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Antimicrobial resistance profiles of *Streptococcus suis* isolated from pigs, wild boars, and humans in France between 1994 and 2020

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ABSTRACT *Streptococcus suis,* an emerging zoonotic pathogen, causes invasive infections and substantial economic losses in the pig industry worldwide. Antimicrobial resistance against 22 antibiotics was studied for 200 *S. suis* strains collected in different geographical regions of France. Most of the strains (86%) showed resistance to at least one antibiotic with a low rate of resistance to fluoroquinolones, penicillins, pleuromutilin, and diaminopyrimidine-sulfonamides, and a higher rate to macrolides-lincosamides and tetracycline. Multi-resistance patterns were observed in 138 strains; three of them being resistant to six antibiotic families. Statistical analyses highlighted a decrease in the resistance to trimethoprim-sulfamethoxazole, in our collection, between the two periods studied—before 2010 and after 2015—as well as an impact of the geographical origin with a higher rate of resistance to macrolides-lincosamides and penicillin in Brittany than in the other French regions. Furthermore, macrolides-lincosamides and tetracycline resistance patterns were more likely to be found in pig isolates than in human and wild boar isolates. A difference in resistance was also observed between serotypes. Most of the penicillin-resistant strains belong to serotypes 1, 5, 9, 11, 12, 15, 27, and 29. Finally, penicillin and pleuromutilin resistances were mostly found in "non-clinical" isolates. The empirical treatment of human and porcine infections due to *S. suis* in France can therefore still be carried out with beta-lactams. However, this study emphasizes the need to monitor antimicrobial resistance in this zoonotic pathogen.

KEYWORDS *Streptococcus suis*, pathogen, antimicrobial resistance, CLSI, disc diffusion method

Streptococcus suis is a zoonotic pathogen that causes pig infections and substantial economic losses. Even though serotype 2 is preponderant, other serotypes can be responsible for infections worldwide (1). In Europe, serotypes 9, 2, and 7 are dominant among clinical pig isolates (61%, 18%, and 7% of the isolates, respectively), whereas serotypes 1/2 and 3 are also frequently found in the rest of the world (2, 3). Together with serotypes, Weinert et al. proposed to group *S. suis* strains into three groups or pathotypes according to their isolation sites: (i) "non-clinical," (ii) "respiratory," or (iii) "systemic" (4). These groups correspond to the (i) commensal, (ii) possibly opportunistic, and (iii) pathogenic groups defined by Estrada et al. (5). These authors also suggested using the serotype to predict the pathotype, as they demonstrated a link between serotypes and pathotypes (5). Human cases of *S. suis* were mainly described in Asia, with three large outbreaks in China reported in 1998, 2005, and 2016 (6). Clinical manifestations of *S. suis* infection include meningitis, septicemia, endocarditis, and arthritis (7). In France, human infections are scarce; the eighth French case since 1995 was reported in 2016 (8) and the

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latest case corresponds to a woman hospitalized for meningitis after preparing wild boar meat, with *S. suis* identified as the causative agent of the disease (7).

Several families of antimicrobials are commonly used in veterinary medicine to treat bacterial infections. These include tetracyclines, beta-lactams, polymyxins, macrolides-lincosamides, aminoglycosides, sulfonamides, or trimethoprim-sulfonamides (TMPSs) (9). The antibiotic families mostly prescribed for gastrointestinal pig infections worldwide are tetracyclines, lincosamides, pleuromutilins, and macrolides (10–13). Chlortetracycline, tetracycline, and amoxicillin are used for treating respiratory and infectious diseases (14, 15). In France, tetracycline, beta-lactams (penicillins), macrolides, and TMPSs are generally used for respiratory infections; polypeptides and tetracycline for sepsis and gastrointestinal infections, respectively (12). Beta-lactams (penicillins) are indicated for treating *S. suis* meningitis, septicemia, and/or arthritis (12). In France, the regulation of antibiotics in veterinary medicine must meet French and European standards. The use of these molecules is subject to marketing authorizations and be indicated in the "summary of product characteristics" sheet. Checks are made on their distribution, prescription, and use in accordance with European laws. In 2010, the European Union initiated a common European program called "One Health" (16). This program is based on the premise that there is a direct link between human health, animal health, and, more generally, environmental health. In 2012, France implemented its first "EcoAntibio" plan in order to reduce the use of antibiotics nationwide by more than 25% in 5 years. This plan was a success, as the use of antibiotics dropped by 37% (9). Resapath (a French surveillance network for antimicrobial resistance in bacteria from diseased animals) is part of the antimicrobial resistance surveillance system for animals setup in France. The Resapath network collects and analyzes data from routine antimicrobial susceptibility tests (disc diffusion method) performed by diagnostic laboratories in the network and publishes an annual report. For *S. suis*, this report includes the proportions of susceptible isolates for 12 antibiotics (amoxicillin, oxacillin, erythromycin, tylosin, spiramycin, lincomycin, streptomycin, kanamycin, gentamicin, tetracycline, doxycycline, and trimethoprim-sulfonamides) (17). Except from Resapath, few data on the resistance of *S. suis* in France are available in the literature (18, 19). Therefore, the aim of this study was to investigate the resistance of a collection of *S. suis* strains isolated in France to a larger number of antimicrobials. This collection of strains was fully characterized regarding serotype, and included strains isolated from humans and wild boars. The statistical associations between resistance/susceptibility and the isolation period, the strain's geographical origin, serotype, host, and pathotype were analyzed.

