Antimicrobial susceptibility of *Streptococcus suis* isolated from diseased pigs, asymptomatic pigs, and human patients in Thailand

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**Abstract**

**Background:** Prophylaxis and treatment of emerging zoonotic *Streptococcus suis* infection in agricultural and healthcare settings mainly rely on antibiotics. However, continued use of antibiotics contributes to emergence and widespread of antibiotic resistant *S. suis* becomes a significant challenge in many endemic countries, including Thailand. Meanwhile, the knowledge of antibiotic susceptibility patterns of bacterial pathogens is required for overcoming the antimicrobial resistance problem, the information of antibiotic susceptibility of *S. suis* strains isolated in Thailand remains limited. This study aims to assess the susceptibility of Thai-isolated *S. suis* strains to different antibiotic classes in order to gain an insight into the distribution of antibiotic-resistant patterns of *S. suis* strains in different regions of Thailand.

**Results:** This study revealed the antimicrobial resistance and multidrug resistance of 262 *S. suis* strains isolated in different regions of Thailand. Susceptibility testing indicated widespread resistance to macrolides and tetracyclines of *S. suis* strains in the country. Beta-lactam antibiotic drugs (including cefotaxime and ceftiofur), vancomycin, chloramphenicol, as well as florfenicol were potential lyth es te f f e ct i v e tr e atm e nt t h e r a p e u t i c d r u g s f o r t h e t r e a t m e n t o f *S. suis* infection in both pigs and humans. High prevalence of intermediate susceptibility of *S. suis* isolated from asymptomatic pigs for penicillin G, gentamicin, enrofloxacin, and norfloxacin could be the premise of the emergence of *S. suis* antibiotic resistance. Resistance was also found in *S. suis* strains isolated from asymptomatic pigs indicating that they could act as reservoirs of antibiotic resistance genes.

**Conclusions:** To the best of our knowledge, this is the first report on antimicrobial resistance of a large collection of *S. suis* strains isolated from pigs and humans in Thailand. It revealed the multidrug resistance of *S. suis* strains in pigs and humans. The information gained from this study raises an awareness and encourage best practices of appropriate antibiotic drug prescribing and use among human health and agriculture sectors.

**Keywords:** *Streptococcus suis*, Zoonosis, Bacterial meningitis, Antimicrobial susceptibility, Antimicrobial resistance, Antibiotic resistance, Multidrug resistance
Background

Streptococcus suis is facultative anaerobic gram-positive α-hemolytic coccus and classified, based on cell-wall antigenic determinants, to be related to Lancefield group D streptococci. It is an important zoonotic bacterial pathogen of pigs worldwide. S. suis naturally colonizes upper respiratory tract of pigs, particularly the tonsils and nasal cavities [1, 2]. It can cause systemic diseases in newborn and, more commonly, weaned piglets, resulting in sepsis, meningitis, endocarditis and arthritis [3, 4]. Moreover, S. suis is an emerging zoonotic pathogen of humans who came in contact with infected pigs or consumed pork products that get contaminated with this pathogenic bacteria [5, 6]. Thirty-five serotypes (serotype 1–34 and serotype 1/2) of S. suis strains have been identified based on polysaccharide capsules by using the coagglutination method [7–9]. However, recent studies, using DNA-based approaches, have shown that serotypes 20, 22, 26, 33, 32, and 34 belong to novel bacterial species [10, 11]. Moreover, novel 9 capsular polysaccharide synthesis (cps) loci (NCLs) of non-typable S. suis strains have been identified based on DNA sequencing [12, 13]. Therefore, strict S. suis species currently comprise of 38 serotypes. Serotype 2 of S. suis is considered as the most common type recovered from both pigs and humans worldwide and other serotypes, such as 1, 3, 5, 7, 8, 9, 14, 16, 21 and 24, are also capable to induce disease in pigs and, some of them, also in humans [7–9, 14, 15]. To prevent or control S. suis infection in pigs and to deliver safer pork products for consumers, antimicrobial agents have long been applied in pig farming industries. However, the increased usage of antimicrobial agents in pigs and humans causes the antimicrobial resistance [16] that has become a global problem in recent years.

The antimicrobial agents and antibiotic classes used for prophylaxis and treatment of S. suis infections in pigs and humans are somewhat similar. Beta-lactam antibiotics (penicillin, ceftriaxone and cefotiofur) and fluoroquinolone antibiotics such as enrofloxacin are used in pigs and humans to treat S. suis infections [16–18]. Generally, three major antibiotics (penicillin, ampicillin and trimethoprim-sulfonamides) are frequently used in S. suis infection [16]. The increasing cases of antimicrobial resistance in S. suis isolated from pigs and humans have been reported from many countries in America, Asia and Europe [19, 20]. Notably, resistant S. suis has been identified as a reservoir for antibiotic resistance genes which can be transferred horizontally to streptococcal human pathogens such as S. pyogenes, S. pneumoniae and S. agalactiae [21].

Acute bacterial infection for humans and animals relies on effective antibiotic treatment. Monitoring the susceptibility pattern of bacterial pathogens to antibiotic drugs is therefore an important tool that provides an evidence-based guidance for further optimizing effective antimicrobial treatment options and following up the emergence of antibiotic drug resistance. The prevalence of antimicrobial resistance (AMR) pattern of particular pathogen is geographically variable. Hence, knowledge of the susceptibility pattern of bacterial pathogen, in certain regions is necessary for controlling the AMR problem. So far, antimicrobial susceptibility data of S. suis isolated in Thailand has not been well reported and available studies have focused on human cases [22–26]. Lakkitjareon et al. investigated the antimicrobial profile of 52 S. suis isolates from healthy pigs in Northern Thailand during 2008 to 2009 by disk diffusion susceptibility test [27]. The results showed high rate of lincomycin and tetracycline resistance but most of the isolates remained susceptible to ceftiofur, ampicillin, amoxicillin, penicillin and enrofloxacin.

The study described herein, aimed to assess the antimicrobial susceptibility of S. suis isolated from both human patients (epidemic and sporadic cases) and pigs (diseased and asymptomatic pigs) in northern, central, and southern regions of Thailand. The information of antimicrobial resistance of Thai-isolated S. suis strains could have implications for optimizing the therapeutic treatment for zoonosis and controlling the emergence of antibiotic-resistant S. suis in the country and worldwide.

