



SHORT COMMUNICATION

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Carriage of the zoonotic organism *Streptococcus suis* in chicken flocks in Vietnam

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Abstract

Streptococcus suis infections are an emerging zoonotic agent causing severe disease in humans and a major pig pathogen worldwide. We investigated the colonization of *S. suis* in healthy chickens in different flocks ($n = 59$) as well as in-contact pigs in farms with *S. suis*-positive chickens ($n = 44$) in the Mekong Delta of Vietnam. *Streptococcus suis* was isolated from 20 (33.9%) chicken flocks and from all pigs investigated. Chicken isolates formed a distinct genotypic cluster compared with pig and human strains, although two chicken isolates (10%) clustered with pig isolates. Chicken isolates had unusually high levels of resistance against tetracycline (100%), clindamycin (100%) and erythromycin (95%); and intermediate resistance against penicillin (35%) and ceftriaxone (15%). Our findings suggest that chickens may potentially represent a source of *S. suis* infection to in-contact humans and pigs.

KEYWORDS

anti-microbial resistance, chicken, genotypic cluster, *Streptococcus suis*

1 | INTRODUCTION

Streptococcus suis (*S. suis*) is a global pathogen of pigs, but also an major zoonotic pathogen that causes severe human disease. Humans may become infected through exposure to live pigs or through ingestion of contaminated pork products (Dutkiewicz et al., 2017). In Vietnam, *S. suis* is the most common cause of bacterial meningitis in adults, mainly due to serotype 2 (Mai et al., 2008). The occurrence of anti-microbial resistance (AMR) in *S. suis* is of additional concern, since it may jeopardize the treatment of severe human infections (Varela et al., 2013). Although *S. suis* is mostly adapted to swine, it has also sporadically been isolated from cattle, sheep, goats, horses, cats, dogs and birds (Staats, Feder, Okwumabua, & Chengappa, 1997).

The domestic chicken (*Gallus gallus*) is numerically the most prevalent livestock species worldwide. In Vietnam, there are ~317 million chickens and small-scale chicken farming is practised in about eight million households (Desvaux, Ton, Hang, & Hoa, 2008). Many such farms also raise pigs, and often birds are unconfined, allowing frequent inter-species contact, as well as contact between animals (and their excreta) and humans. While a number of studies have reported *S. suis* in pig and pork products in Vietnam (Hoa et al., 2013; Huong et al., 2014; Ngo et al., 2011), no studies have to date investigated the role of the chicken species as a reservoir of this organism. The aims of this study were (a) to investigate the prevalence of *S. suis* in chickens and its potential association with pigs; and (b) to characterize phenotypic AMR profile and potential association with anti-microbial use (AMU) in the same flocks.

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2 | MATERIALS AND METHODS

We investigated chickens raised in Dong Thap Province (Mekong Delta), a region characterized by a high density of small-scale farms. Healthy chickens (3–5 months old, one per flock) were selected from 59 flocks (median 255 [interquartile range (IQR), 200–500] chickens per flock) which were randomly recruited as part of a larger study (Carrique-Mas & Rushton, 2017). Chickens were euthanized following humane procedures (Leary, Regensten, Shearer, Smith, & Golab, 2016), and swabs were collected from the infraorbital sinus in aseptic conditions. In all farms investigated, the closest distance between the chicken house/pen and any pigs (in the same or in a neighbouring farm) was recorded, alongside AMU by week. In farms testing positive for *S. suis* in chickens where pigs were also raised, tonsil swabs were collected from 1 to 5 live pigs. All chicken and pig samples were collected between February and July 2018.

Pig and chicken swabs were cultured on blood agar (Oxoid) and were incubated at 37°C at 5% CO₂ for 24 hr. Colonies showing typical *S. suis* morphology were confirmed by MALDI-TOF (Bucker). All isolates were investigated for their serotype 2 identity using the slide agglutination test (Statens Serum Institut) and for their susceptibility against 10 anti-microbials by Vitek (BioMerieux). We compared the phenotypic AMR pattern between chicken and pig isolates, as well as with all published *S. suis* anti-microbial susceptibility data for the period 2000–2019. The probability colonization of *S. suis* in chicken and in relation to the presence of pigs was investigated by logistic regression (Pinheiro, Bates, Debroy, Sarkar, & R Core Team, 2017). Whole-genome library preparation for chicken and pig isolates was carried out using Nextera XT and 100PE sequenced on HiSeq4000 Illumina platform (Macrogen). To compare our sequences with published data, sequences of 261 pig (Vietnam and UK) and 153 human patient (Vietnam) isolates reported in a previous publication (Weinert et al., 2015) were downloaded from ENA. Data were processed as previously described (Ashton et al., 2019) with alignment to *S. suis* P1/7 reference genome (NCBI accession AM946016). The resulting 286,039 variant positions were used as input to the *dudi.pca* function of the 'adequenet' R package (Jombart, 2008; R Core Team, 2019) to perform principle component analysis (PCA).