MATERIALS AND METHODS

Bacterial strains

Two hundred isolates of *S. suis* were selected from the ANSES strain collection (n = 1,619), resulting from different research programs, in order to get a set of strains representative of the diversity of serotypes, geographical origins, and hosts available. All strains isolated from human cases or wild boars were selected. This set included 21 strains of serotype 1, 20 of serotype 1/2, 32 of serotype 2, 30 of serotype 3, 30 of serotype 7, 33 of serotype 9, and 34 strains of other serotypes (six strains of serotype 4 and one or two strains of each of the following serotypes: 5, 8, 10, 11, 12, 14, 15, 16, 18, 21, 24, 27, 28, 29, and 31) (Table S1). In France, more than 50% of national swine production is based in Brittany, so we chose to include 130 strains collected in Brittany and 70 from other regions of France. Most of the strains were isolated from pigs (n = 178); the remaining 22 were isolated from humans (n = 13) or wild boars (n = 9). Strains were allocated to two groups according to the isolation period (88 strains for the "before 2010" group and 112 for the "after 2015" group) to examine the potential impact of the French EcoAntibio plan. Finally, strains were classified into three pathotypes. The "systemic" group contained 127 strains isolated from samples of the nervous system, blood, or joint when the causes of death were attributed to an infection by this pathogen. The "respiratory" group

hosted 31 strains isolated from pulmonary samples obtained either from sick or clinically healthy animals on farms that had had outbreaks of *S. suis* infections. The strains in the "non-clinical" group (n = 36) were from the upper respiratory tract with no sign of *S. suis* infection in animals, and six strains were not classified due to a lack of information. More details can be found in the supplementary data (Table S1). All the strains used were confirmed by serotyping using the PCR method described by Okura et al. (20).

Culture conditions and antimicrobial susceptibility testing

All the strains were stored at −80°C in a buffered peptone water solution with glycerol (20%). Each strain was subcultured on Columbia agar with 5% sheep blood (BIO-RAD, Marne la Coquette, France) and incubated overnight at 37°C under 5% CO_2. Antimicrobial susceptibility was tested using the disc (agar) diffusion method, according to Clinical Laboratory Standards Institute (CLSI) standards (VET01-A and VET01-S) (21, 22). Briefly, an inoculum of 1×10^8 CFU/mL was prepared for each strain and used to inoculate a Mueller-Hinton plate containing 5% of sheep blood (120 mm square plates; BIO-RAD) before the application of antimicrobial discs (BIO-RAD, Marne-La-Coquette, France, or Mast Diagnostics, Amiens, France). The plates were incubated for 22 h ± 1 h at 35°C, and, after bacterial growth, inhibition zone diameters were measured with a sliding calliper. The reference strain *Streptococcus pneumoniae* ATCC 49619 was used as a quality control according to CLSI recommendations.

As *S. suis* is a zoonotic bacterium, the antimicrobials studied belong to either the veterinary or human medicine list, or to both. For strains found resistant to penicillins and fluoroquinolones by disc diffusion method, the minimal inhibitory concentration (MIC) was determined using Micronaut-S microplates (from Merlin, Bruker). These microplates were used according to the manufacturer's instructions.

Strain categorization

Antimicrobial susceptibility test results were used to differentiate between strains without or with acquired resistance mechanisms [wild type (WT) or non-wild type (NWT), respectively] using cut-off values (COWT). The normalized resistance interpretation (NRI) method was applied for each antibiotic to determine these COWT values (23, 24). The NRI method applied to the disc diffusion antimicrobial susceptibility testing was precisely described by Kronvall and Smith (23) and the related excel program is freely accessible at the following website link: http://www.bioscand.se/nri/. The clinical interpretative criteria specified in CLSI (22) were also used when available. When the COWT calculation was not possible, the strain was categorized using the threshold diameters described in the literature and observations of the measured diffusion diameters (22, 25–27). In this study, the WT term will be expressed as susceptible to the antibiotic tested, and the NWT will be expressed as resistant to the antibiotic tested.

Statistical analyses

The strains were classified according to different resistance profiles (Table S1) in proportion to their resistance/susceptibility to the antibiotics tested. In total, 24 resistance profiles were identified following the analyses carried out. The first (univariate) step entailed using a Chi-squared test and Fisher correction (required for limited expected counts) to explore for each antibiotic possible links with other antimicrobial resistance rates together with links between each antimicrobial resistance profile and the isolation period, serotype, geographical origin, host, and/or pathotype. The second (multivariate) step considered all the antimicrobial resistances. First, a multiple correspondence analysis (MCA) was performed to illustrate whether the antimicrobial resistance rates for the different antibiotics or the antimicrobial resistance profiles depend on the isolation period, serotype, geographical origin, host, and/or pathotype (the latter variables being considered supplementary in the analysis). Then, a multiple factor analysis (MFA) was used to determine whether there is a correlation between the

antimicrobial resistance observed for the antibiotics of the same family and antibiotics of different families. For this purpose, each family was considered a block of variables. In order to ensure the robustness of the various tests and analyses, statistical analyses were performed only on groups with at least five representative strains. All the analyses were performed using the R software with the FactoMineR package (28). Only results with a *P*-value equal to or lower than 0.05 were considered significant and subsequently interpreted.

