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Association between the Phenotypes and Genotypes of Antimicrobial Resistance in *Haemophilus parasuis* Isolates from Swine in Quang Binh and Thua Thien Hue Provinces, Vietnam

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ABSTRACT

Haemophilus parasuis (*H. parasuis*) is one of the bacterial pathogens of great concern as it causes huge economic losses to the swine industry worldwide. One of the reasons why the control of *H. parasuis* has failed is the increase in antimicrobial resistance (AMR). The country of Vietnam has the second-largest pig production in Asia. However, there is still a lack of data about the AMR prevalence of *H. parasuis* in Vietnam. The purpose of this study is to investigate the prevalence of AMR and analyze the association between AMR and AMR genes (ARGs). The *H. parasuis* strains used in this research were isolated from swine in the Quang Binh and Thua Thien Hue Provinces, Central Vietnam, as reported in our previous study. All of the strains were tested for AMR against 25 antibacterial agents using the broth microdilution method and for the presence of ARGs using the polymerase chain reaction (PCR) method. The tested strains were shown to have a high frequency of resistance to trimethoprim/sulfamethoxazole (94.6%), followed by resistance to colistin, chloramphenicol, gentamicin, penicillin, lincomycin, and amoxicillin. The most prevalent ARGs in these strains were bla_{TEM-1} (94.6%), *int* (76.8%), *gyrA* (58.9%), and *rmtD* (50.0%). Cefuroxime, chloramphenicol, and tobramycin resistances were strongly correlated with the presence of the ARGs bla_{ROB-1} (odds ratio (OR) = 26.3, 95% confidence interval (CI) 2.7–255.7, $p = 0.002$), *catI* (OR = 25.1, 95% CI 2.4–258.9, $p = 0.004$), and *strB* (OR = 23.5, 95% CI 2.6–212.6, $p = 0.001$), respectively. This study reveals for the first time the current situation of *H. parasuis* AMR in Central Vietnam, which is helpful for the clinical control of this disease, as well as for the development of policies and clinical practice guidelines to reduce AMR in swine production in Central Vietnam.

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1. Introduction

Haemophilus parasuis (*H. parasuis*) is a nicotinamide adenine dinucleotide (NAD)-dependent Gram-negative bacterium that causes Glässer's disease including pneumonia, arthritis, serofibrinous polyserositis, and meningitis in swine, leading to huge economic losses in the swine industry worldwide [1–3]. In the last few years, the number of outbreaks caused by *H. parasuis* has been reported to increase dramatically in Europe [4,5], Asia [6,7], and North America [8]. The high prevalence of non-typable strains and the serotype diversity of *H. parasuis* hinder the development of effective cross-protecting vaccines [2].

Antimicrobial treatment as an effective therapy continues to be indispensable for the control of Glässer's disease. A number of antimicrobial agents, including β-lactams, aminoglycosides, macrolides, phenicols, tetracyclines (TETs), fluoroquinolones, and sulfonamides, have been used to control this disease through feed, water, or injection in swine. Antimicrobials at low dosages have

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also been used for growth promotion and disease prevention during swine production [9]. However, the extensive use of antimicrobials has been deemed the major cause of antimicrobial resistance (AMR) accumulation [10–12]. The AMR and AMR genes (ARGs) of *H. parasuis* in different geographical regions have been reported previously [13–17]. It has been reported that *H. parasuis* possesses β-lactams resistance (bla_{ROB-1}, bla_{TEM-1}) [18], TETs resistance (*tetB*, *tetC*, *tetH*) [17], sulfonamides resistance (*sulI*, *sulII*) [19], and fluoroquinolones resistance (*qnrA1, qnrB6, aac(6′)-Ib-cr, gyrA, gyrB*) [20].

Vietnam has the fifth-highest swine production in the world. However, there is no information about the prevalence of AMR phenotypes and ARGs or about their correlation, in *H. parasuis* isolates from Vietnam. In this study, we investigated the antimicrobial susceptibility profiles against 25 antimicrobial agents and detected the presence of ARGs in 56 strains of *H. parasuis* isolated from swine in the Quang Binh and Thua Thien Hue Provinces, Vietnam. The associations between different AMR phenotypes and genotypes were analyzed. These results provide a first glimpse of the prevalence and epidemiology of AMR in *H. parasuis* in Central Vietnam, which is useful for the clinical control of Glässer's disease, as well as for the development of policies and clinical practice guidelines to reduce AMR in swine production in Vietnam.

2. Materials and methods

2.1. Bacterial isolates

A total of 56 *H. parasuis* strains were used in this study. The strains were isolated from swine in swine farms and slaughterhouses in Central Vietnam (Quang Binh Provinces and Thua Thien Hue) from June to September 2017, as characterized in our previous study [21]. The bacteria were cultured using tryptic soy agar (TSA; BD Difco™, BD Biosciences, USA) containing 10 $\mu g \cdot mL^{-1}$ of NAD (Sigma-Aldrich, Inc., USA) and 5% of bovine serum.

2.2. Antimicrobial susceptibility testing

The broth microdilution method suggested by the Clinical and Laboratory Standards Institute (CLSI) was used to test the antimicrobial minimum inhibitory concentrations (MICs) of the *H. parasuis* strains [22]. A total of 25 antimicrobial agents, including gentamicin (GEN), kanamycin (KAN), streptomycin (STR), tobramycin (TOB), spectinomycin (SPE), amoxicillin (AMX), cephalexin (CFL), cefuroxime (CFX), penicillin (PEN), enrofloxacin (ERF), norfloxacin (NOR), ciprofloxacin (CIP), tiamulin (TIA), tylosin (TYL), erythromycin (ERY), lincomycin (LIN), chloramphenicol (CHL), florfenicol (FFC), sulfamethazine (SDM), trimethoprim/sulfametoxazole (TXT), doxycycline (DOX), TET, chlortetracycline (CTET), and colistin (CL), were used in the MIC test. The breakpoints for the antimicrobial agents were set according to the CLSI guidelines [22]. The ranges of resistance were recorded, along with the MIC_{50}s and the MIC_{90}s of the strains. *Heomophilus influenzae* ATCC 49247 was used as the quality-control strain. The strains that were resistant to at least two different types of antimicrobials were classified as multi-drug resistant (MDR).