Impacts

- This paper reports a high prevalence of infection with *Streptococcus suis* (33.9%) in chickens in the Mekong Delta of Vietnam.
- Results from this study should help increase awareness of good farming practices to limit transmission of *S. suis* from animals to humans.

3 | RESULTS

Of the 59 chicken farms investigated, 10 (16.9%) raised both pigs and chickens; the median distance between the chicken house/pen and the nearest pig pen/s was 10 m [IQR 6–19]. In 29 (49.2%) farms, pigs were not raised in the farm itself, but there were nearby pig farms (located within 1 km). The remaining 20 flocks (33.9%) were located >2 km from a pig farm. A total of 20/59 (33.9%) chicken and 44/44 (100%) pig samples (from the 10 farms raising both pigs and chickens) yielded *S. suis* (20 chicken and 160 pig isolates). Of the 160 pig isolates, 39 (24.4%) were isolated from 23 pigs in the 10 farms where *S. suis* was also isolated from chickens. There was no difference in the prevalence of *S. suis* in chickens from pig-raising farms (5/10; 50%) and those in farms without pigs (15/49; 30.6% if nearby and remote farms combined; overall $p = .26$; Table 1).

None of the 59 (20 chicken, 39 pig) isolates investigated were serotype 2. PCA of whole-genome sequence data (available in ENA BioProject PRJNA626534) showed that the majority of chicken isolates formed a distinct population compared with pigs, and there was less overall diversity among chicken isolates. Despite this, two chicken isolates grouped near pig isolates (Figure 1a). Our chicken isolates formed a distinct cluster from pig and human isolates reported previously (Figure 1b). Furthermore, the PCA distance between chicken and pig isolates in same farm was similar to the distance between chicken and pig isolates from different farms (Figure 1c), suggesting that direct contact with pigs is not the main source of infection to chickens.

Among chicken isolates, the highest prevalence of resistance corresponded to clindamycin (100%), tetracycline (100%) and

Type of flock (n)	Age in weeks [IQR]	No. of positive/no. of tested	Prevalence (%) [95% CI]	Odd ratio (p-value)
Chicken flocks in farms with pigs (n = 10)				
Chicken	13 [3–6]	5/10	50 [19–80]	1.0
Pig	16 [10–21]	44/44	100	
Chicken flocks with neighbouring pig farms (n = 29)				
Chicken	13 [9–16]	7/29	24 [12–42]	0.32 (.13)
Chicken flocks without neighbouring pig farms (n = 20)				
Chicken	15 [9–17]	8/20	40 [20–64]	0.67 (.60)

Odds ratios (OR) were derived from logistic regression models.

TABLE 1 Prevalence of *Streptococcus suis* by types of farm in Dong Thap, Vietnam