RESULTS

Determination of cut-off values using the NRI method

The NRI method applied to the distributions of inhibition zone diameters can be used to define two groups corresponding to the two observed populations divided by the COWT (Table 1). Only one population was observed for gentamicin, chloramphenicol, florfenicol, linezolid, quinupristin/dalfopristin, and vancomycin. Two were observed for ampicillin, penicillin, cefotaxime, ceftiofur, imipenem, clindamycin, lincomycin, erythromycin, tilmicosin, tylosin, enrofloxacin, levofloxacin, norfloxacin, and trimethoprim-sulfamethoxazole. Finally, a complex pattern was observed for tiamulin and tetracycline. In the absence of CLSI clinical interpretative criteria, the COWT value was therefore determined manually for tiamulin. The human CLSI's interpretive criteria for *Streptococcus* spp. were used for tetracycline. For trimethoprim-sulfamethoxazole, the human CLSI's interpretive criteria for *S. pneumoniae* was used since no criteria was available for *Streptococcus* spp.

Antibiotic susceptibility of the strains

No resistance was observed for the following antibiotics: gentamicin (aminoglycosides) (to high concentration levels), linezolid (oxazolidinones), quinupristin/dalfopristin (streptogramins), and vancomycin (glycopeptides) (Table 1). For phenicols (chloramphenicol and florfenicol), the NRI COWT and the breakpoints proposed by the CLSI differed by only 2 mm and 1 mm, respectively, which could lead to different interpretations for 18 strains. It was decided to use the cut-off calculated by the NRI method and to classify all the strains as susceptible to this family of antibiotics. Indeed, no strain differed from the wild-type population for these two antimicrobials.

The resistance rates observed are reported in Fig. 1. Less than 1% of the strains tested were resistant to fluoroquinolones (this single strain was resistant to enrofloxacin, levofloxacin, and norfloxacin). Five percent of the strains tested showed beta-lactam resistance with 10 strains resistant to penicillin G. Of these, three were also resistant to ampicillin. These results were confirmed by MIC determination of penicillin G: 2 µg/mL for three strains, 1 µg/mL for four strains, 0.5 µg/mL for one strain, and 0.25 µg/mL for two strains. For ampicillin, MIC was 1 µg/mL for three strains. The breakpoints used were >0.12 µg/mL for penicillin G and >0.25 µg/mL for ampicillin (CLSI M100) (28). No strain was resistant to cefotaxime, ceftiofur, or imipenem. For pleuromutilin (tiamulin) and diaminopyrimidine-sulfonamides (TMPS), the proportions of resistant strains were 8% (n = 16) and 16% (n = 32), respectively. One of the highest resistance percentages observed was for macrolides-lincosamides, with more than 71% of the strains tested having resistance profiles [to erythromycin, 69% (n = 137); tilmicosin, 69% (n = 137); tylosin, 69% (n = 138); clindamycin, 71% (n = 142); and lincomycin, 71% (n = 142)]. Finally, 81% of the tested strains were resistant to tetracycline (n = 162).

In total, 86% of the strains tested were resistant to at least one antibiotic (n = 171). Among these isolates, 138 can be classified as multiresistant as they are resistant to three families of antibiotics. Thirty-five, eight, and three strains were resistant to, respectively, four, five, or six families of antibiotics. The resistance patterns are included in the supplementary data (Table S2). The most common multi-resistance patterns observed in this study are for macrolides (erythromycin, tilmicosin, and tylosin) associated with lincosamides (clindamycin and lincomycin), and tetracycline (97 strains, profile P11),

TABLE 1 Normalized resistance interpretation cut-off value obtained with the disc diffusion method for 200 *S. suis* strains

Class	Antimicrobial agent	Disc load (µg)	Distribution pattern	NRI cut-off value (mm)[a]	CLSI (mm)[b]	Wild-type population (mm)	Non-wild-type population (mm)[c]
Aminoglycosides	Gentamicin	500	Unimodal	23		23–39	NA[2]
Beta-lactams	Ampicillin	2	Multi-modal	23		24–38	13–22
	Penicillin G	1	Multi-modal	19		19–32	6–17
	Cefotaxime	30	Multi-modal	24		24–38	NA[2]
	Ceftiofur	30	Multi-modal	24	≥21 (S) *S. suis*	24–36	NA[2]
	Imipenem	10	Multi-modal	30		32–44	NA[2]
Phenicols	Chloramphenicol	30	Unimodal	19	≥21 (H) *Streptococcus* spp.	19–30	NA[2]
	Florfenicol	30	Unimodal	21	≥22 (S) *S. suis*	21–34	NA[2]
Lincosamides	Clindamycin	2	Multi-modal	20	≥19 (H) Streptococci	20–29	6–19
	Lincomycin	15	Multi-modal	22		22–32	6–21
Macrolides	Erythromycin	15	Multi-modal	22	≥21 (H) Streptococci	32–24	6–17
	Tilmicosin	15	Multi-modal	10		10–21	6
	Tylosin	30	Multi-modal	18		18–27	6–16
Fluoroquinolones	Enrofloxacin	5	Multi-modal	18		20–34	14
	Levofloxacin	5	Multi-modal	18		18–32	14
	Norfloxacin	10	Multi-modal	13		16–29	6–10
Pleuromutilins	Tiamulin	30	Multi-modal	NA[1]		14–29	6–13
Oxazolidinones	Linezolid	30	Unimodal	22		24–37	NA[2]
Streptogramins	Quinupristin/dalfopristin	15	Unimodal	15		15–27	NA[2]
Tetracycline	Tetracycline	30	Multi-modal	NA[1]	≥23 (H) *Streptococcus* spp.	23–33	6–22
Diaminopyrimidines-sulfonamides	Trimethoprim-sulfamethoxazole	25	Multi-modal	NA[1]	≥19 (H) *S. pneumoniae*	19–39	6–18
Glycopeptides	Vancomycin	30	Unimodal	18	≥17 (H) *Streptococcus* spp.	20–32	NA[2]