2.3. ARG amplification

Simplex polymerase chain reaction (PCR) assays were used to detect the presence of 32 ARGs that confer resistance to aminoglycosides (eight genes), β-lactams (two genes), fluoroquinolones (seven genes), macrolides (four genes), phenicols (five genes), sulfonamides (two genes), and TET (four genes). The ARGs, primers, and sizes of each amplified product are listed in Table 1 [17,19,23–29]. The PCR mixture had a total volume of 25 μL and included 12.5 μL of 2 × Taq PCR master mix (CW Biotech, China), 1 μL of each primer (25 $\mu mol \cdot L^{-1}$), 8.5 μL of ultrapure H_2O, and 2 μL of genomic DNA (gDNA) for each strain (at > 10 $ng \cdot \mu L^{-1}$). All PCR assays were performed using an Eppendorf thermal cycler, with cycling conditions that were optimized for each target gene (Table 1). The PCR products were confirmed using 1% agarose gel electrophoresis and were visualized under ultraviolet light.

2.4. Data analysis

Descriptive analysis was performed, and variables were recorded as necessary for statistical modeling using SPSS software (IBM SPSS Statistics version 18.0, IBM, USA). The primary outcome and response variables of interest included the individual resistance genes with prevalence rates of more than 2%. Strains with phenotype resistance to two or more antimicrobial agents were defined as multiple-AMR strains, and strains with two or more resistance genes in a single strain were defined as multiple-ARGs strains. The associations between specific ARGs and the AMR phenotype were calculated using Chi-square and Fisher's extract tests. Statistically significant associations were shown as odds ratios (ORs) with 95% confidence intervals (CI). An OR of > 1 (a positive association) indicated the increasing probability of the co-occurrence of the genotype (or phenotype) being studied with the measured phenotype (or genotype), while an OR of < 1 (a negative association) indicated the decreasing probability of the co-occurrence of the genotype (or phenotype) being studied with the measured phenotype (or genotype). An association was significant if the p value was lower than 0.05.

3. Results

3.1. AMR phenotypes of H. parasuis strains

The susceptibility of the 56 *H. parasuis* strains to the 25 antimicrobials is shown in Table 2. According to the MIC breakpoint of each antimicrobial agent, the strains showed high resistance to all of the antimicrobial agents except fluoroquinolones, TIA, DOX, and FFC. Among all of the strains tested, the highest resistance rate was observed for TXT (94.6%), followed by resistance to CL (91.1%), CHL (91.1%), PEN (85.7%), GEN (83.9%), LIN (82.1%), AMX (78.6%), CFL (71.4%), ERY (69.6%), CTET (67.9%), and TYL (66.1%). None of the *H. parasuis* strains were resistant to DOX. Moreover, the results revealed that all of the strains were MDR; in fact, a strain from nasal swab of swine in a slaughterhouse was surprisingly resistant to 18 antimicrobials (Table 3).

3.2. Associations among AMR phenotypes

We next analyzed the relationship among the 16 AMR phenotypes. As shown in Table 4, almost every AMR was significantly associated with at least one other AMR ($p < 0.05$). The strongest association, which showed an increased probability of observing the outcome resistance in the presence of the predictor resistance, was found to be between TYL and CTET (OR = 93.3, $p < 0.0001$), followed by the association between TYL and ERY (OR = 65.6, $p < 0.0001$), and that between AMX and PEN (OR = 60.2, $p < 0.0001$). Strong associations between resistance to LIN and CTET as well as between resistance to CFL and PEN were also observed.

3.3. Amrgenotypes of H. parasuis strains

Table 5 presents the findings regarding the presence of each ARG in the 56 *H. parasuis* strains. The bla_{TEM-1} gene was the most

Table 1
Primers used for ARG amplification.