Results

The antimicrobial susceptibility of 262 Thai S. suis isolated strains was determined using 20 antibiotic drugs with different modes of inhibition. It is noted that multi-drug resistance (MDR) is defined as resisting to at least three different classes of agents [32]. The result showed that there were 144 distinct antimicrobial resistance (AMR) patterns (Additional file 1: Table S1). None of Thai S. suis isolated strains used in this study exhibited drug susceptibility to all tested 20 antibiotic drugs. Overall, 99.3% (260/262) of Thai S. suis strains resisted to at least one antibiotic drug. Two out of 262 strains isolated from diseased or asymptomatic pigs shared the same antimicrobial susceptibility profile with susceptibility to 19 tested antibiotic drugs and intermediate susceptibility to norfloxacin (AMR pattern No. 1). A similar AMR pattern (AMR pattern No. 78) was observed in S. suis serotype 2 strains isolated from both human patients and asymptomatic pigs during 2006–2007. In addition, S. suis serotype 2 strains isolated from human patients and asymptomatic pigs from Northern Thailand (during 2006–2007) shared a similar AMR pattern (AMR pattern No. 79) with S. suis serotype 2 strains isolated from diseased pigs from central regions of Thailand (during 2012–2015).

The MDR S. suis strains were isolated from pigs only. Out of 235 pig-isolated S. suis strains, 118 strains...
isolated from asymptomatic pigs (118/253, 50.2%) and 20 strains isolated from diseased pigs (20/235, 8.5%) are MDR S. suis strains, displaying 90 different AMR patterns (Additional file 1: Table S1). Most of the MDR S. suis strains were AA (63 strains) and followed by non-serotype 2 (42 strains), NT (24 strains), and serotype 2 (9 strains). Two MDR S. suis strains, resisting to 17 out of 20 antibiotic drugs (AMR pattern No. 136) were isolated from two diseased pigs during 2006–2007. They were found in the central regions of the country where there have been intensive swine farming and production. The most predominant MDR S. suis strains isolated from diseased pigs resistant to AZM/CLI/DOX/ERY/GEN/TET/TIA/NOR/SXT (AMR pattern No. 97, total 12 strains) were found in different isolation periods and different regions of the country.

A total of 110 Thai S. suis strains, including 27 human-isolated strains, 30 strains isolated from diseased pigs, and 53 strains isolated from asymptomatic pigs, were susceptible to all 6 antibiotics drugs inhibiting cell-wall synthesis. One S. suis strain isolated from southern diseased pig resisted to all these 6 antibiotic drugs (AMR pattern No. 139, Additional file 1: Table S1). A total of 260 strains resisted to at least one antibiotic drug acting on protein synthesis, suggesting less effectiveness of these particular drugs for the treatment of S. suis infection in both human patients and pigs. Intermediate susceptibility to at least one antibiotic drug inhibiting DNA synthesis was observed for 118 Thai S. suis isolated strains, including 23 human-isolated strains, 24 strains isolated from diseased pigs, and 71 strains isolated from asymptomatic pigs, suggesting the emerging of antimicrobial resistance (AMR) for these antibiotic drugs.

The distribution of antimicrobial susceptibility of Thai S. suis isolated strains is summarized in Table 1. Thai S. suis isolated strains showed high level of antimicrobial susceptibility to CTX (93.1%), CTF (94.7%), VAN (96.6%), and FFC (92.4%). Susceptibility of Thai S. suis isolated strains to CLI (6.5%), DOX (9.2%), TET (5.0%) and TIA (2.3%) indicates the high prevalence of antibiotic-resistant S. suis against these drugs. Intermediate level of antibiotic susceptibility was relatively high for PEN (33.2%), GEN (23.3%), ENR (21.4%), and NOR (27.9%), suggesting the emergence of AMR problem for these antibiotic drugs in S. suis.

Table 1 Antimicrobial susceptibility of Thai Streptococcus suis

| Antibiotic drugs | Thai-isolated S. suis (262 strains) | Susceptible | Intermediate | Resistant |
|------------------|-------------------------------------|-------------|--------------|----------|
| Ampicillin (AMP) | 192 (73.3%)                         | 25 (9.5%)   | 45 (17.2%)   |
| Cephalexin (CFL) | 165 (63.0%)                         | 5 (1.9%)    | 92 (35.1%)   |
| Cefotaxime (CTX) | 244 (93.1%)                         | 4 (1.5%)    | 14 (5.3%)    |
| Cefiofur (CTF)   | 248 (94.7%)                         | 5 (1.9%)    | 9 (3.4%)     |
| Penicillin G (PEN) | 119 (45.4%)                      | 87 (33.2%)  | 56 (21.4%)   |
| Vancomycin (VAN) | 253 (96.6%)                         | 4 (1.5%)    | 4 (1.5%)     |
| Azithromycin (AZM) | 40 (15.3%)                        | 4 (1.5%)    | 218 (83.2%)  |
| Chloramphenicol (CHL) | 229 (87.4%)                     | 17 (6.5%)   | 16 (6.1%)    |
| Clindamycin (CLI) | 17 (6.5%)                          | 0 (0%)      | 245 (93.5%)  |
| Doxycycline (DOX) | 24 (9.2%)                          | 5 (1.9%)    | 233 (88.9%)  |
| Erythromycin (ERY) | 43 (16.4%)                        | 7 (2.7%)    | 212 (80.9%)  |
| Florfenicol (FFC) | 242 (92.4%)                        | 2 (0.8%)    | 18 (6.8%)    |
| Gentamicin (GEN) | 100 (38.2%)                         | 61 (23.3%)  | 101 (38.5%)  |
| Tetracycline (TET) | 13 (5.0%)                          | 7 (2.7%)    | 242 (92.4%)  |
| Tiamulin (TIA) | 6 (2.3%)                            | 45 (17.2%)  | 211 (80.5%)  |
| Ciprofloxacin (CIP) | 155 (59.2%)                      | 36 (13.7%)  | 71 (27.1%)   |
| Enrofloxacin (ENR) | 143 (54.6%)                        | 56 (21.4%)  | 63 (24.0%)   |
| Norfloxacin (NOR) | 65 (24.8%)                         | 73 (27.9%)  | 124 (47.3%)  |
| Levofloxacin (LEV) | 202 (77.1%)                        | 6 (2.3%)    | 54 (20.6%)   |
| sulfamethoxazole /Trimethoprim (SXT) | 95 (36.3%)                        | 10 (3.8%)   | 157 (59.9%)  |

The antimicrobial susceptibility of 262 Thai S. suis isolated strains was determined using 20 antibiotic drugs with different modes of inhibition, including beta-lactams (ampicillin, cephalexin, cefotaxime, cefiofur, penicillin G), glycopeptide (vancomycin), aminoglycoside (gentamicin), tetracyclines (doxycycline, tetracycline), amphenicols (chloramphenicol, florfenicol), pleuromutilin (tiamulin), macrolides (azithromycin, erythromycin), lincomamide (clindamycin), fluoroquinolones (ciprofloxacin, enrofloxacin, levofloxacin), quinolone (norfloxacin), and folate inhibitors (sulfamethoxazole/trimethoprim). S: susceptible; I: intermediate; R: resistant
The distribution of antibiotic susceptibility according to the sources of *S. suis* isolation is presented in Table 2 and Additional file 2: Figure S1. The statistical analysis revealed no significant correlation between the source of bacterial isolation and susceptibility of the bacteria towards antibiotic drugs acting on protein synthesis, including AZM, CHL, DOX, and TET. The results suggested that, among those three sources, the antibiotic resistant patterns of the isolated *S. suis* were similar. On the contrary, for the other drugs, there were associations between the resistant pattern and the source of isolation.