[a]NRI cut-off calculated for 22 antibiotics. NA[1], no diameter cut-off available.
[b]Interpretive criteria provided by the CLSI (22) for swine (S) or human (H).
[c]NA[2], no values for the non-wild-type clusters.

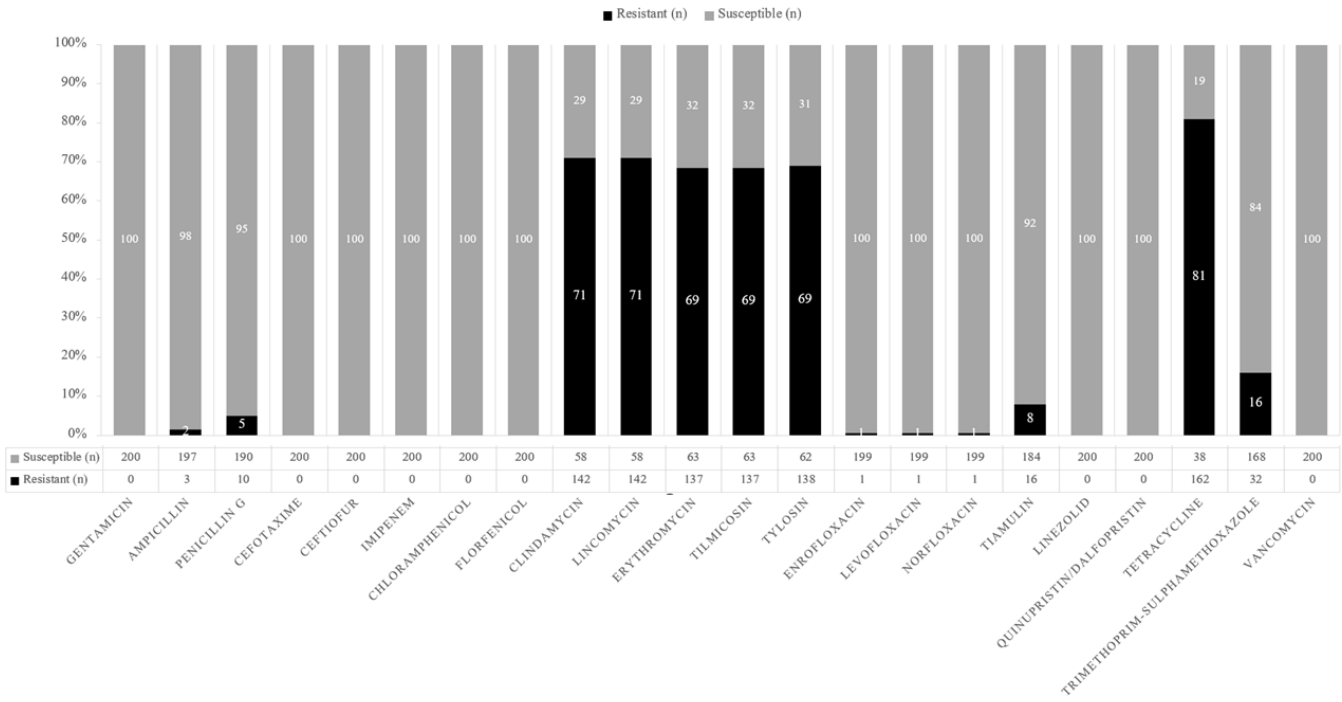

FIG 1 Resistance rates observed for 200 *S. suis* strains. The percentage of strains without or with resistance mechanisms (in light gray or black, respectively) was represented for each antibiotic tested. The antibiotics are classified according to the antimicrobial family to which they belong.

followed by macrolides-lincosamides, tetracycline, and trimethoprim-sulfamethoxazole (20 strains, profile P15).

Prevalence of antimicrobial resistance according to the isolation period, serotype, geographical origin, host, and pathotype

A significant decrease in resistance to TMPS occurred between the two periods with a proportion of resistant strains dropping from 23.5% (20/85) before 2010 to 10.4% (12/115) after 2015 ($P < 0.035$).

Serotype 2 was over-represented among lincomycin-susceptible strains with 20 strains out of 61, i.e., 33% ($P = 0.001$). Interestingly, penicillin resistance was associated ($P < 0.001$) with less common serotypes (two strains of serotypes 11 and 12, and one strain of serotypes 1, 5, 9, 15, 27, and 29). A similar result was observed for tiamulin-resistant strains, there being an over-representation of the serotypes classified under "others," with seven strains out of 16 from serotype 1 or 9 (three and four strains, respectively) compared with nine strains from serotypes 5, 12, 15, 16, 27, 29, and 31 (1, 2, 1, 1, 1, 1, and 2 strains, respectively) ($P < 0.001$). Strains with these serotypes are less frequent and thus less studied than the others. Finally, among the 32 TMPS-resistant strains, there was an over-representation ($P \leq 0.01$) of serotypes "1/2" (nine strains), "others" (seven strains), and serotype 3 (six strains).