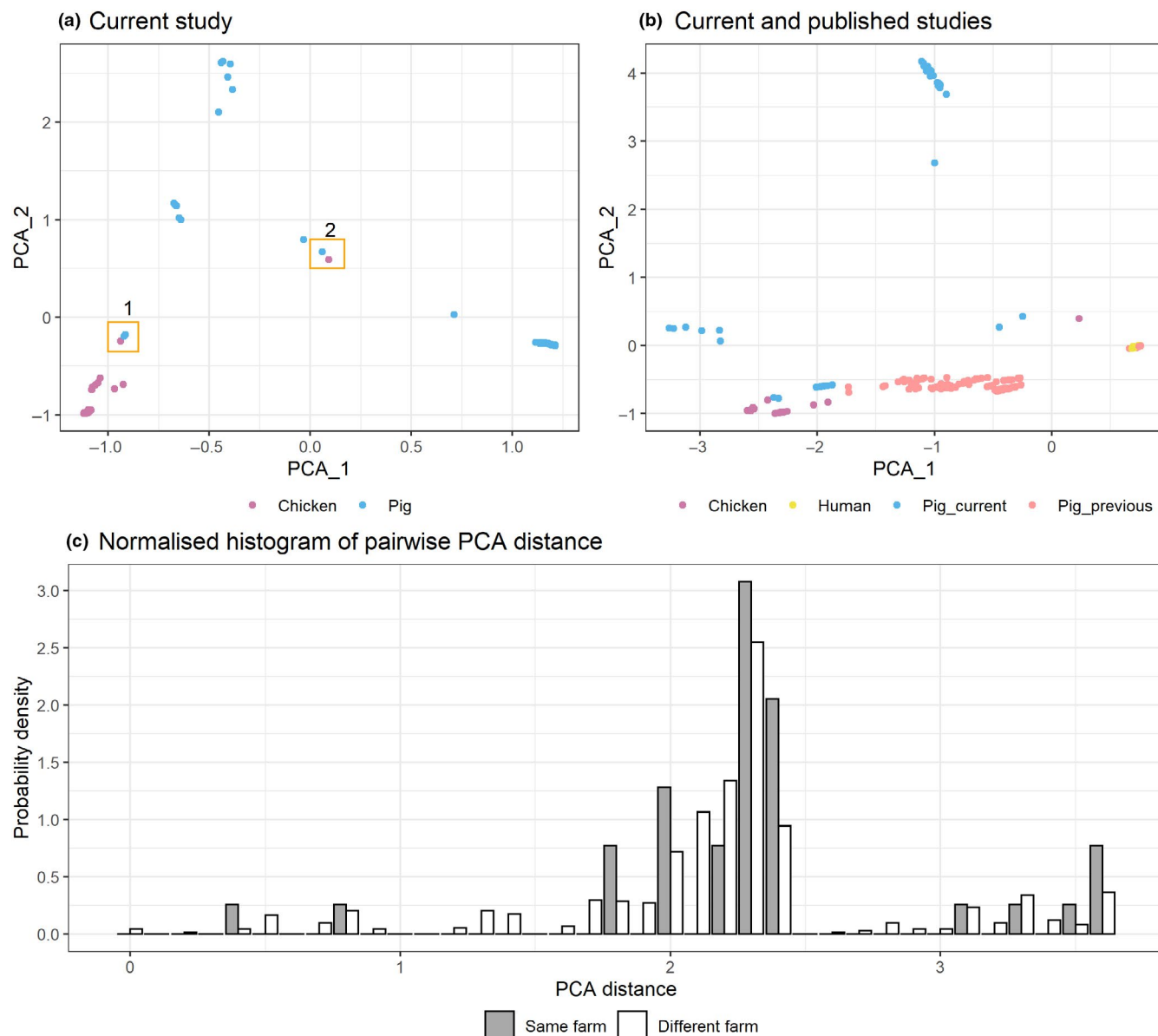


FIGURE 1 (a) Principle component analysis (PCA) of the variant positions from the novel isolates obtained as part of this study (20 chicken and 39 pig isolates). Shows that chicken *Streptococcus suis* is generally distinct from pig *S. suis*, but there are two chicken isolates close to pig isolates. (b) PCA of variant positions including isolates from this study and from Weinert et al. (2015) (including 261 pig isolates from the UK and Vietnam and 153 patient isolates from central and southern Vietnam, 2000–2010). This shows that there is no overlap between the human and chicken *S. suis* populations sampled here. (c) Normalized histogram of pairwise distance between pairs of isolates where one of the pair was a chicken and the other was a pig, either from the same farm or different farms (indicated by bar fill). The distance is the distance between the isolates in the first two dimensions of the PCA. This shows that pairs of pigs/chickens from different farms have a very similar distribution of pairwise distances to those from the same farm, indicating frequent transmission between farms or from an external reservoir [Colour figure can be viewed at wileyonlinelibrary.com]

erythromycin (95%). A total of 35% and 15% of isolates showed intermediate resistance to penicillin and ceftriaxone, respectively. In contrast, all chicken isolates were susceptible to ampicillin, cefotaxime, levofloxacin, linezolid and vancomycin (Table 2). Overall, we did not find evidence of differences in the prevalence of multidrug resistance between chicken (95%) and pig isolates (87%) ($p = .63$). Chicken isolates were fully resistant against three of 10 anti-microbials tested, in contrast to pig isolates (eight anti-microbials). Notably, chicken isolates had higher levels of resistance against levofloxacin