All 27 strains of *S. suis* isolated from human patients showed the highest level of antimicrobial susceptibility (100%) to AMP, CFL, CTX, CTF, PEN, VAN, LEV, and SXT. The data supports that these antibiotic drugs could still be effective drugs for treating *S. suis* infection in human patients. High-level sensitivity to CTX (82.6 and 94.7%), CTF (84.8 and 96.3%), and VAN (91.3 and 97.4%) were also observed in *S. suis* isolated from diseased and asymptomatic pigs. Although *S. suis* strains isolated from pigs remained highly sensitive to CTX, CTF, and VAN, their resistance against all these drugs showed the highest level of antimicrobial susceptibility.

Table 2 Antimicrobial susceptibility of Thai *Streptococcus suis* isolated from human patients (27 strains), diseased pigs (46 strains), and asymptomatic pigs (189 strains)

| Antibiotic drugs | Human patients 27 strains | Diseased pigs 46 strains | Asymptomatic pigs 189 strains |
|------------------|---------------------------|--------------------------|-------------------------------|
|                  | S  | I  | R  | S  | I  | R  | S  | I  | R  | P-value |
| AMP              | 27 (100%) | 0 (0%) | 0 (0%) | 40 (86.9%) | 2 (4.4%) | 4 (8.7%) | 125 (66.1%) | 23 (12.2%) | 41 (21.7%) | 0.001* |
| CFL              | 27 (100%) | 0 (0%) | 0 (0%) | 34 (73.9%) | 1 (2.2%) | 11 (23.9%) | 104 (55.0%) | 4 (2.1%) | 81 (42.9%) | < 0.001* |
| CTX              | 27 (100%) | 0 (0%) | 0 (0%) | 38 (82.6%) | 2 (4.4%) | 6 (13.0%) | 179 (94.7%) | 2 (1.1%) | 8 (4.2%) | 0.029* |
| CTF              | 27 (100%) | 0 (0%) | 0 (0%) | 39 (84.8%) | 3 (6.5%) | 4 (8.7%) | 182 (96.3%) | 2 (1.1%) | 5 (2.6%) | 0.018* |
| PEN              | 27 (100%) | 0 (0%) | 0 (0%) | 33 (71.7%) | 8 (17.4%) | 5 (10.9%) | 59 (31.2%) | 79 (41.8%) | 51 (27.0%) | < 0.001* |
| VAN              | 27 (100%) | 0 (0%) | 0 (0%) | 42 (91.3%) | 1 (2.2%) | 3 (6.5%) | 184 (97.4%) | 4 (2.1%) | 1 (0.5%) | 0.042* |
| AZM              | 5 (18.5%) | 0 (0%) | 22 (81.5%) | 9 (19.6%) | 0 (0%) | 37 (80.4%) | 26 (13.8%) | 4 (2.1%) | 159 (84.1%) | 0.619 |
| CHL              | 24 (88.9%) | 3 (11.1%) | 0 (0%) | 37 (80.4%) | 4 (8.7%) | 5 (10.9%) | 168 (88.9%) | 10 (5.3%) | 11 (5.8%) | 0.254 |
| CLI              | 5 (18.5%) | 0 (0%) | 22 (81.5%) | 5 (10.9%) | 0 (0%) | 41 (89.1%) | 7 (3.7%) | 0 (0%) | 182 (96.3%) | 0.006* |
| DOX              | 0 (0%) | 0 (0%) | 27 (100%) | 2 (4.4%) | 2 (4.4%) | 42 (91.3%) | 22 (11.6%) | 3 (1.6%) | 164 (86.8%) | 0.114 |
| ERY              | 4 (14.8%) | 4 (14.8%) | 19 (70.4%) | 9 (19.6%) | 1 (2.2%) | 36 (78.2%) | 30 (15.9%) | 2 (1.1%) | 157 (83.0%) | 0.001* |
| FFC              | 26 (96.3%) | 1 (3.7%) | 0 (0%) | 36 (78.2%) | 0 (0%) | 10 (21.8%) | 180 (95.3%) | 1 (0.5%) | 8 (4.2%) | < 0.001* |
| GEN              | 22 (81.5%) | 3 (11.1%) | 2 (7.4%) | 11 (23.9%) | 9 (19.6%) | 26 (56.5%) | 67 (35.5%) | 49 (25.9%) | 73 (38.6%) | < 0.001* |
| TET              | 0 (0%) | 0 (0%) | 27 (100%) | 3 (6.5%) | 2 (4.4%) | 41 (89.1%) | 10 (5.3%) | 5 (2.6%) | 174 (92.1%) | 0.552 |
| TIA              | 0 (0%) | 23 (85.2%) | 4 (14.8%) | 4 (8.7%) | 5 (10.9%) | 37 (80.4%) | 2 (1.1%) | 17 (9.0%) | 170 (89.9%) | < 0.001* |
| CIP              | 20 (74.1%) | 5 (18.5%) | 2 (7.4%) | 32 (69.5%) | 5 (10.9%) | 9 (19.6%) | 103 (54.5%) | 26 (13.8%) | 60 (31.7%) | 0.048* |
| ENR              | 15 (55.6%) | 12 (44.4%) | 0 (0%) | 23 (50.0%) | 15 (32.6%) | 8 (17.4%) | 105 (55.6%) | 29 (15.3%) | 55 (29.1%) | < 0.001* |
| NOR              | 5 (18.5%) | 19 (70.4%) | 3 (11.1%) | 13 (28.3%) | 13 (28.3%) | 20 (43.4%) | 47 (24.9%) | 41 (21.7%) | 101 (53.4%) | < 0.001* |
| LEV              | 27 (100%) | 0 (0%) | 0 (0%) | 41 (89.1%) | 1 (2.2%) | 4 (8.7%) | 134 (70.9%) | 5 (2.6%) | 50 (26.5%) | 0.003* |
| SXT              | 27 (100%) | 0 (0%) | 0 (0%) | 18 (39.1%) | 6 (13.0%) | 22 (47.9%) | 50 (26.5%) | 4 (2.1%) | 135 (71.4%) | < 0.001* |

AMP ampicillin, AZM azithromycin, CTX cefotaxime, CTF cefotirix, CFL cepalexin, CHL chloramphenicol, CIP ciprofloxacin, CLI clindamycin, DOX doxycycline, ENR enrofloxacin, ERY erythromycin, FFC florfenicol, GEN gentamycin, LEV levofloxacin, NOR norfloxacin, PEN penicillin G, SXT sulfamethoxazole/trimethoprim, TET tetracyclins, TIA tiamulin, VAN: vancomycin. S susceptible; I intermediate; R resistant. The asterisk indicates statistical significance with *P*-value < 0.05.
In addition, the data clearly showed that tiamulin which has a long history of use in veterinary medicine was significantly less effective for strains isolated from pigs.

