *Ann. Rev. Genet.* 49, 577–601. doi: 10.1146/annurev-genet-112414-055018
- Juhas, M., Power, P. M., Harding, R. M., Ferguson, D. J. P., Dimopoulou, I. D., Elamin, A. R. E., et al. (2007). Sequence and functional analyses of *Haemophilus* spp. genomic islands. *Genome Biol.* 8:R237. doi: 10.1186/gb-2007-8-11-r237
- Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. doi: 10.1093/molbev/msw054
- Lancashire, J. F., Terry, T. D., Blackall, P. J., and Jennings, M. P. (2005). Plasmid-encoded tet B tetracycline resistance in *Haemophilus parasuis*. *Antimicrob. Agents Chemother.* 49:1927. doi: 10.1128/AAC.49.5.1927-1931.2005
- Lei, C. W., Zhang, A. Y., Wang, H. N., Liu, B. H., Yang, L. Q., and Yang, Y. Q. (2016). Characterization of SXT/R391 integrative and conjugative elements in *Proteus mirabilis* isolates from food-producing animals in China. *Antimicrob. Agents Chemother.* 60:1935. doi: 10.1128/AAC.02852-15
- Li, B., Zhang, Y., Wei, J., Shao, D., Liu, K., Shi, Y., et al. (2015). Characterization of a novel small plasmid carrying the florfenicol resistance gene floR in *Haemophilus parasuis*. *J. Antimicrob. Chemother.* 70, 3159–3161. doi: 10.1093/jac/dkv230
- Li, R., Xie, M., Dong, N., Lin, D., Yang, X., Wong, M. H. Y., et al. (2018). Efficient generation of complete sequences of MDR-encoding plasmids by rapid assembly of MinION barcoding sequencing data. *Gigascience* 7, 1–9. doi: 10.1093/gigascience/gix132
- Li, Y., Li, Y., Crespo, R. F., Leanse, L. G., Langford, P. R., and Bosse, J. T. (2018). Characterization of the *Actinobacillus pleuropneumoniae* SXT-related integrative and conjugative element ICEApl2 and analysis of the encoded FloR protein: hydrophobic residues in transmembrane domains contribute dynamically to florfenicol and chloramphenicol efflux. *J. Antimicrob. Chemother.* 73, 57–65. doi: 10.1093/jac/dkx342
- Molerer, J., Santos-López, A., Lázaro, I., Labairu, J., Prat, C., Ardanuy, C., et al. (2015). Novel blaROB-1-bearing plasmid conferring resistance to β -lactams in *Haemophilus parasuis* isolates from healthy weaning pigs. *Appl. Environ. Microbiol.* 81, 3255–3267. doi: 10.1128/AEM.03865-3814
- Mullins, M. A., Register, K. B., Brunelle, B. W., Aragon, V., Galofré-Mila, N., Baylese, D., et al. (2013). A curated public database for multilocus sequence typing (MLST) and analysis of *Haemophilus parasuis* based on an optimized typing scheme. *Vet. Microbiol.* 162, 899–906. doi: 10.1016/j.vetmic.2012.11.019
- Mulvey, M., Martin, M., Alfa, M., and Ng, R. M. K. (2001). Multiplex PCR for the detection of tetracycline resistant genes. *Mol. Cell Probes.* 15, 209–215. doi: 10.1006/mcpr.2001.0363
- Oliveira, S., Galina, L., and Pijoan, C. (2001). Development of a PCR test to diagnose *Haemophilus parasuis* infections. *J. Vet. Diagn. Invest.* 13, 495–501. doi: 10.1177/104063870101300607
- Oliveira, S., and Pijoan, C. (2004). *Haemophilus parasuis*: new trends on diagnosis epidemiology and control. *Vet. Microbiol.* 99, 1–12. doi: 10.1016/j.vetmic.2003.12.001
- Robinson, E., Didelot, X., Hood, D., and Crook, D. (2013). Comparative phylogenetics of ICEHin1056 family reveals deep evolutionary associations of mobile genetic elements responsible for transfer of antibiotic resistance genes. *Lancet* 381(Suppl.1):S93. doi: 10.1016/S0140-6736(13)60533-4
- Tegetmeyer, H. E., Jones, S. C. P., Langford, P. R., and Baltes, N. (2008). ISAp11, a novel insertion element of *Actinobacillus pleuropneumoniae*, prevents ApxIV-based serological detection of serotype 7 strain AP76. *Vet. Microbiol.* 128, 342–353. doi: 10.1016/j.vetmic.2007.10.025
- Toleman, M. A., and Walsh, T. R. (2011). Combinatorial events of insertion sequences and ICE in Gram-negative bacteria. *FEMS Microbiol. Rev.* 35, 912–935. doi: 10.1111/j.1574-6976.2011.00294.x
- Wick, R. R., Judd, L. M., Gorrie, C. L., and Holt, K. E. (2017). Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput. Biol.* 13:e1005595. doi: 10.1371/journal.pcbi.1005595
- Wright, L. D., Johnson, C. M., Grossman, A. D., and Viollier, P. H. (2015). Identification of a single strand origin of replication in the integrative and conjugative element ICEBs1 of *Bacillus subtilis*. *PLoS Genet.* 11:e1005556. doi: 10.1371/journal.pgen.1005556
- Yang, S. S., Sun, J., Liao, X. P., Liu, B. T., Li, L. L., Li, L., et al. (2013). Co-location of the erm(T) gene and blaROB-1 gene on a small plasmid in *Haemophilus parasuis* of pig origin. *J. Antimicrob. Chemother.* 68, 1930–1932. doi: 10.1093/jac/dkt112

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Sun, Cui, Liu, Li, Yi, Pan, Wu, Yuan, Hu and He. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.