than pig isolates, but this should be interpreted cautiously since these analyses ignored farm clustering. In one of the two chicken-pig clusters (cluster 1, Figure 1a), isolates from both species had identical AMR profile although chicken and pig isolates came from different farms. In contrast, AMR profile of pig isolates had considerable diversity even if they clustered together (data not shown), suggesting that AMR profiles are probably driven by differences in plasmid composition. No significant associations between AMU in flocks and phenotypic AMR in chicken *S. suis* were found (Table A1).

TABLE 2 Antimicrobial susceptibility of 20 *Streptococcus suis* isolates from chickens and 39 isolates from pigs in Dong Thap, Vietnam

Anti-microbial	Chicken isolates (n = 20)			Pig isolates (n = 39)			p-value
	MIC (median [IQR])	No. of intermediate resistant ^a (%)	No. of fully resistant (%)	MIC (median [IQR])	No. of intermediate resistant (%)	No. of fully resistant (%)	
Penicillin	≤0.06 [≤0.06–0.25]	7 (35)	0 (0)	0.25 [≤0.12–1]	14 (36)	5 (13)	.47
Ampicillin	All ≤ 0.25	0 (0)	0 (0)	≤0.25 [≤0.25–≤0.25]	5 (13)	2 (5)	.11
Ceftriaxone	0.25 [≤0.12–1]	3 (15)	0 (0)	0.5 [≤0.12–2]	4 (10)	6 (15)	.55
Cefotaxime	≤0.12 [≤0.12–1]	0 (0)	0 (0)	0.25 [≤0.12–2]	3 (8)	6 (15)	.05
Clindamycin	All ≥ 1	0 (0)	20 (100)	≥1 [≥1–≥1]	0 (0)	37 (95)	.79
Erythromycin	≥8 [≥ 8–≥8]	0 (0)	19 (95)	≥8 [4– ≥8]	0 (0)	33 (85)	.46
Levofloxacin	≤0.25 [≤0.25–0.5]	0 (0)	0 (0)	1 [0.5–8]	3 (8)	11 (28)	<.01
Linezolid	All ≤ 2	0 (0)	0 (0)	All ≤ 2	0 (0)	0 (0)	NC
Tetracycline	All ≥ 16	0 (0)	20 (100)	≥16 [≥16–≥16]	0 (0)	36 (92)	.52
Vancomycin	All ≤ 0.12	0 (0)	0 (0)	≤0.12 [≤0.12–≤0.12]	0 (0)	0 (0)	NC
MDR			19 (95)			34 (87)	.63

Note: Chi-square tests are only crude approximation since they ignore clustering of host species within farms.

Abbreviations: IQR, interquartile range; MDR, multidrug resistance; MIC, minimum inhibitory concentration (μg/μl); NC, not calculated.

^aBased on CLSI breakpoints for *Streptococcus* spp. viridans group (Clinical & Laboratory Standards Institute, 2016).

Data on AMR of 260 *S. suis* strains from patients and 5,696 strains from pigs have been reported in a total of 19 publications (Table A2, summarized in Table A3). Compared to pig and human isolates, chicken isolates had a significantly higher prevalence of resistance against penicillin (p -values = .02 and <.01, respectively), clindamycin (both p -values < .01) and erythromycin (both p -values < .01).

4 | DISCUSSION

The relatively high prevalence of *S. suis* (~34%) in chickens even in the absence of pigs demonstrates that chickens can be a self-sustaining reservoir of *S. suis*. The prevalence of AMR in chickens was higher than observed in human patients, notably with regard to penicillin, ceftriaxone, erythromycin and clindamycin, which is of concern, given that β -lactams (including penicillin and ceftriaxone) are currently the most effective anti-microbials to treat human infections (Dutkiewicz et al., 2017). The lack of association between AMU in flocks and the observed profiles suggests that chickens may not harbour these organisms long-term and may acquire *S. suis* from additional non-pig source. Whole-genome sequencing analyses showed that most (18/20) chicken isolates formed a distinct cluster, while two were genetically linked to pig isolates. Given that small farms are prevalent across Southeast Asia, and that animals are typically raised in conditions of limited biosecurity and biocontainment, chickens may potentially represent a source of *S. suis* infection to in-contact humans as well as to pigs. This suggests that in small-scale farms typical of the Mekong Delta, raising mixed species present opportunities for inter-species transmission. Since slaughtering of birds is often performed with low levels of personal protection, this therefore puts a considerable fraction of the rural population in Southeast