Antimicrobial class	ARGs	PCR primer sequence (5′–3′)	Annealing temperature (°C)	Product size (bp)	Ref.
Aminoglycosides	*strA*	F: CCTGGTGATAACGGCAATTC R: CCAATCGCAGATAGAAGGC	55	546	[23]
	strB	F: ATCGTCAAGGGATTGAAACC R: GGATCGTAGAACATATTGGC	56	509	
	aadA	F: GTGGATGGCGGCCTGAAGCC R: AATGCCCAGTCGGCAGCG	68	525	
	amtA	F: ATTCTGCCTATCCTAATTGG R: ACCTATACTTTATCGTCGTC	55	315	[24]
	rmtB	F: GCTTTCTGCGGGCGATGTAA R: ATGCAATGCCGCGCTCGTAT	55	173	
	rmtD	F: CGGCACGCGATTGGGAAGC R: CGGAAACGATGCGACGAT	55	401	
	aacC2	F: TCGGTTGGATGACAAAGC R: TCTCAAGATAGGTGACGC	54	572	[17]
	aadA1	F: TTTGCTGGTTACGGTGAC R: GCTCCATTGCCCAGTCG	56	497	[19]
β-lactams	bla_{TEM-1}	F: GAGTATTCAACATTTTCGT R: ACCAATGCTTAATCAGTGA	50	856	[25]
	bla_{ROB-1}	F: CATTAACGGCTTGTTCGC R: CTTGCTTTGCTGCATCTTC	54	852	[23]
Fluoroquinolones	*qnrA1*	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	53	516	[26]
	qnrB1	F: GATCGTGAAAGCCAGAAAGG R: ATGAGCAACGATGCCTGGTA	53	476	
	aac(6′)-Ib	F: TATGAGTGGCTAAATCGA R: CCCGCTTTCTCGTAGCA	56	394	[19]
	gyrA	F: AGCGTTACCAGATGTGCGAGATG R: TTGCCACGACCTGTACGATAAGC	55	620	[17]
	gyrB	F: TACATACGCTGTAGGTTCAAGGA R: CAAGATAATACGGAAATGGAGC	55	500	
	parC	F: AACTTCAACATTACCACTTAGCCCTC R: GTACCTCACCAAGCCTCGCCATCT	55	1445	
	parE	F: CGATAATTCCCTTGAAGTCGTTG R: ATTGATCTGCTCGCCACCCTCTG	55	609	
Macrolides	*erm*(A)	F: ATGAACCAGAAAAACCCTAAAG R: TTAGTGAAACAATTTGTAACTATTG	50	732	[25]
	erm(B)	F: GAAAAGGTACTCAACCAAATA R: AGTAACGGTACTTAAATTGTTTAC	50	616	
	erm(C)	F: AATCGGCTCAGGAAAAGG R: ATCGTCAATTCCTGCATG	50	562	
	lnu (C)	F: TCTTGATGGTGGCTGGGGTG R: CATTTTCATCAAACTCGTATCCC	50	365	[29]
Phenicols	*catI*	F: TTTATCCGGCCTTTATTCACATTC R: ACCACCTTGTCGCCTTGC	56	388	[19]
	cmlA	F: TGCCAGCAGTGCCGTTTAT R: CACCGCCCAAGCAGAAGTA	56	900	
	flor	F: GGCTTTCGTCATTGCGTCTC R: ATCGGTAGGATGAAGGTGAGGA	56	679	
	int	F: CCTCCCGCACGATGATC R: TCCACGCATCGTCAGGC	55	280	[27]
	cfr	F: TGAAGTATAAAGCAGGTTGGGAGTCA R: ACCATATAATTGACCACAAGCAGC	48	746	[28]
Sulfonamides	*sul1*	F: GTGACGGTGTTCGGCATTCT R: TCCGAGAAGGTGATTGCGCT	56	779	[19]
	sul2	F: TTCGGCATCGTCAACATAACCT R: CGTGTGTGCGGATGAAGTCAG	56	727	
TET	*tetA*	F: GGCGGTCTTCTTCATCATGC R: CGGCAGGCAGAGCAAGTAGA	64	210	[23]
	tetB	F: CATTAATAGGCGCATCGCTG R: TGAAGGTCATCGATAGCAGG	64	640	[25]
	tetC	F: GCCGGAAGCGAGAAGAATCA R: GCTGTAGGCATAGGCTTGGT	54	888	
	tetH	F: TTATACTGCTGATCACCG R: CATCCCAATAAGCGACGC	54	1080	

F: forward; R: reverse.

frequently detected ARG (94.6%), followed by *int* (76.8%), *gyrA* (58.9%), and *rmtD* (50.0%). The *parE* and *flor* genes had the lowest detection rates (3.6% for both). However, none of the strains were positive for *gyrB*, *cfr*, or *tetC*. Most of the strains (55 strains, 98.2%) carried at least one of the ARGs (Table 3).

3.4. Associations among resistance genes

Positive associations (ordered by OR) were observed between the following gene pairs: *tetB*/*tetH* (OR = 153, $p = 0.001$), *tetA*/*tetB* (OR = 75, $p = 0.001$), *tetH*/*aac(6′)-Ib* (OR = 49, $p = 0.001$),

Table 2
MICs for 25 antimicrobial agents of *H. parasuis* strains.