The susceptibility test using fluoroquinolones, antibiotic drugs acting on DNA synthesis, demonstrated that LEV was the most effective drug against *S. suis* strains isolated from both human patients and pigs. The highest numbers of strains with intermediate susceptibility to fluoroquinolones of *S. suis* isolated from human patients and diseased pigs was observed for ENR (44.4 and 32.6%, respectively) and NOR (70.4 and 28.3%, respectively). As a preferable veterinary medicine, enrofloxacin is commonly prescribed for the treatment of streptococcal infection and also used against gram-negative bacterial infections in pigs [40]. Therefore, the observations of intermediate susceptibility to fluoroquinolones in pig-isolated *S. suis* strains in Thailand suggest that continued administration of fluoroquinolones could eventually lead to widespread of resistance to this class of compounds.

Comparison of antibiotic resistance of *S. suis* strains isolated from diseased pigs in two discrete periods (Additional file 3: Table S2 Additional file 4: Figure S2) revealed the associations between isolation period and susceptibility of *S. suis* for CFL, PEN, AZM, CHL, ERY, CIP, and ENR. The resistance to antibiotic drugs inhibiting on protein synthesis, including AZM and ERY increased in 2012–2015. The results also showed significant increases in susceptibility of Thai *S. suis* isolated strains to CFL, PEN, CIP, and ENR in 2012–2015. For fluoroquinolones, high prevalence of *S. suis* strains susceptible to NOR (100%). Nonetheless, intermediate susceptibility against LEV (21.7%) and ENR (43.5%) was observed in 2012–2015. In addition, the result showed that the susceptibility of *S. suis* against CHL was relatively high; however, the increasing cases of intermediate susceptibility could be found in the isolation year of 2012–2015. Taken together, the data suggest a tendency of reduced efficacy of these antibiotic drugs for the treatment of *S. suis* infection in the future.

The prevalence of antibiotic resistance of *S. suis* was determined according to capsular serotype of *S. suis*, including serotype 2, non-serotype 2, AA, and NT (Table 3 and Additional file 5: Figure S3). Based on statistical analysis, there were significant associations between bacterial serotypes and the susceptibility patterns towards AMP, CFL, PEN, ERY, GEN, TET, TIA, CIP, ENR, LEV, and SXT. The results showed that most of the serotype 2 *S. suis* strains were highly sensitive to antibiotic drugs acting on cell-wall synthesis, AMP (98.3%), CFL (98.3%), CTX (98.3%), CTF (98.3%), PEN (96.6%), and VAN (100%), and antibiotic drugs inhibiting DNA synthesis, CIP (79.6%), and LEV (100%). The overall data implied that serotype 2 *S. suis* strains were prone to be susceptible to more antibiotic drugs, compared to the other *S. suis* serotypes.

Among antibiotic drugs inhibiting protein synthesis used in this study, a high susceptibility to CHL (82.1–91.2%) and FFC (88.1–95.9%) was observed for all serotypes; however, high-level of intermediate susceptibility to CHL (12.8%) and FFC (2.6%) was found in the NT *S. suis* strains. The result obtained from this study was also consistent with other reports on the resistance to tetracyclin and macrolide drugs of *S. suis* in pig isolates worldwide [16]. The highest percentage of resistance to AZM (69.2–91.5%), CLI (87.2–96.7%), DOX (82.1–100%), ERY (69.2–87.7%), and TET (79.5–100%) was observed for all serotypes. In addition, the percentage of resistance to AZM observed from this study was higher than reported in other countries (49% and 69% for Brazil and China, respectively) [16, 20, 38].

Among the serotypes described, serotype 2 is the most virulent and frequently isolated from both diseased pigs and human patients. Focusing on the serotype 2 *S. suis* isolated from the groups of human patients and diseased pigs (Additional file 6: Table S3 and Additional file 7: Figure S4), no correlation between the sources of bacterial strains and the susceptibility patterns of *S. suis* was observed for AMP, CFL, CTX, CTF, PEN, VAN, CHL, DOX, TET, CIP, ENR, and LEV. All serotype 2 *S. suis* strains were completely sensitive to VAN and LEV and resistant to tetracyclins (DOX and TET). The susceptibility test showed that all serotype 2 *S. suis* isolated strains in Thailand remained sensitive to beta-lactams. Although most of the serotype 2 strains isolated from both human patients and diseased pigs still exhibited sensitive susceptibility towards AMP, CFL, CTX, CTF, and PEN, the cases of intermediate susceptibility or resistance against these drugs was found in the strains isolated from diseased pigs in central regions of the country in 2012–2015, raising a concern about the emerging resistance of serotype 2 *S. suis* to these drugs in the country.

Although susceptibility to CIP was relatively high in serotype 2 *S. suis*, high frequency of intermediate
Table 3 Antimicrobial susceptibility of Thai Streptococcus suis, serotype 2 (59 strains), non-serotype 2 (73 strains), autoagglutinating (91 strains), and non-typable (39 strains)\\