The resistance profiles of the strains can also be correlated with their geographical origin. An over-representation of resistance to the macrolide and lincosamide families and penicillin was observed in strains collected in Brittany (erythromycin, $P = 0.017$; tilmicosin, $P = 0.017$; tylosin, $P = 0.012$; clindamycin, $P = 0.019$; lincomycin, $P = 0.019$; and penicillin, $P = 0.016$).

Furthermore, strains isolated from humans or wild boars appear to have fewer acquired resistance mechanisms to macrolides (erythromycin, $P < 0.001$; tilmicosin, $P < 0.001$; tylosin, $P < 0.001$), lincosamides (clindamycin, $P = 0.002$; lincomycin, $P = 0.001$), and tetracycline ($P = 0.003$) than strains isolated from pigs. However, these results must be taken with caution since, in the collection, the number of strains isolated from humans or wild boar ($n = 22$) is much lower than the number of strains isolated from pigs ($n = 178$).

Finally, regarding the correlations between resistance profiles and pathotypes, penicillin and TMPS resistances are more frequently found in "non-clinical" or "respiratory" isolates ($P < 0.001$, $P = 0.011$, respectively) than in systemic isolates.

The MFA, confirmed by Chi-squared test, revealed an association between antimicrobial resistances to tetracycline, macrolides (tylosin, erythromycin, and tilmicosin), and lincosamides (lincomycin and clindamycin) ($P < 0.001$) and between resistances to penicillin and tiamulin ($P < 0.001$) (Fig. 2A). An association between TMPS resistance and lincosamides (lincomycin and clindamycin) resistance was also observed, but to a lesser extent ($P = 0.042$).

The MCA (Fig. 2B) and Chi-squared test revealed a statistically significant link between a systemic pathotype and an antimicrobial resistance to macrolides and/or lincosamides and/or tetracyclines. A significant link was also found between the serotype "others," the geographical origin "Brittany," the pathotypes "non-clinical" or "respiratory," and resistance to beta-lactams and pleuromutilin. The Chi-squared test also showed the influence of serotypes and pathotypes on the resistance profiles observed in this study ($P < 0.001$ and $P < 0.001$, respectively).

DISCUSSION

In this study, the NRI method was applied to calculate COWT values in order to distinguish the *S. suis* strains with or without acquired resistance to 22 antibiotics (non-wild type: NWT or wild type: WT, respectively). When the COWT calculation was not possible, the strain was categorized using the threshold diameters described in the literature (22, 25–27) and observations of the measured diffusion diameters. Five antibiotics are documented with MICs for *S. suis* in the CLSI's VET01S (22) (penicillin G, ceftiofur, enrofloxacin, florfenicol, and tetracycline), but interpretation diameters are given for only two of them (ceftiofur and florfenicol). The COWT values obtained with the NRI method for ceftiofur and florfenicol in this work do not differ more than 2 mm from the interpretation diameters indicated in the CLSI's VET01S for *S. suis* (22). Likewise,