AMs	Number of strains														MIC_{50} ($\mu g \cdot mL^{-1}$)	MIC_{90} ($\mu g \cdot mL^{-1}$)	Resistance rate
	MIC = 0.13 $\mu g \cdot mL^{-1}$	MIC = 0.25 $\mu g \cdot mL^{-1}$	MIC = 0.5 $\mu g \cdot mL^{-1}$	MIC = 1 $\mu g \cdot mL^{-1}$	MIC = 2 $\mu g \cdot mL^{-1}$	MIC = 4 $\mu g \cdot mL^{-1}$	MIC = 8 $\mu g \cdot mL^{-1}$	MIC = 16 $\mu g \cdot mL^{-1}$	MIC = 32 $\mu g \cdot mL^{-1}$	MIC = 64 $\mu g \cdot mL^{-1}$	MIC = 128 $\mu g \cdot mL^{-1}$	MIC = 256 $\mu g \cdot mL^{-1}$	MIC = 512 $\mu g \cdot mL^{-1}$	MIC = 1024 $\mu g \cdot mL^{-1}$			
GEN	3	1	0	2	1	1	1 |	7	9	13	5	7	1	5	64	512	83.9%
KAN	4	0	1	3	1	0	6	12	2 |	9	5	5	0	8	32	1024	48.2%
STR	1	3	0	0	3	1	5 |	11	8	6	5	7	1	5	32	512	—
TOB	6	1	0	2	9	15	5	3	4	2	3	5	0	1	4	256	32.1%
SPE	2	1	0	2	4	5	3	16	1	4 |	8	3	7		16	512	32.1%
AMX				5	2	5 |	1	4	4	5	4	7	1	18	128	1024	78.6%
CFL		3	1	2	0	5	4	1 |	6	9	11	14			64	256	71.4%
CFX	8	3	8	7	5	5	7 |	1	4	2	6				2	128	23.2%
PEN	3	3	1	1 |	3	3	1	5	5	16	9	4	1	1	64	256	85.7%
ERF	20	18	13	4	0 |	1									0.25	0.5	1.8%
NOR	6	3	7	5	5	26	3 |	1							4	4	1.8%
CIP	19	4	12	16	4 |	1									0.5	1	1.8%
TIA	6	0	0	0	3	2	19	24	2						8	16	3.6%
TYL	9	5	2	1	2 |	0	0	0	1	2	4	2	28	0	256	512	66.1%
ERY	9	2	1	3	1	1 |	1	0	1	11	0	0	1	25	64	1024	69.6%
LIN		2	1	2	5 |	2	2	1	11	21	5	2	1	1	64	128	82.1%
CHL	1	2	0	0	1	1 |	1	0	7	23	0	0	13	7	64	1024	91.1%
FFC	10	5	1	1	3	12	19 |	2	2	1					4	8	8.9%
SDM									3	3	0	26	1	23	256	1024	—
CTET		0	3	4	6	2	3 |	2	19	8	6	1			64	256	67.9%
DOX	12	3	9	17	13	2	|								1	2	0
TET	11	0	0	1	5	13 |	16	10							4	16	46.4%
CL	2	0	1	1	1 |	2	3	17	8	9	0	3	4	5	32	512	91.1%
TXT								1[a]	2[b]	1[c]	8[d]	15[e]	2[f]	27[g]	608/32	1216/64	94.6%

Breakpoints of resistance used are indicated with vertical black lines when available. Number of strains with MICs of trimethoprim/sulfamethoxazole (TXT) = 0.15/0.008, 0.30/0.016, 0.6/0.032, 1.2/0.064, 2.4/0.125, 4.8/0.25, and 9.5/0.5 are not available. MIC_{50}, and MIC_{90}: the lowest concentration of AM agents capable of inhibiting the growth of 50% and 90% of strains, respectively.

[a] MIC of TXT = 19 $\mu g \cdot mL^{-1}$/1 $\mu g \cdot mL^{-1}$.
[b] MIC of TXT = 38 $\mu g \cdot mL^{-1}$/2 $\mu g \cdot mL^{-1}$.
[c] MIC of TXT = 76 $\mu g \cdot mL^{-1}$/4 $\mu g \cdot mL^{-1}$.
[d] MIC of TXT = 152 $\mu g \cdot mL^{-1}$/8 $\mu g \cdot mL^{-1}$.
[e] MIC of TXT = 304 $\mu g \cdot mL^{-1}$/16 $\mu g \cdot mL^{-1}$.
[f] MIC of TXT = 608 $\mu g \cdot mL^{-1}$/32 $\mu g \cdot mL^{-1}$.
[g] MIC of TXT = 1216 $\mu g \cdot mL^{-1}$/64 $\mu g \cdot mL^{-1}$.

Table 3
Numbers of AMRs and ARGs in *H. parasuis* strains.

Number of AMR or ARGs	Number strains positive for	
	AMR phenotype	AMR genotype
0	0	1
1	0	2
2	0	0
3	3	7
4	3	8
5	1	7
6	3	8
7	0	3
8	0	1
9	3	8
10	7	3
11	9	3
12	11	31
13	5	1
14	4	2
15	1	1
16	5	0
18	1	0

sulII/*aac (6′)-Ib* (OR = 23, $p = 0.003$), and *tetB*/*aadA1* (OR = 23, $p = 0.01$) (Table 6). However, *amtA*, bla_{TEM-1}, *parE*, and *flor* were not associated with any of the other genes. Most of the associations between ARGs were positive (OR > 1), but the associations between the four ARG pairs (*qnrA1*/*gyrA*, *qnrB1*/*int*, *parC*/*int*, and *lnu(C)*/*int*) were negative (OR < 1) (Table 6).