| Antibiotic drugs | Serotype 2 59 strains | Non-serotype 2 73 strains | Autoagglutinating 91 strains | Non-typable 39 strains | P-value |
|------------------|-----------------------|---------------------------|-----------------------------|------------------------|---------|
| Ampicillin (AMP) | 58 (98.3%) 1 (1.7%) 0 (0%) | 50 (68.5%) 6 (8.2%) 17 (23.3%) | 56 (61.5%) 13 (14.3%) 22 (24.2%) | 28 (71.8%) 5 (12.8%) 6 (15.4%) | < 0.001* |
| Cephalaxin (CFL) | 58 (98.3%) 0 (0%) 1 (1.7%) | 41 (56.2%) 4 (5.5%) 28 (38.3%) | 46 (50.5%) 0 (0%) 45 (49.5%) | 20 (51.3%) 1 (2.6%) 18 (46.1%) | < 0.001* |
| Cefotaxime (CTX) | 58 (98.3%) 0 (0%) 1 (1.7%) | 65 (89.0%) 2 (2.7%) 6 (8.3%) | 88 (96.7%) 1 (1.1%) 2 (2.2%) | 33 (84.6%) 1 (2.6%) 5 (12.8%) | 0.078 |
| Ceftiofur (CTF) | 58 (98.3%) 0 (0%) 1 (1.7%) | 69 (94.5%) 1 (1.4%) 3 (4.1%) | 88 (96.7%) 1 (1.1%) 2 (2.2%) | 33 (84.6%) 3 (7.7%) 3 (7.7%) | 0.053 |
| Penicillin G (PEN) | 57 (96.6%) 1 (1.7%) 1 (1.7%) | 22 (30.1%) 34 (46.6%) 17 (23.3%) | 26 (28.5%) 35 (48.5%) 30 (33.0%) | 14 (35.9%) 17 (43.6%) 8 (20.5%) | < 0.001* |
| Vancomycin (VAN) | 59 (100%) 0 (0%) 0 (0%) | 69 (94.6%) 2 (2.7%) 2 (2.7%) | 90 (98.9%) 1 (1.1%) 0 (0%) | 35 (89.8%) 2 (5.1%) 2 (5.1%) | 0.072 |
| Azithromycin (AZM) | 5 (8.5%) 0 (0%) 0 (0%) | 54 (91.5%) 8 (11.0%) 0 (0%) | 65 (89.0%) 16 (23.3%) 3 (4.1%) | 11 (28.2%) 1 (2.6%) 27 (69.2%) | 0.063 |
| Chloramphenicol (CHL) | 53 (89.8%) 6 (10.2%) 0 (0%) | 61 (83.6%) 5 (6.8%) 7 (9.6%) | 83 (91.2%) 1 (1.1%) 7 (7.7%) | 32 (82.1%) 5 (12.8%) 2 (5.1%) | 0.032* |
| Tetracycline (TET) | 0 (0%) 0 (0%) 59 (100%) | 8 (11.0%) 0 (0%) 64 (87.6%) | 19 (20.9%) 1 (1.1%) 71 (98.9%) | 12 (30.8%) 0 (0%) 27 (69.2%) | 0.002* |
| Levofloxacin (LEV) | 59 (100%) 0 (0%) 0 (0%) | 48 (65.8%) 4 (5.5%) 21 (27.7%) | 67 (73.6%) 1 (1.1%) 23 (25.3%) | 28 (71.8%) 1 (2.6%) 10 (25.6%) | < 0.001* |
| Sulfadimethoxine/trimethoprim (SXT) | 56 (93.5%) 2 (3.4%) 11 (18.6%) | 22 (30.1%) 3 (4.1%) 48 (65.8%) | 16 (17.6%) 2 (2.2%) 73 (86.2%) | 11 (28.2%) 3 (7.7%) 25 (64.1%) | < 0.001* |

*Ampicillin, AZM azithromycin, CTX cefotaxime, CTF ceftiofur, CFL chloramphenicol, CIP ciprofloxacin, CLU clindamycin, DOX doxycycline, ENR enrofloxacin, ERY erythromycin, FPC florfenicol, GEN gentamicin, LEV levofloxacin, NOR norfloxacin, PEN penicillin G, SXT sulfamethoxazole/trimethoprim, TET tetracycline, TIA tiamulin, VAN: vancomycin. S: susceptible; I: intermediate; R: resistant. The asterisk indicates statistical significance with P-value < 0.05.
susceptibility to CIP was found in human-isolated serotype 2 S. suis strains and CIP-resistant serotype 2 S. suis strains were determined from pig-isolated strains. Among the serotype 2 S. suis strains, the results also showed that the prevalence of serotype 2 S. suis strains resisting to AZM, CLI, ERY, FFC, GEN, TIA, NOR, and SXT was higher in the group of pig-isolated strains. This information suggest that pigs could be a significant reservoir for antibiotic-resistant serotype 2 S. suis.

Discussion

Monitoring of antimicrobial susceptibility of S. suis is conducted worldwide, particularly in the countries with an intensive swine production. Resistance of S. suis to many classes of antimicrobial agents such as lincosamides, macrolides, sulphonamides and tetracycline showed high prevalence [20]. In Northern America and European countries, the resistance of lincosamides and macrolides has been increasing both for strains isolated from pigs and humans [16, 20]. A high prevalence of tetracycline resistance was reported for S. suis isolates in many countries including those of North America, Asia and some from Europe [16, 19, 20]. A significant increase in tetracycline resistance was found in meningitis patients from Asia [41–43] and high prevalence of tetracycline-resistant S. suis isolated from pigs was clearly found in different regions of China [44]. Resistance to cephalosporin was reported in both China and Europe [45–47]. However, among common antibiotics used for the treatment of S. suis infection, the prevalence of S. suis strains resistant to penicillin (0–27%), ampicillin (0.6–23%) and ceftiofur (0–23%) was still low in many countries [20].

This study revealed the antimicrobial susceptibility of S. suis strains isolated in Thailand. Similar AMR patterns determined from S. suis strains isolated from different sources and in discrete periods of time could suggest a zoonotic transmission of AMR S. suis between pigs and humans and widespread of antibiotic-resistant S. suis across the country. The AMR patterns of Thai S. suis isolated strains also revealed that only S. suis strains isolated from pigs exhibited MDR and most of the MDR S. suis strains were isolated from asymptomatic pigs. This finding confirmed that asymptomatic pigs could potentially serve as reservoirs for MDR S. suis. As a result, a narrow spectrum of effective antibiotic drugs can be used for the treatment of S. suis infection in both pigs and humans.

It is important to note that S. suis isolates used in our study were classified by serotyping method, as described [28]. Under this circumstance, S. suis serotypes 22, 34 (19 and 4 strains, respectively) and all NT (39 strains) were still included for the susceptibility test and data analysis in this study. The result showed that none of the serotype 2 S. suis strains displayed MDR pattern and high prevalence of MDR patterns were observed for AA S. suis strains. The antimicrobial resistance pattern showed that serotype 22 and 34 S. suis strains exhibited different MDR patterns and the most MDR S. suis strains belonged to serotype 22. This finding suggests that precise bacterial classification methods are necessary for the AMR surveillance study of this bacterial species.