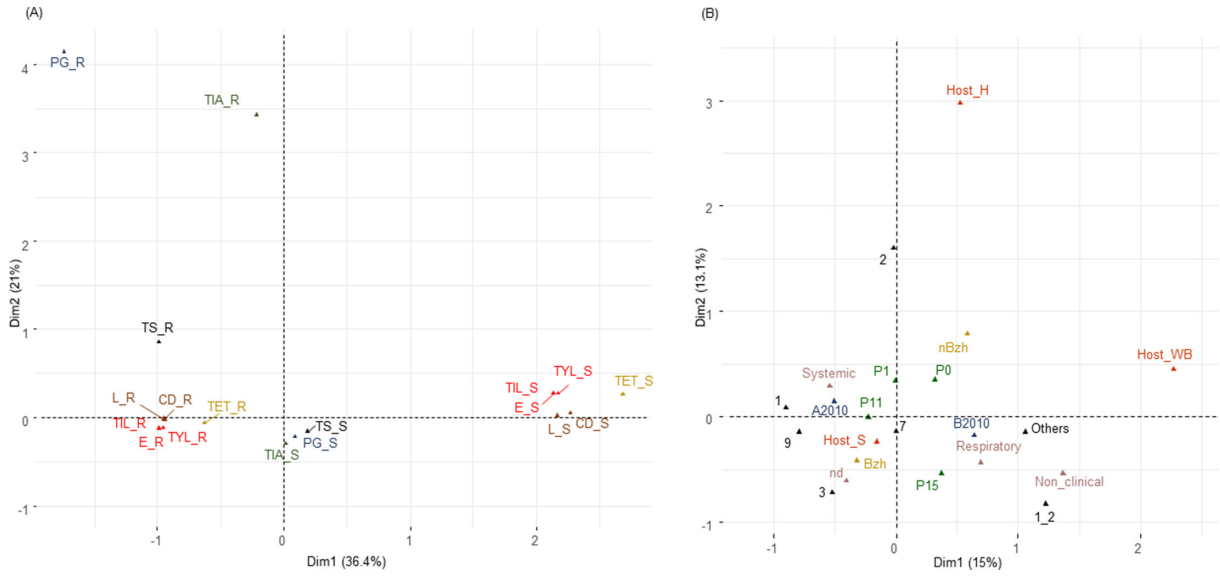

FIG 2 Comparative analysis of the resistance profiles of 200 strains of *S. suis* isolated in France against antibiotics belonging to different families. (A) Multiple factor analysis of antibiotic resistances: the different antibiotics belonging to the same family are represented using the same color code. Yellow was attributed to tetracycline, brown to lincosamides, red to macrolides, and blue, gray, and black to penicillin, tiamulin, and trimethoprim-sulfamethoxazole, respectively. Resistances are noted as "_R" and susceptibilities as "_S". TET, tetracycline; L, lincomycin; CD, clindamycin; E, erythromycin; TIL, tilmicosin; TYL, tylosin; PG, penicillin G; TIA, tiamulin; TS, trimethoprim-sulfamethoxazole. (B) Multiple correspondence analysis of all variables; in blue, black, yellow, red, and pink are active variables (which were used to build the MCA): isolation periods before 2010 (B2010) and after 2015 (A2010), serotypes (1, 1/2, 2, 3, 7, 9, Others), geographical origins (Brittany: Bzh, other regions of France: nBzh), hosts (swine, S; wild boars, WB; humans, H), and pathotypes (systemic, respiratory, and non-clinical), respectively; in green are additional variables (resistance profiles) (the correspondence table of the resistance profiles is available as additional data, Table S2).

the interpretation diameters indicated for *Streptococcus* spp. only differ by 2 mm at most from the COWT values obtained with the NRI method for chloramphenicol and vancomycin.

No strain appeared resistant to linezolid (oxazolidinone), quinupristin-dalfopristin (streptogramins A + B), vancomycin (glycopeptides), and gentamicin (to a high concentration level). Oxazolidinone (linezolid) appears to be very efficient against most Gram-positive bacteria, including pathogens of importance for human health (29). To our knowledge, *S. suis* resistance to linezolid and more broadly to the oxazolidinone family has only been reported in Asia in recent years (30–34). Two genes conferring two types of resistance mechanisms are known in the literature, the *cfr* gene (coding an rRNA methyltransferase) and the *optrA* gene (coding an ABC transporter) (35). The absence of resistance to linezolid in our collection of strains confirms that this resistance is not present or very rare in *S. suis* in France. Streptogramins are used against numerous highly resistant pathogens and are therefore classified as antibiotics for last-resort human therapy (36, 37). Streptogramin A resistance in *S. suis* conferred by the *cfr* gene and *lsa(E)* gene (coding an ABC transporter) has only been described in a few genomics studies (29, 30, 34, 38, 39). These genes are combined with an *erm(B)* gene in order to provide resistance to the quinupristin-dalfopristin combination tested in our work. Vancomycin is among the last-resort antimicrobial agents for the treatment of multidrug-resistant Gram-positive bacterial infections (40). Resistance to vancomycin has been reported in China but not yet in Europe, and more specifically in France, to our knowledge (32, 40). This can be explained by the difference in use specific to each country and the nature of this resistance, as resistance is due to the presence of the VanG-type resistance operon (40). *S. suis* has a naturally low level of resistance to aminoglycosides. This family of antibiotics is currently used in combination with beta-lactams in veterinary practice and is among the critically important antimicrobials (37). High-level resistance to aminoglycosides is due to several genes encoding three types of target modification enzymes leading to their inactivation, and are encoded by the *aac*, *aph,* and *ant* genes (35). The Resapath network indicated the presence, in France, of a low rate (<2%) of high-level resistance in *S. suis* isolated from diseased pigs in 2019 (2% for streptomycin, 4% for kanamycin, and 0% for gentamicin) (17). Our results are therefore in accordance with Resapath findings.

Using the NRI cut-off, none of the *S. suis* strains appeared resistant to the two phenicols tested (chloramphenicol and florfenicol). The resistance mechanisms to phenicols are mediated by the *cfr* and *optrA* genes discussed above, which confer resistance to linezolid. Another resistance mechanism is chloramphenicol acetyl-transferase mediated by the *cat* gene (35). In Europe, phenicol resistance in *S. suis* is less common than in Asia (35). Thus, the absence of resistance observed in this study is consistent with the observations reported in the literature (18, 26).

Only one strain resistant to fluoroquinolones was identified in our collection. The resistance was confirmed by MIC determination. This serotype-5 strain, isolated in 2009, has a multiresistance profile as it is also resistant to macrolides-lincosamides, tetracycline, pleuromutilin, diaminopyrimidine-sulfonamide, and penicillins. In Asia, the reported resistance rate to enrofloxacin is higher (21–32.8% before 2010 and 28.8% after 2010) than in both Europe (2% before 2010 and 1–5.3% after 2010) and North America (1.4% between 2006 and 2016) (25, 27, 41–43). The resistance rate measured in our work is consistent with the rate measured in Europe. Fluoroquinolone usage represents 2.4% of the total use of antibiotics in global pig production (12). Two types of resistance mechanisms have been described in the literature as follows: mutations in the targets of fluoroquinolones (DNA gyrase and topoisomerase IV) and efflux by an ABC transporter [*sat(A)* and *sat(B)* genes] (35).