3.5. Associations between phenotypes and genotypes of antimicrobial resistance

Resistance genes can be linked to genetic elements, and the use of a particular antimicrobial can select for resistance not only to its own, but also potentially to a variety of other antimicrobials [30]. The strong association between the phenotypes and genotypes of AMR in bacteria supports the hypothesis that antimicrobial use selects for bacteria with novel resistance determinants [31]. Significant associations were found between AMR phenotypes and genotypes in the *H. parasuis* strains (Table 7). Among the 47 strains resistant to GEN, 33 strains (70.2%) carried one to seven corresponding ARGs, and this phenotype was strongly associated with the presence of *rmtD* (OR = 10.8, $p = 0.02$). For the 27 strains resistant to KAN, *strA* was detected in six strains (22.2%), *strB* in seven (25.9%), *aadA* in 16 (59.3%), *amtA* in one (3.7%), *rmtB* in 10 (37.0%), *rmtD* in 22 (81.5%), *aacC2[aac(3)-lic]* in seven (25.9%), and *aadA1* in eight (29.6%). However, it is worth noting that one resistant strain did not carry any of the related resistance genes (3.7%). KAN resistance was associated with the presence of *aadA* (OR = 19.6, $p < 0.0001$), *rmtD* (OR = 16.9, $p < 0.0001$), *aadA1* (OR = 11.8, $p = 0.01$), both *strB* and *aacC2[aac(3)-lic]* (OR = 9.8, $p = 0.02$), *strA* (OR = 8.0, $p = 0.048$), and *rmtB* (OR = 7.9, $p = 0.01$). Most strains (17/18, 94.4%) that were resistant to TOB contained the corresponding ARGs, including *strB*, *aadA*, *rmtB*, *rmtD*, and *aadA1*, among which *strB* (OR = 23.5, $p = 0.001$) and *rmtB* (OR = 11.7, $p = 0.001$) were found to have much stronger correlation than the others. Among the 18 strains resistant to SPE, *strA* was detected in six strains (13.3%), *strB* in eight (17.8%), *aadA* in 17 (37.8%), *amtA* in two (4.4%), *rmtB* in 11 (24.4%), and *aadA1* in seven (15.6%). SPE resistance was associated with *aadA* (OR = 29.8, $p < 0.0001$), *rmtB* (OR = 22.5, $p < 0.0001$), and (*rmtD*) (OR = 9.6, $p = 0.001$). Thirteen strains were classified as CFX resistant, and all of these were found to carry bla_{TEM-1}, while five (38.5%) also carried bla_{ROB-1}. In contrast, 40/53 (75.5%) strains carried bla_{TEM-1}, and one out of six strains positive for bla_{ROB-1} did not show phenotypes resistant to CFX. CFX resistance was strongly associated with bla_{ROB-1} (OR = 26.3, $p = 0.002$). Twenty-five (67.6%) of the 37 TYL-resistant strains were positive for at least one corresponding TYL-resistance gene. Of these strains, six (16.2%) carried *erm(A)*, 20 (54.1%) carried *erm(B)*, 11 (29.7%) carried *erm(C)*, and two (5.4%) carried *lnu (C)*. TYL resistance was associated with *erm(B)* (OR = 10.0, $p = 0.03$), *erm(C)* (OR = 7.6, $p = 0.04$),

Table 4
Outcome predictor association between AMR phenotypes of *H. parasuis* strains.

Outcome	Predictor	Outcome predictor association		
		OR	95% CI	*p*
GEN	CFL	14.7	2.6–83.5	0.001
	ERY	6.5	1.4–30.6	0.01
	CTET	5.8	1.3–27.0	0.02
KAN	CFL	22.4	2.7–189.3	0.0003
TOB	SPE	4.7	1.4–15.8	0.015
	AMX	0.2	0.1–0.9	0.04
	CFL	28.2	5.1–156.5	< 0.0001
SPE	TOB	4.7	1.4–15.8	0.015
	CFX	8.5	2.1–34.1	0.002
	TET	7.6	2.1–27.9	0.002
AMX	TOB	0.2	0.1–0.9	0.04
	CFL	5.4	1.4–21.2	0.01
	PEN	60.2	6.1–594.9	< 0.0001
	ERY	4.7	1.2–18.3	0.03
	LIN	5.5	1.3–24.4	0.02
CFL	GEN	14.7	2.6–83.5	0.001
	AMX	5.4	1.4–21.2	0.01
	PEN	30.3	3.3–278.5	0.0003
	TY	3.8	1.1–13.1	0.03
	ERY	12.4	3.2–48.9	0.0002
	LIN	5.4	1.3–22.9	0.02
	CHL	13	1.3–127.7	0.02
	CL	13	1.3–127.7	0.02
CFX	KAN	22.4	2.7–189.3	0.0003
	TOB	28.2	5.1–156.5	< 0.0001
	SPE	8.5	2.1–34.1	0.002
PEN	AMX	60.2	6.1–594.9	< 0.0001
	CFL	30.3	3.3–278.5	0.0003
	TYL	8.07	1.4–45.2	0.01
	ERY	10.1	1.8–57.3	0.007
TYL	CFL	3.8	1.1–13.1	0.03
	PEN	8.1	1.4–45.2	0.01
	ERY	65.6	10.8–397.8	< 0.0001
	LIN	32.4	3.7–287.1	0.0001
	CTET	93.3	14.2–614.4	< 0.0001
	TET	5.5	1.5–19.9	0.01
ERY	GEN	6.5	1.4–30.6	0.01
	AMX	4.7	1.2–18.3	0.03
	CFL	12.4	3.2–48.9	0.0002
	PEN	10.1	1.8–57.3	0.007
	TYL	65.6	10.8–397.8	< 0.0001
	CTET	22.1	5.1–95.3	< 0.0001
	TET	28.5	3.4–238.9	0.0001
	CL	11.7	1.2–114.3	0.02
LIN	AMX	5.5	1.3–24.4	0.02
	CFL	5.4	1.3–22.9	0.02
	TYL	32.4	3.7–287.1	0.0001
	CHL	9.4	1.3–66.9	0.03
	CTET	37	4.1–330.8	0.0001
CHL	CFL	13	1.3–127.7	0.02
	LIN	9.4	1.3–66.9	0.03
	SDM	33.3	2.3–480.5	0.01
SDM	CHL	33.3	2.3–480.5	0.01
CTET	GEN	5.8	1.3–27.0	0.02
	TYL	93.3	14.2–614.4	< 0.0001
	ERY	22.1	5.1–95.3	< 0.0001
	LIN	37	4.1–330.8	0.0001
	TET	4.8	1.3–17.4	0.02
	CL	10.5	1.1–102.9	0.03
TET	TYL	5.5	1.5–19.8	0.01
	ERY	28.5	3.4–238.9	0.0001
	CTET	4.8	1.3–17.4	0.02
	SPE	7.6	2.1–27.9	0.002
CL	CFL	13	1.3–127.7	0.02
	ERY	11.6	1.2–114.3	0.02
	CTET	10.5	1.1–102.9	0.03

Table 5
Distribution of ARGs in *H. parasuis* strains.