Although the findings from this study are consistent with previous literatures reporting the surveillance of S. suis susceptibility to beta-lactam antibiotics [16–18], high prevalence of intermediate-susceptibility S. suis strains against penicillin were observed and the prevalence of penicillin resistance was highest in asymptomatic pig-isolated S. suis strains, inferring pigs being a main reservoir for penicillin resistance of S. suis. Hence, a proper use of penicillin for S. suis infection in pigs is recommended to avoid further spread of penicillin-resistant S. suis.

The third-generation cephalosporin, ceftiofur has been the most effective antibiotic drug for both humans and pigs until now. Nonetheless, recent evidences from China and Europe showed the emergence of resistance to the third-generation cephalosporins [39, 41, 42]. Our study also revealed the presence of ceftiofur-resistant S. suis in Thailand. The evidence raise an awareness of long-term administration of this antibiotic drug inducing the spread of cephalosporins resistance in S. suis and therefore of the need of a surveillance of the susceptibility pattern of this zoonotic pathogen.

In this study, the periodic comparison of S. suis strains was performed for small number of strains that were isolated from diseased pigs only and the isolation sources of two sample groups were considerably different. Although the heatmap result demonstrated the increase of S. suis strains susceptible to beta-lactams in 2012–2015 (Additional file 4: Fig. S2), this finding might not markedly reflect a declining trend of AMR situation for S. suis in the country. To evaluate the progression of AMR situation and guide for prevention and control of AMR problem in the country, AMR surveillances of S. suis isolates in different regions and in consecutive years needs to be continuously carried out.

Overall, the result obtained from this study confirm that beta-lactams are the current highly-effective antibiotics whereas tetracyclines and macrolides failed to treat S. suis infection. Our finding also supports that chloramphenicol remains the most potent antibiotic among the protein synthesis inhibitors; however, due to toxicity of this compound, its usage has been limited in humans and prohibited to be used in food-producing animals in many countries, including Thailand.
Among Thai-isolated S. suis strains, a wide-range resistance to drugs acting on protein synthesis was observed in both diseased and asymptomatic pigs. Macrolides have a long history of intensive use in swine industries for prophylaxis and treatment of zoonotic streptococcal diseases [34]. Therefore, overuse and misuse of these antibiotics over many years could introduce drug resistance. In this study, the high prevalence of both AZM and ERY of S. suis strains observed in pig population suggests a cross-resistant mechanism of these two drugs, which needs to be further investigated.

Resistance to macrolides is mainly due to erythromycin ribosomal methylase encoded by erm genes or by macrolide efflux protein encoded by mef genes. Previous studies have identified the gene erm(B) associated with macrolide-lincosamide-streptogramin B (MLS) resistance in S. suis isolated from pigs and humans [48, 49]. Recently, our preliminary data analysis of sequenced genome has shown that erm(B) is the most common gene found in macrolide-resistant Thai S. suis strains and erm(T) and erm(A) are resistant determinants of pig-isolated ERY/AZM-resistant S. suis strains (unpublished data). The mef(A) gene associated in efflux-mediated erythromycin resistance for 14- and 15-membered macrolides (known as M phenotype) and msr(D) encoding macrolide-efflux pump were determined in Thai S. suis strains (unpublished data). Nonetheless, macrolide-resistant Thai S. suis strains without these resistant genes were found, suggesting that other resistance mechanisms could occur and need to be further investigated.

Tetracycline resistance mechanism in Streptococcus species is mainly due to tetracycline-resistant ribosomal protection protein and tetracycline efflux protein, encoded by tet genes. In S. suis, tet(B), tet(40), tet(L), tet(M), tet(O), tet(W), and mosaic tet(O/W/32/O) have been identified [21, 50]. The tet(W) associates with a transposable chromosomal element and carries elements in S. suis isolates. Characterization of tet(W)-carrying elements revealed that two genetic elements, both carrying erm(B) besides tet(W), were completely different, one was almost identical to a genomica island of S. suis genome and another one resembling a phage that also carried other antibiotic (macrolide, aminoglycoside, and streptothricin) and heavy metal (cadmium) resistance genes [51]. A 14,741-bp unstable genetic element associated with tet(O/W/32/O) has been detected. This element can also carry macrolide erm(B) and aminoglycoside (aadE, aphA) resistance genes. In the integrated form, this unstable genetic element could be found inside an integrative and conjugative elements (ICE) which is transferable at high frequency to pathogenic Streptococcus species [50]. Our preliminary results, obtained from analysis of sequenced genome of tetracycline-resistant S. suis strains isolated from pigs and humans, have determined tet(M), tet(O), mosaic tet(O/W/32/O), tet(L), and mosaic tet(W/N/W) (unpublished data) which need to be further validated and their mobile genetic elements must be investigated.

Mobile genetic elements (MGEs), including ICEs, transposons, plasmids, insertion sequences, integrons, prophages, and other genomic islands, play a crucial role in dissemination of AMR determinants. Recently, comprehensive analysis of AMR-associated mobilome among Streptococcus species showed that several AMR genes mediating resistance to antibiotics were carried by their corresponding MGEs [52]. Among the MGEs, ICEs play a major role in bacterial adaptation and S. suis has high rates of ICEs. Compared to other pathogenic Streptococcus species, S. suis has higher prevalent and greater diversity of MGEs. These evidences support that S. suis potentially serves as MGEs reservoir for playing a key role in intra- and interspecies horizontal transfer of AMR genes to other Streptococcus species.

**Conclusions**

The data obtained from this study support that multi-drug resistance of S. suis strains occurs in Thailand and pigs could serve as reservoirs for the spread of antibiotic-resistant S. suis strains. Beta-lactam antibiotic drugs remain the most effective therapeutic drugs for the treatment of S. suis infection in both humans and pigs in Thailand; however, a high prevalence of intermediate susceptibility of Thai-isolated S. suis to different antibiotic drugs indicates a tendency for AMR problems in the future. In addition, the presence of high resistance for macrolides is raising an awareness of long-term and over use of antibiotics inducing antibiotic resistance of S. suis. Therefore, an appropriate and careful selection of antibiotic drug choice for prophylactic and empirical treatments of zoonotic streptococcal disease are highly recommended. To tackle the AMR problem in S. suis, antibiotic resistance surveillance activities in both swine industries and healthcare sector are needed to guide decisions on appropriate antibiotic use. Intensive research aiming at understanding AMR mechanism including the identification of drug-resistant biomarkers, mechanism of resistant-associated gene transfer, and development of rapid diagnostics for S. suis identification, are urgently needed.

**Methods**

**Bacterial strains**

A total of 239 strains of S. suis isolated from diseased pigs, healthy pigs (or so-called asymptomatic pigs), and human patients (epidemic and sporadic cases), in northern, central, and southern regions of Thailand during 2006–2007, and 23 strains of S. suis isolated in central regions of the country during 2012–2015 were subjected...
to antimicrobial susceptibility test. Diseased pigs were pigs died with clinical symptoms of septicemia and meningitis whereas asymptomatic pigs were pigs did not present any clinical signs of \textit{S. suis} disease.