Among the 200 strains tested, 5% was resistant to at least one antibiotic of the beta-lactam family. In France, the Resapath network registered a resistance rate to beta-lactams lower than 5% in 2019 (17). In Asia, the resistance rate to ampicillin (0.6–23% before 2010) is higher than both North America (2.3–3% before 2010 and 0.6%

after 2010) and South America (0.5%) (25, 27, 44). Resistance to ampicillin was reported to be lower than resistance to penicillin or ceftiofur (35). This is the case in our study for penicillin with three strains resistant to ampicillin and 10 strains resistant to penicillin G. A decrease in penicillin G resistance has been observed in North America (14–14.5% before 2010 dropping to 6.9% after 2010), whereas the resistance rate remains stable in Asia and Europe (respectively 0.9–27% and 13.5% in Asia, and 0–13% and 2.6–5% in Europe before and after 2010) (25, 43). In this study, no difference between the two time periods was observed, in accordance with the literature. Overall, the resistance rate measured in this study is similar to the rate reported in Europe (Denmark, England, and Sweden) but lower than the rate measured in North America and Asia (25–27, 41, 43). Penicillins—and especially amoxicillin—are the molecules used for first-line treatment of *S. suis* infections. Thus, investigations were pursued on the 10 *S. suis* strains resistant to beta-lactams. The determination of the MIC indicated that 10 strains were resistant to penicillin. All the strains resistant to penicillin were isolated from pigs in Brittany, and were categorized as "non-clinical" for five strains, "respiratory" for one strain, and "systemic" for one strain, while the pathotype of three other strains was unknown. The resistant strains belong to various serotypes and were isolated at different periods of time: serotypes 1 (one strain), 5 (one strain), 9 (one strain), 11 (two strains), 12 (two strains), 27 (two strains), and 29 (one strain). In this study, none of the strains tested were resistant to cephalosporins. This is in accordance with the much lower level of resistance to cephalosporin described in Europe (0.7% before 2010 and 0% after 2010) than in Asia and North America (0.6–23% before 2010 and 0.9% after 2010 for Asia and 1–23.1% before 2010 and 9.3% after 2010 for North America) (25). Resistance to beta-lactams is due to modifications within the signal peptide region of certain penicillin-binding proteins (*mraY* gene) (39).

Some of the strains tested showed acquired resistance mechanisms against pleuromutilins (8% of the strains are resistant to tiamulin). No data are available from Resapath for comparison. Resistance of *S. suis* to pleuromutilins has been observed worldwide (1.9% in Brazil and >10% in North America and Denmark) (27, 41, 44). In our study, the serotype seems to influence the pleuromutilins resistance profiles observed with three strains of serotype 1, four strains of serotype 9, and nine strains of serotype "others" (one strain of serotype 5, two strains of serotype 12, one each of serotypes 15, 16, 27, and 29, and two strains of serotype 31). Tiamulin is the third most used drug in pig production (12, 41), being prescribed for swine dysentery, colitis or ileitis, and pneumonia (45). Resistance mechanisms are encoded by two genes, the *cfr* gene and the ABC-transporter *Isa(E)* (33). Trimethoprim-sulfamethoxazole resistance was encountered in 16% of the studied strains (13% registered by the Resapath network) (17). Before 2010, North America had a low resistance rate (1–1.2%), while in Asia and Europe, resistance rates were variable (0–59.1% and 0–51.5%, respectively) (25). After 2010, the resistance rate in North America (2%) was still low (27). In Europe, the resistance rate appears to be around 10% (8.6% in Denmark and 12% in England), i.e., in the same range as observed in our strain collection (26, 41). Trimethoprim-sulfamethoxazole is an old antibiotic combination used in pig production (12). In our study, the resistance rate to these antibiotics differs according to the isolation period with a significant reduction in resistance between the two time periods. One third of the resistant strains were of serotype 1/2. Finally, among the 30 strains classified as "respiratory," 11 were TMPS resistant.

A high proportion of strains resistant to macrolides-lincosamides was observed (71%). In France, the Resapath annual report indicated in 2019 a figure of 60% for resistance to macrolides and lincosamides (54% for erythromycin, 65% for tylosin, 58% for spiramycin, and 61% for lincomycin) (17). The resistance rate measured in this study is thus above that reported by Resapath but in the range described in Europe (up to 75% for erythromycin and 8%–87.4% for clindamycin) (25). The erythromycin resistance rate increased in Asia after 2010 (96.8% in China) (43) but appears to have decreased in some European countries (48% in Denmark and 46% in England) (26, 41). Variations in the rate

of resistance to macrolides-lincosamides are observed worldwide, possibly explained by the usage of macrolides in pig production (12). In our study, differences in resistance were observed according to the geographical origin of the strains and the host, with a significant concentration of resistance in porcine isolates from Brittany.