Antimicrobials class	ARGs	Strains positive	Percentage (%)	Number of strains positive in a class	Percentage (%)
Aminoglycosides	*strA*	7	12.5	45	80.4
	strB	8	14.3		
	aadA	18	32.1		
	amtA	3	5.4		
	rmtB	12	21.4		
	rmtD	28	50.0		
	aacC2[aac(3)-Iic]	8	14.3		
	aadA1	9	16.1		
β-lactams	bla_{TEM-1}	53	94.6	53	94.6
	bla_{ROB-1}	6	10.7		
Fluoroquinolones	*qnrA1*	13	23.2	43	76.8
	qnrB1	10	17.9		
	aac(6′)-Ib	6	10.7		
	gyrA	33	58.9		
	gyrB	0	0.0		
	parC	4	7.1		
	parE	2	3.6		
Macrolides	*erm(A)*	9	16.1	37	66.1
	erm(B)	22	39.3		
	erm(C)	12	21.4		
	lnu (C)	9	16.1		
Phenicols	*catI*	11	19.6	48	85.7
	cmlA	13	23.2		
	flor	2	3.6		
	int	43	76.8		
	cfr	0	0.0		
Sulfonamides	*sul1*	8	14.3	13	23.2
	sul2	8	14.3		
TET	*tetA*	5	8.9	7	12.5
	tetB	4	7.1		
	tetC	0	0.0		
	tetH	4	7.1		

and *lnu(C)* (OR = 0.1, $p = 0.005$). However, TYL resistance was only positively associated with two resistance genes (*erm(B)* and *erm(C)*). Regarding resistance to the phenicol group, 51 and five strains were found to be resistant to CHL and FFC, respectively. Forty-five (45/51, 88.2%) of the CHL -resistant strains carried at least one corresponding ARG. A positive association was observed between CHL resistance and *int* (OR = 18.7, $p = 0.01$), and FFC resistance and *catI* (OR = 25.1, $p = 0.004$) and *cmlA* (OR = 18.7, $p = 0.01$). However, other antimicrobials, including AMX, CFL, and PEN, were not associated with any corresponding resistance gene.

4. Discussion

In contrast to several other reports showing that most *H. parasuis* strains isolated from swine in the United Kingdom, Germany, and Denmark had lower levels of AMR [13,15,32], the data in this study revealed a high prevalence of AMR. Moreover, compared with the high-frequency resistance to both CIP and ERF that was found in the *H. parasuis* isolates from swine in Denmark [14] and the high prevalence of strains resistant to at least three antimicrobials (i.e., ERF, TXT, and CIP) in the strains (68.2%) from China [16] and Spain [15], our study suggests that the *H. parasuis* strains isolated from swine in Central Vietnam possess a lower rate of resistance to both CIP and ERF, as well as to at least three other antimicrobials. Our results are consistent with the results of a previous study by Nedbalcová and Kučerová [32], which showed that the *H. parasuis* strain isolated from swine in the Czech Republic possessed a low ratio of resistance to fluoroquinolones. Therefore, our findings suggest that fluoroquinolones, TIA, FFC, and DOX may be effective in controlling Glässer's disease in swine produced in Central Vietnam. Moreover, the results of this study (Table 4) will be useful for antimicrobial users in practical applications.

Aminoglycoside resistance is conferred by the presence of *amtA*, *rmtB*, and *rmtD* encoding 16S rRNA-methyltransferase [33]; *strA* and *strB* encoding phosphotransferase [34,35]; and *aadA*, and *addA1* encoding adenylyltransferase [36,37]. Our results showed that the *H. parasuis* strains carried *strA*, *strB*, *aadA*, and *amtA* genes. This is very similar to the results of a previous study [17], which reported that the *H. parasuis* isolates from swine in China carried *rmtB* (11.9%), *rmtD* (0.7%), *aacC2[aac(3)-Iic]* (4.2%) and *aadA1* (20.8%), but does not align with other studies on the isolates from swine in Australia [25]. The highest detection rate of bla_{TEM-1} (94.5%) in the *H. parasuis* strains explained the high AMR phenotype to PEN (85.7%), AMX (78.6%), and CFL (71.4%). This resistance phenotype caused by the presence of bla_{TEM-1} and bla_{ROB-1} (both encoding β-lactamases) has been reported previously [38–40]. The results of this study are similar to those reported for *H. parasuis* isolates from swine in China [18] and other countries [17,25,38], suggesting a wide spread of β-lactam resistance in *H. parasuis*. Previous studies have revealed that fluoroquinolone resistance is spreading and increasing rapidly in bacteria, because most *qnr* genes are located on a Tn-like sequence or integron on a conjugative plasmid [26,41]. In this study, although the frequency of strains resistant to fluoroquinolones was truly low, the bacterial strains carrying *qnrA1* and *qnrB1* could not be ignored. Furthermore, the macrolides resistance genes *ermA*, and *ermB* in *H. parasuis* have been reported to be at a low frequency in other studies [17,19]. However, our study revealed that the bacterial strains not only carried these genes but also carried other macrolides resistance genes such as *ermC* and *lnuC*. These genes are responsible for the ribosomal binding sites modification that is the most important macrolides resistance mechanism [42]. The emergence of FFC resistance in *H. parasuis* strains is attributable to a novel small plasmid pHPSF1 carrying *flor* [17], which explains the low frequency of the strains (in this study) carrying *flor* and FFC resistance capacity. Resistance to sulfonamide involves the presence of

Table 6
Pairwise statistical correlation between ARGs of *H. parasuis* strains.