The isolation of \textit{S. suis} has previously been described in [28]. Briefly, \textit{S. suis} strains isolated from human patients were collected from blood and cerebrospinal fluid (CSF), prior to an outbreak (2006 to March 2007) and during the outbreak (April–May 2007). \textit{S. suis} strains isolated from diseased pigs during 2006–2007 were collected from blood. \textit{S. suis} strains isolated from diseased pigs during 2012–2015 were collected from lungs and mesenteric lymph nodes. \textit{S. suis} strains isolated from asymptomatic pigs were obtained from whole tonsil swab of pigs at slaughterhouses.

Bacterial identification of all \textit{S. suis} isolated strains used in this study were conducted using conventional biochemical tests and PCR-based approaches [28]. Serotyping of \textit{S. suis} isolated strains was performed by coagglutination test using serotype-specific anti-sera for all 35 serotypes at the Reference Laboratory for \textit{S. suis} typing of swab of pigs at slaughterhouses.

Antibiotic susceptibility test

To assess the antibiotic susceptibility profile of \textit{S. suis} strains isolated from Thailand, the antibiotic susceptibility test was carried out by disk diffusion method according to a standard protocol of Clinical and Laboratory Standards Institute [29]. \textit{S. suis} was grown overnight on Columbia agar (Sisco Research Laboratories, New Mumbai, India) supplemented with 5% defibrinated sheep blood at 37°C in 5% CO₂. Subsequently, colonies from the overnight culture were selected and suspended in Todd Hewitt broth (Oxoid Limited, Hampshire, England). The bacterial cell suspension was adjusted to be a 0.5 McFarland standard, equivalent to 10⁶ colony-forming units per milliliter (cfu/mL). The adjusted cell suspension was spread on 4-mm depth Mueller Hinton agar supplemented with 5% defibrinated sheep blood. The disks containing standardized known amount of antibiotic agent were placed on the bacterial agar plate. Approximately, 5–6 disks were placed per plate using a disk dispenser (BioRad, Hercules, California USA). The plates were then incubated at 37°C in 5% CO₂ for 18 h. During the plate incubation, the antibiotic agents diffused around the disk and inhibited the growth of bacteria, generating a clear zone known as “zone of inhibition”.

The diameter of inhibition zone of \textit{S. suis} strains, control strain, and reference strain was measured and interpreted as susceptible (S), intermediate (I), or resistant (R), according to CLSI supplement M100S [29] for cefotaxime (CTX), azithromycin (AZM), chloramphenicol (CHL), clindamycin (CLI), doxycycline (DOX), erythromycin (ERY), tetracycline (TET), levofloxacin (LEV), and sulfamethoxazole/trimethoprim (SXT). The inhibition zone for ceftiofur (CTF), florfenicol (FFC), ciprofloxacin (CIP), enrofloxacin (ENR) and norfloxacin (NOR) was interpreted according to Soares TCS., et al. 2014 [20]. The diameter breakpoint for ampicillin (AMP), cepalexin (CFL), penicillin G (PEN), vancomycin (VAN), gentamicin (GEN), tiamulin (TIA) was taken from EUCAST and CLSI-potency Neo-Sensitabs™ User’s Guide [30] (Additional file 9: Table S5).

Statistical analysis

The Pearson’s Chi-square (χ²) test was performed to determine the independence between antibiotic susceptibility and the four categorical variables of interest, including bacterial serotype, source of bacterial isolation, health status of the source, and year of isolation. The null hypothesis was stated as no association between antibiotic susceptibility and the testing categorical variable whereas the alternative hypothesis was that the susceptibility of each testing antibiotic drug was significantly associated with the testing variables. The Chi-square formula is shown as followed.

$$\chi^2 = \sum \frac{(f_{ij} - e_{ij})^2}{e_{ij}}$$

where \(f_{ij}\) is the observed frequency count of events belonging to both \(i^{th}\) of category X and \(j^{th}\) of category Y
and $e_{ij}$ is the corresponding expected count if $X$ and $Y$ are independent. Antibiotic susceptibility (category Y) was denoted as “sensitive” (S), “intermediate sensitive” (I) and “resistance” (R). For each category $X$, bacterial serotype included “serotype 2”, “non-serotype 2”, “autoagglutinating (AA)”, and “non-typable (NT)”; source of bacterial isolation comprises “human patients”, “diseased pigs”, and “asymptomatic pigs”; health status of source consisted of “diseased pigs” and “asymptomatic pigs”; year of isolation was defined as the period between “2006-2007” and “2012-2015”.

The analysis was performed using function chisq.test of R package version 3.4.3 [31]. The null hypothesis of the independence assumption is to be rejected if the $P$-value of the Chi-squared test was less than a given significance level $\alpha = 0.05$ ($P$-value < 0.05).

### Additional files

**Additional file 1:** Table S1. Antimicrobial resistance patterns of Thai $S$. suis isolated strains. AMP: ampicillin, AZM: azithromycin, CTX: cefotaxime, CFT: cefotior, CFP: cepalexin, CHL: chloramphenicol, CIP: ciprofloxacin, CLI: clindamycin, DOX: doxycycline, ENR: enrofloxacin, ERY: erythromycin, FCC: florfenicol, GEN: gentamicin, LEV: levofloxacin, NOR: norfloxacin, PEN: penicillin G, SXT: sulfamethoxazole/trimethoprim, TET: tetracyclin, TIA: tiamulin, VAN: vancomycin. $S$. suis strains isolated from human patients, diseased pigs during 2006–2007 and 2012–2015 were named as Hpxx, DP6xxx and DP15xx, respectively, when x was the identification number. (TIF 602 kb)

**Additional file 2:** Figure S1. Heatmap illustrates susceptibility, i.e. susceptible, intermediate, and resistant, of $S$. suis isolated from human patients ($n=27$), diseased pigs ($n=46$) and asymptomatic pigs ($n=189$) grouped towards testing antibiotic drugs. The isolated bacteria were clustered based on the isolation sources. Associations between source of isolation and susceptibility of each antibiotic drug were analyzed using Pearson’s Chi-square dependent test. The asterisk indicates that null hypothesis of the Chi-square test was rejected ($P$-value < 0.05), suggesting a significant association. (TIF 268 kb)