A high proportion of resistance was also observed for tetracycline (81%) in accordance with the data from the Resapath network (17), which reported a resistance rate of 79% to tetracycline for *S. suis* isolated from pigs in 2019. In North America and Asia, resistance rates measured for tetracycline (87–100% and 86.9–96%, respectively) were higher for strains isolated before 2010. In Europe, the resistance rate before 2010 was relatively heterogeneous, ranging from 48% in the Netherlands to 95.4% in Spain (25). After 2010, tetracycline resistance rates in Asia (97.8% in China) and Europe (75% in Denmark and 91% in England) were still high (26, 41, 43). In North America, resistance to oxytetracycline was also high (95.4%) (27). These observations are explained by the worldwide use of tetracyclines in all pig production stages (first class of antibiotics used) (12). In our study, no change was observed in the rate of tetracycline resistance between the two time periods studied. As for macrolides-lincosamides, resistance to tetracyclines was higher in pigs than in humans or wild boars. The mechanisms of tetracycline resistance have been well studied. They include efflux pumps and ribosome protection proteins (35). The existence of numerous resistance genes may explain the diversity of the inhibition diameters observed.

In our study, 138 strains were classified as multi-resistant. Among them, the most common resistance pattern observed was the macrolides-lincosamides-tetracycline pattern (97 strains). This agrees with the association between resistance to tetracycline and macrolides-lincosamides detected by MFA. This resistance pattern is the most frequently described in the literature and generally results from a co-localisation of a tetracycline resistance gene with an *erm(B)* gene on mobile genetic elements (25–27, 35, 41, 42, 44, 46, 47). Macrolides and tetracyclines are among the most frequently used antibiotics in pig production worldwide (12). The World Health Organization has also defined them, among others, as critically (macrolides) or highly (lincosamides and tetracyclines) important antimicrobials for human medicine (36). In our study, antibiotics used exclusively in veterinary or human medicine as well as antibiotics used in both spheres were tested against strains isolated from swine, wild boars, and humans. There do not appear to be differences in the resistance profiles of these strains in terms of the type of medical field in which these antibiotics are used. The strain collection used for our study includes a small number of human (n = 13) and wild boar (n = 9) isolates, and it would be beneficial to test more strains in order to confirm these results. However, this shows the presence of these resistance genes as well as their persistence given the lack of selection pressure for some molecules. Among all the strains tested (200), three showed resistance to six antimicrobial families: beta-lactams, macrolides, lincosamides, pleuromutilins, tetracyclines, and trimethoprim-sulfamethoxazole. These strains belong to the least represented serotypes (5, 27, and 29) in our collection and were of non-determined pathotype "not determined," "non-clinical," and "respiratory" isolates, respectively.

In order to observe the potential impact of the French EcoAntibio plan intended to reduce the prescription, sale, and consumption of antibiotics, we analyzed strains isolated before 2010 and strains isolated after 2015. A significant change (P = 0.035) in the TMPS resistance rate was observed (63% of resistant strains before 2010 and 38% after 2010) in our collection. Between 2011 and 2019, the exposure of pigs to this antimicrobial fell by 60.6% in France (17), which could have a positive impact on the reduction of TMPS resistance in *S. suis*. In France, more than half of the national pig production is concentrated in Brittany. Overall, strains from this region appear significantly more resistant than those from other French regions. In the literature, the impact of the close proximity of pigs on the spread of resistance has already been studied at the scale of a few herds (48) or in a country, and the link between the intensity of animal production and the spread of resistance has been proven (27, 39, 41).

Some resistances were associated with particular serotypes. This is the case for penicillin resistance found in infrequent serotypes. Further studies are needed to explain these associations. It has been suggested that the serotype could predict the pathotype of *S. suis* strains (5). The significant link observed in our study between "non-clinical" or "respiratory" and serotypes other than serotypes 1, 2, 7, and 9 (serotypes called "other") supports this hypothesis. In addition, Hadjirin et al. pointed out a higher MIC in non-clinical isolates (39). In our study, a statistically significant link was observed between the pathotypes "non-clinical" or "respiratory" and resistance to penicillin and pleuromutilins. This agrees with a previous report on resistance to beta-lactams mainly found in commensal strains (1). It would therefore be beneficial to study these strains, which could serve as a reservoir of resistance genes. Weinert et al. suggested that the respiratory tract is more ecologically diverse than systemic locations (4). This environment could facilitate the appearance of resistance mutations and the horizontal gene transfer of mobile genetic elements. Since most resistance genes are carried by mobile genetic elements in *S. suis* (49), this would lead to an enrichment of "non-clinical" strains in resistance genes. To effectively combat antibiotic resistance, it appears vital to improve understanding of antimicrobial resistance in all serotypes, but especially those not frequently studied, but which can act as a reservoir of resistance genes for other bacteria.

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DIRECT CONTRIBUTION

M.D-T., E.J., C.M-C., V.L., and S.P. contributed to funding acquisition, study design, and conceptualization. M.D-T., C.M-C., V.L., S.P., and M-H.B-A. contributed to strains selections and supply. M.D-T., E.J., C.M-C., V.L., S.P., and S.B. contributed to investigation and data curation. S.B., C.C., and M.D-T. contributed to statistical analyses. M.D-T. contributed to formal analysis and writing the original draft. C.M-C., V.L., S.P., E.J., S.B., C.C., and M-H.B-A. contributed to validation. M.D-T., C.M-C., V.L., S.P., E.J., S.B., C.C., and M-H.B-A. contributed to writing, reviewing, and editing. All authors have read and agreed to the published version of the manuscript.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Table S1 (JCM00164-23-S0001.xlsx). Antibiotic susceptibility of *S. suis* strains (n = 200) collected in France.
Table S2 (JCM00164-23-S0002.xlsx). Antibiotic susceptibility/resistance profiles of *S. suis* strains (n = 200) collected in France.

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