Outcome ARG	Predictor ARG	Outcome predictor association		
		OR	95% CI	*p*
strA	*aadA*	6.9	1.2–40.2	0.03
strB	*rmtB*	9.7	1.9–50.4	0.008
	bla_{ROB-1}	9.0	1.4–57.1	0.03
aadA	*strA*	6.9	1.2–40.2	0.03
	rmtB	6.8	1.7–28.4	0.007
	rmtD	9.6	2.4–39.4	0.001
rmtB	*strB*	9.7	1.9–50.4	0.008
	aadA1	7.1	1.5–33.3	0.01
	qnrB1	5.6	1.3–24.4	0.03
	ermA	7.1	1.5–33.3	0.02
	tetB	14.3	1.3–154.0	0.03
	tetH	14.3	1.3–154.0	0.02
rmtD	*aadA*	9.6	2.4–39.4	0.001
aacC2[aac(3)-Iic]	*catI*	5.9	1.9–29.0	0.04
aadA1	*rmtB*	7.1	1.5–33.3	0.01
	ermA	6.7	1.4–33.6	0.03
	tetB	23.0	2.1–258.1	0.01
bla_{ROB-1}	*strB*	9.0	1.4–57.1	0.03
qnrA1	*qnrB1*	4.8	1.1–20.4	0.04
	parC	12.6	1.2–134.2	0.03
	gyrA	0.2	0.1–0.8	0.02
	catI	6.5	1.6–27.4	0.01
	sulII	8.3	1.7–42.1	0.01
qnrB1	*rmtB*	5.6	1.3–24.4	0.03
	qnrA1	4.8	1.1–20.4	0.04
	lnu(C)	10.5	2.1–52.5	0.006
	catI	6.7	1.5–30.1	0.01
	int	0.2	0.1–0.9	0.04
	tetA	9.4	1.3–66.9	0.03
	tetB	19.3	1.8–212.4	0.01
	tetH	19.3	1.8–212.4	0.01
aac(6′)-Ib	*erm(A)*	7.3	1.2–44.9	0.04
	sulII	23.0	3.2–166.8	0.003
	tetH	49.0	3.8–624.9	0.003
gyrA	*qnrA1*	0.2	0.1–0.8	0.02
parC	*qnrA1*	12.6	1.2–134.2	0.03
	erm(A)	23.0	2.1–158.1	0.01
	int	0.1	0.01–0.9	0.04
erm(A)	*rmtB*	7.1	1.5–33.3	0.02
	aadA1	6.7	1.4–33.6	0.03
	aac(6′)-Ib	7.3	1.2–44.9	0.04
	parC	23.0	2.1–158.1	0.01
erm(B)	*erm(C)*	4.3	1.1–16.7	0.04
erm(C)	*erm(B)*	4.3	1.1–16.7	0.04
lnu(C)	*qnrB1*	10.5	2.1–52.5	0.006
	int	0.1	0.02–0.4	0.003
	sulI	8.6	1.6–45.5	0.01
catI	*qnrA1*	6.5	1.6–27.4	0.01
	qnrB1	6.7	1.5–30.1	0.01
	cmlA	11.4	2.5–50.9	0.002
cmlA	*catI*	11.4	2.5–50.9	0.002
	sulII	8.3	1.7–42.1	0.01
	tetH	12.6	1.2–134.2	0.04
int	*qnrB1*	0.2	0.05–0.9	0.04
	parC	0.1	0.01–0.9	0.04
	lnu(C)	0.1	0.02–0.4	0.003
sulI	*lnu(C)*	8.6	1.6–45.5	0.01
sulII	*qnrA1*	8.3	1.7–42.1	0.01
	aac(6′)-Ib	23.0	3.2–166.8	0.003
	cmlA	8.3	1.7–42.1	0.01
	tetA	13.8	1.8–103.3	0.01
tetA	*qnrB1*	9.4	1.3–66.9	0.03
	sulII	13.8	1.8–103.3	0.01
	tetB	75.0	5.2–1081.1	0.001
	tetH	16.3	1.7–159.8	0.03
tetB	*rmtB*	14.3	1.3–154.0	0.03
	aadA1	23.0	2.1–258.1	0.01
	qnrB1	19.3	1.8–212.4	0.01
	tetA	75.0	5.2–1081.1	0.001
	tetH	153.0	7.6–3092.9	0.001
tetH	*rmtB*	14.3	1.3–154.0	0.02
	aac(6′)-Ib	49.0	3.8–624.9	0.003
	qnrB1	19.3	1.8–212.4	0.01
	cmlA	12.6	1.2–134.2	0.04
	tetA	16.3	1.7–159.8	0.03
	tetB	153.0	7.6–3092.9	0.001

the *sulI* and *sulII* genes (encoding dihydropteroate synthases) associating with an integron system and a conjugative plasmid [43]. This is consistent with the results of this study, in which the two genes *sulI* and *sulII* (both sharing 8/56, 14.8%) were found in the *H. parasuis* strains. In addition, our investigation found that *tetA*, *tetB*, and *tetH* existed in the *H. parasuis* strains, which explains the phenotypic resistance to TETs, with the exception of DOX. Previous studies have shown that both *tetB* and *tetH* encoding the efflux pump are responsible for TET resistance in *H. parasuis* isolates from Australia [25,44]. The inactivation of TETs against the bacterial pathogen is involved in the presence of both *tetB* and *tetH* in the action of the efflux pump protein expelling the antimicrobial out of the cells.