**Additional file 3:** Table S2. Antimicrobial susceptibility ofThai $S$. suis strains isolated from diseased pigs during 2006–2007 and 2012–2015. During 2006–2007, the $S$. suis strains were isolated from different regions of the country (northern, 11 strains; central, 6 strains, and southern, 6 strains). All of the $S$. suis strains isolated during 2012–2015 (23 strains) were collected from central regions of the country: AMP: ampicillin, AZM: azithromycin, CTX: cefotaxime, CFT: cefotior, CFP: cepalexin, CHL: chloramphenicol, CIP: ciprofloxacin, CLI: clindamycin, DOX: doxycycline, ENR: enrofloxacin, ERY: erythromycin, FCC: florfenicol, GEN: gentamicin, LEV: levofloxacin, NOR: norfloxacin, PEN: penicillin G, SXT: sulfamethoxazole/trimethoprim, TET: tetracyclin, TIA: tiamulin, VAN: vancomycin. $S$. suis susceptible; I: intermediate; R: resistant. The asterisk indicates statistical significance with $P$-value < 0.05. (TIF 597 kb)

**Additional file 4:** Figure S2. Heatmap illustrates susceptibility, i.e. susceptible, intermediate, and resistant, of $S$. suis isolated from diseased pigs during the two different periods of time toward testing antibiotic drugs. The isolated bacteria were clustered, based on the period of isolation, i.e. 2006–2007 ($n=23$) and 2012–2015 ($n=23$). Associations between the period of isolation and susceptibility of each antibiotic drug were analyzed using Pearson’s Chi-square dependent test. The asterisk indicates that null hypothesis of the Chi-square test was rejected ($P$-value < 0.05), suggesting a significant association. (TIF 244 kb)

**Additional file 5:** Figure S3. Heatmap illustrates susceptibility, i.e. susceptible, intermediate, and resistant, of $S$. suis isolated from different regions of the country (northern, 11 strains; central, 6 strains, and southern, 6 strains). All of the $S$. suis strains isolated during 2012–2015 (23 strains) were collected from central regions of the country: AMP: ampicillin, AZM: azithromycin, CTX: cefotaxime, CFT: cefotior, CFP: cepalexin, CHL: chloramphenicol, CIP: ciprofloxacin, CLI: clindamycin, DOX: doxycycline, ENR: enrofloxacin, ERY: erythromycin, FCC: florfenicol, GEN: gentamicin, LEV: levofloxacin, NOR: norfloxacin, PEN: penicillin G, SXT: sulfamethoxazole/trimethoprim, TET: tetracyclin, TIA: tiamulin, VAN: vancomycin. $S$. suis strains were isolated from human patients and pigs, towards testing antibiotic drugs. The isolated bacteria were clustered based on host, i.e. human patients ($n=27$) and pigs ($n=32$) including asymptomatic and diseased pigs. Associations between source of isolation and susceptibility of each antibiotic drug were analyzed using Pearson’s Chi-square dependent test. The asterisk indicates that null hypothesis of the Chi-square test was rejected ($P$-value < 0.05), suggesting a significant association. (DOC 3450 kb)

**Additional file 7:** Figure S4. Heatmap illustrates susceptibility, i.e. susceptible, intermediate, and resistant, of serotype 2 $S$. suis isolated from human patients and pigs, towards testing antibiotic drugs. The isolated bacteria were clustered based on the isolation sources. Associations between source of isolation and susceptibility of each antibiotic drug were analyzed using Pearson’s Chi-square dependent test. The asterisk indicates that null hypothesis of the Chi-square test was rejected ($P$-value < 0.05), suggesting a significant association. (DOC 3449 kb)

**Additional file 8:** Table S3. Source of $S$. suis isolated strains and numbers of strains used in this study. Thai $S$. suis strains used in this study were isolated during 2006–2007, except 23 strains of serotype 2. $S$. suis obtained from central regions #2 (Nakhon Pathom) were isolated during 2012–2015. Non-serotype 2 $S$. suis isolated from diseased pigs includes serotype 1 (1 strain), 14 (1 strain), 16 (1 strain), 22 (2 strains), 23 (1 strain), 25 (1 strain), and 34 (1 strain). Non-serotype 2 $S$. suis isolated from asymptomatic pigs includes serotypes 1 (1 strain), 3 (5 strains), 5 (3 strains), 7 (2 strains), 9 (7 strains), 12 (2 strains), 15 (1 strain), 16 (5 strains), 19 (2 strains), 21 (1 strain), 22 (17 strains), 24 (2 strains), 25 (1 strain), 27 (1 strain), 28 (2 strains), 29(4 strains), 30 (6 strains), and 34 (3 strains). (DOC 3934 kb)

**Additional file 9:** Table S5. Inhibition zone diameter and zone interpretation of antibiotic drugs. Antibiotic drugs used in this study were classified into four different modes of inhibition including cell-wall synthesis inhibitors (6 drugs), protein synthesis inhibitors (9 drugs), DNA synthesis inhibitors (4 drugs), and antimetabolite (1 drug). The zone of inhibition was interpreted as susceptible (S), intermediate (I), or resistant (R), according to a standard protocol of Clinical & Laboratory Standards Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Ref. [20]. The interpretation according to EUCAST and CLSI 2013 was used for veterinary practice, described in EUCAST and CLSI-potency Neo-Sensitabs™ User’s Guide 2013, rev. date 11-04-2013 [38]. (DOC 3917 kb)

### Abbreviations

AA: Autoagglutinating; AMP: Ampicillin; AMR: Antimicrobial resistance; ATCC: American Type Culture Collection; AZM: Azithromycin; CFP: Cepalexin; CFU: Colony-forming unit; CHL: Chloramphenicol; CIP: Ciprofloxacin; CLI: Clindamycin; CO₂: Carbon dioxide; CSF: Cerebrospinal fluid; CFT: Cefotior; CFX: Cefotaxime; DOX: Doxycycline; ENR: Enrofloxacin; ERY: Erythromycin; FCC: Florfenicol; GEN: Gentamicin; hr.: Hour(s); LEV: Levofloxacin; MDR: Multidrug resistance; NOR: Norfloxacin; NT: Nontypable; PCR: Polymerase chain reaction; PEN: Penicillin G; S. suis: Streptococcus suis; SXT: Sulfamethoxazole/trimethoprim; TET: Tetracyclin; TIA: Tiamulin; VAN: Vancomycin; χ²: Chi-square

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All data supported these findings are present within the manuscript.

Authors’ contributions
SY, KM, and BA performed antimicrobial susceptibility tests. SY and YM conducted statistical analysis. SY was a major contributor in data analysis and manuscript preparation. SY, KM, YM, PS, and MG participated in revising the manuscript. PS, MG, and WV critically reviewed the scientific content of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate
All experimental procedures were conducted according to the IBC Protocol. Ethics approval and consent to participate

Consent for publication
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