Asia in an additional, previously unassessed, risk of zoonotic infection with *S. suis*.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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APPENDIX 1

TABLE A1 Association between anti-microbial use (AMU) on farms and the prevalence of phenotypic resistance in 18 chicken flocks in Dong Thap, Vietnam (there were two flocks where AMU data were not available)

Anti-microbial class used	Anti-microbial tested	Used		Did not use		p-value
		No. of flocks	No. of R ^a (%)	No. of flocks	No. of R ^a (%)	
Beta-lactams	Penicillin	6	1 (16.7)	12	5 (42)	.39
	Ampicillin	6	0 (0)	12	0 (0)	NC
	Ceftriaxone	6	0 (0)	12	3 (25)	.50
	Cefotaxime	6	0 (0)	12	0 (0)	NC
Lincosamides	Clindamycin	1	1 (100)	17	17 (100)	NC
Macrolides	Erythromycin	8	8 (100)	10	9 (90)	.99
Quinolones	Levofloxacin	4	0 (0)	14	0 (0)	NC
Tetracyclines	Tetracycline	12	12 (100)	6	6 (100)	NC
Polypeptides	Vancomycin	13	0 (0)	5	0 (0)	NC

Abbreviation: NC, not calculated.

^aR, resistance, including intermediate resistance, p-values derived from chi-square test.

APPENDIX 2

TABLE A2 Prevalence of anti-microbial resistance by country and host

Publication	DOI	Country	Host	No. of isolates
Martel et al. (2001)	https://doi.org/10.1016/S0378-1135(01)00426-6	Belgium	Diseased pigs	87
Marie et al. (2002)	https://doi.org/10.1093/jac/dkj099	France	Diseased pigs	83
Tian et al. (2004)	https://doi.org/10.1016/j.vetmic.2004.07.009	Denmark	Diseased pigs	103
Wisselink et al. (2006)	https://doi.org/10.1016/j.vetmic.2005.10.035	Europe	Diseased pigs	384
Hoa et al. (2013)	https://doi.org/10.3201/eid1902.120470	Vietnam	Diseased pigs	9
Zhang et al. (2015)	https://doi.org/10.1155/2015/284303	China	Diseased pigs	34
Gurung et al. (2015)	https://doi.org/10.1128/JCM.00123-15	Korea	Diseased pigs	56
Yongkiettrakul. (2019)	https://doi.org/10.1186/s12917-018-1732-5	Thailand	Diseased pigs	46
Hernandez-Garcia et al. (2017)	https://doi.org/10.1016/j.vetmic.2017.06.002	England	Diseased pigs	93
Hernandez-Garcia et al. (2017)	https://doi.org/10.1016/j.vetmic.2017.06.002	England	Diseased pigs	117
Chen et al. (2013)	https://doi.org/10.1292/jvms.12-0279	China	Diseased pigs	106
van Hout et al. (2016)	https://doi.org/10.1016/j.vetmic.2016.03.014	The Netherlands	Diseased pigs	1,163
Vela et al. (2005)	https://doi.org/10.1016/j.vetmic.2004.10.009	Spain	Diseased pigs	151
Marie et al. (2002)	https://doi.org/10.1093/jac/dkj099	France	Healthy pigs	27
Hendriken et al. (2008)	https://doi.org/10.1186/1751-0147-50-19	Denmark	Healthy pigs	557
Hendriken et al. (2008)	https://doi.org/10.1186/1751-0147-50-19	England	Healthy pigs	34
et al. (2008)	https://doi.org/10.1186/1751-0147-50-19	England	Healthy pigs	53
Hendriken et al. (2008)	https://doi.org/10.1186/1751-0147-50-19	The Netherlands	Healthy pigs	762
Hendriken et al. (2008)	https://doi.org/10.1186/1751-0147-50-19	Poland	Healthy pigs	111
Hendriken et al. (2008)	https://doi.org/10.1186/1751-0147-50-19	Poland	Healthy pigs	150
Hendriken et al. (2008)	https://doi.org/10.1186/1751-0147-50-19	Poland	Healthy pigs	151
Hendriken et al. (2008)	https://doi.org/10.1186/1751-0147-50-19	Portugal	Healthy pigs	24
Zhang et al. (2008)	https://doi.org/10.1016/j.vetmic.2008.04.005	China	Healthy pigs	421
Hoa et al. (2011)	https://doi.org/10.1371/journal.pone.0017943	Vietnam	Healthy pigs	45
Lakkitjaroen et al. (2011)	Not available	Thailand	Healthy pigs	52
Soares et al. (2014)	Not available	Brazil	Healthy pigs	260
Zhang et al. (2015)	https://doi.org/10.1155/2015/284303	China	Healthy pigs	62
Gurung et al. (2015)	https://doi.org/10.1128/JCM.00123-15	Korea	Healthy pigs	171
Hernandez-Garcia et al. (2017)	https://doi.org/10.1016/j.vetmic.2017.06.002	England	Healthy pigs	66
Hernandez-Garcia et al. (2017)	https://doi.org/10.1016/j.vetmic.2017.06.002	England	Healthy pigs	129
Yongkiettrakul. (2019)	https://doi.org/10.1186/s12917-018-1732-5	Thailand	Healthy pigs	189
Marie et al. (2002)	https://doi.org/10.1093/jac/dkj099	France	Humans	25
Chu et al. (2009)	https://doi.org/10.1016/j.ijantimicag.2009.01.007	Hong Kong	Humans	33
Hoa et al. (2011)	https://doi.org/10.1186/1471-2334-11-6	Vietnam	Humans	175
Yongkiettrakul. (2019)	https://doi.org/10.1186/s12917-018-1732-5	Thailand	Humans	27