Previous studies have demonstrated that the mechanisms of associations between resistance genes can be confirmed by molecular investigations [45,46]. An increased level of associations between resistance genes may result from the co-location of resistance genes on a single mobile genetic element such as a plasmid, transposon, or integron [43,47,48]. Our results showed many strong associations among ARGs which aligns with the opinion that there might be a linkage between many of these resistance genes on mobile elements. This opinion agrees with the results of a study by Rosengren et al. [31], which reported that *qnrB1* had an increased association with eight other ARGs including *rmtB*, *qnrA1*, *lnu(C)*, *catI*, *int*, *tetA*, *tetB*, and *tetH*. In addition, associations between *sulI* and *lnu(C)*, and between *sulII* and *tetA*, are parts of integrons; the association between *tetB* and *tetH* is a requirement for the high frequency of strains resistant to CTET. The results strongly support the finding that the resistance genes are associated with a mobile DNA, such as plasmids and transposons, which enables horizontal gene spreading. Strong associations between the phenotypes and genotypes of AMR were found in our study, such as between GEN and *rmtD*, CFX and bla_{ROB-1}, and CHL and *int*, indicating that the resistance to a given antimicrobial was caused in some cases by a single gene. This finding is similar to the results of previous studies [30,31]. However, interestingly, we found that some strains possessed resistance phenotypes but did not have the corresponding ARGs and *vice versa*; for example, some strains showed resistance to AMX, but did not carry any corresponding genes. This finding is similar to the results reported by Rosengren et al. [31]. A possible explanation is that resistance phenotypes can be expressed upon the stimulation of many different genetic factors, and that each factor may present a unique epidemiological character [23,49]. Thus, the mechanisms of AMR in *H. parasuis* isolates from swine produced in Vietnam deserve further investigation.

5. Conclusion

This study is the first investigation on the prevalence of AMR and ARGs of *H. parasuis* isolates from swine in Central Vietnam. The strains tested were resistant to a broad range of antimicrobial agents with high MIC values, and high rates of multi-resistance were observed. The distribution of the most common resistance genes in the *H. parasuis* isolates included bla_{TEM-1}, *int*, *gyrA*, and *rmtD*. This study also identified a number of ARGs that are clearly correlated with most of the AMR phenotypes observed in the *H.*

Table 7
Associations between phenotypes and genotypes of AMR in *H. parasuis* strains.

AMR	Characteristics of strain					Agreement between resistance phenotype and genotype (n = 56)		
	n-Pr[a]	ARGs	n-Gp[b]	P+/G−[c]	P−/G+[d]	OR	95% CI	p[e]
GEN	47	*rmtD*	28	20	1	10.8	1.3–93.4	0.02
KAN	27	*strA*	7	21	1	8.0	0.9–71.6	0.048
		strB	8	20	1	9.8	1.1–86.0	0.02
		aadA	18	11	2	19.6	3.9–100.1	< 0.0001
		rmtB	12	17	2	7.9	1.6–40.7	0.01
		rmtD	28	5	6	16.9	4.5–63.3	< 0.0001
		aacC2[aac(3)-Iic]	8	20	1	9.8	1.1–86.0	0.02
		aadA1	9	19	1	11.8	1.4–102.1	0.01
TOB	18	*strB*	8	11	1	23.5	2.6–212.7	0.001
		aadA	18	7	7	7.0	2.0–24.4	0.002
		rmtB	12	9	3	11.7	2.6–52.2	0.001
		rmtD	28	4	14	6.0	1.7–21.8	0.01
		aadA1	9	12	3	5.8	1.3–27.0	0.02
SPE	18	*aadA*	18	4	4	29.8	6.6–136.0	< 0.0001
		rmtB	12	8	2	22.5	4.1–123.2	< 0.0001
		rmtD	28	3	13	9.6	2.3–39.4	0.001
CFX	13	bla_{ROB-1}	6	8	1	26.3	2.7–255.7	0.002
TYL	37	*erm(B)*	22	17	2	10.0	2.0–49.6	0.003
		erm(C)	12	26	1	7.6	0.9–64.3	0.04
		lnu(C)	9	35	7	0.1	0.02–0.5	0.01
ERY	39	*erm(B)*	22	19	2	7.9	1.6–39.2	0.01
LIN	46	*lnu(C)*	9	42	5	0.1	0.02–0.5	0.01
CHL	51	*int*	43	9	1	18.7	1.9–187.4	0.01
FFC	5	*catI*	11	1	7	25.1	2.4–258.9	0.004
		cmlA	13	1	9	18.7	1.9–187.4	0.01

[a] n-Pr: number of strains expressing phenotype resistant to the indicated antimicrobial agents.
[b] n-Gp: number of strains carrying the indicated resistance genes.
[c] P+/G−: number of phenotypically resistance strains (P+) with no resistance genes (G−) for antimicrobials identified.
[d] P−/G+: number of phenotypically susceptible strains (P−) with one or more resistance genes (G+) for antimicrobials identified.
[e] Only the results for AMR phenotype that displayed an association with those genotype at a $p < 0.05$ are shown.

parasuis strains. The results suggest that the use of some of the antimicrobials that show a high resistance frequency should be limited, while fluoroquinolones, TIA, FFC, and DOX can still be used in clinics in Vietnam to control Glässer's disease. Moreover, the network of associations identified in this study will be useful for the development of policy and clinical practice guidelines to minimize AMR in Vietnam. It has been suggested that current attempts to limit the spread of AMR based on the prudent use of antimicrobials may prevent the selection of genes conferring resistance. Hence, assessment of AMR at the genetic level and the identification of associations between the phenotype and genotype resistance are critical tools in devising guidelines for the control of AMR.

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Compliance with ethics guidelines

Chao Nguyen Van, Lijun Zhang, Tam Vu Thi Thanh, Hung Pham Hoang Son, Tuan Tran Ngoc, Qi Huang, and Rui Zhou declare that they have no conflict of interest or financial conflicts to disclose.

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