Erythromycin	Penicillin	Tetracycline	Clindamycin	Ampicillin	Vancomycin	Ceftriaxone	Cefotaxime	Levofloxacin
71								
74.7	1.2							
40.8	0	24.3						
	0	75						
		66						
5.9	0	82.3	5.9	0				
	37.5		87.5	19.6				
80.4	28.3	93.5	89.1	13.1	8.7		17.4	10.9
45	3	92						
55	3	97						
67.9		99.1	67.9			0.9		2.8
	1.2	86.7	49.3	0.5				
90.7	4	95.4	87.4					
88.9	0							
29.1	0.9	52.2						
36	0	68						
50	0	68						
35	0	48						
30.6	8.1	64						
	10.6	73.3						
	7.9	55						
75	13	92						
73.2	51.8	92.9	73.2	34				
51	0	100			0			
	39	96		26.9				
52.3	51.2	97.7	93.7	33.1				37.7
56.4	3.2	91.9	56.4	0				
	62.6		98.2	17				
39	12	100						
42	17	100						
84.1	68.8	94.7	96.3	33.9	2.6		5.3	29.1
28	0							
21.2		100	21.2					
22.2	0	90.9			0	0		
85.2	0	100	81.5	0	0		0	0

APPENDIX 3

TABLE A3 Summary results of the prevalence of anti-microbial resistance in *Streptococcus suis* isolates by host origin from 19 previously published studies

	Current study	Published studies							
	Chicken isolates	Pig isolates				Human isolates			
Anti-microbial	% R ^a	No. of studies	No. of tested	% R	<i>p</i> -value	No. of studies	No. of tested	% R	<i>p</i> -value
Penicillin	35	14	5,494	13.7	.02	3	227	0	<.01
Ampicillin	0	7	2,454	13.0	.16	1	27	0	NC
Ceftriaxone	15	1	106	9.4	.72	1	175	0	<.01
Cefotaxime	0	1	235	7.6	.41	1	27	0	NC
Clindamycin	100	8	2,659	46.7	<.01	2	60	48.3	<.01
Erythromycin	95	12	3,560	50.7	<.01	4	260	29.2	<.01
Levofloxacin	0	3	601	26.8	.02	1	27	0	NC
Tetracycline	100	14	5,272	77.9	.04	3	235	93.1	.47
Vancomycin	0	3	280	3.2	.89	2	202	0	NC

Note: Reported *p*-values correspond to comparisons with 20 chicken *S. suis* isolates in this study; chi-square tests are only crude approximation, since they ignore clustering by study and the different study sizes.

Abbreviation: NC, not calculated.

^aR, resistance, including intermediate resistance.