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

Manon Dechêne-Tempier, Eric Jouy, Marie-Hélène Bayon-Auboyer, Stéphanie Bougeard, Claire Chauvin, Virginie Libante, Sophie Payot, Corinne Marois-Créhan

### ► To cite this version:

Manon Dechêne-Tempier, Eric Jouy, Marie-Hélène Bayon-Auboyer, Stéphanie Bougeard, Claire Chauvin, et al.. Antimicrobial resistance profiles of *Streptococcus suis* isolated from pigs, wild boars, and humans in France between 1994 and 2020. *Journal of Clinical Microbiology*, 2023, 61 (9), pp.e0016423. 10.1128/jcm.00164-23 . anses-04228454

**HAL Id: anses-04228454**

**<https://anses.hal.science/anses-04228454>**

Submitted on 18 Oct 2023

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1 Research Articles

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

5

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14

15 Running Head: Antimicrobial resistance patterns of *S. suis* in France

16

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18

19 **Abstract**

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

38 **Keywords:** *Streptococcus suis*, pathogen, antimicrobial resistance, CLSI, disc diffusion  
39 method.

40 **1. Introduction**

41 *Streptococcus suis* is a zoonotic pathogen causing pig infections and substantial economic  
42 losses. Even though serotype 2 is preponderant, other serotypes can be responsible for  
43 infections worldwide (1). In Europe, serotypes 9, 2 and 7 are dominant among clinical pig

44 isolates (61%, 18% and 7% of the isolates, respectively) whereas serotypes 1/2 and 3 are also  
45 frequently found in the rest of the world (2, 3). Together with serotypes, Weinert et al. proposed  
46 to group *S. suis* strains into three groups or pathotypes according to their isolation sites: [1]  
47 “non-clinical”, [2] “respiratory” or [3] “systemic” (4). These groups correspond to the [1]  
48 commensal, [2] possibly opportunistic, and [3] pathogenic groups defined by Estrada et al. (5).  
49 These authors also suggested using the serotype to predict the pathotype, as they demonstrated  
50 a link between serotypes and pathotypes (5). Human cases of *S. suis* were mainly described in  
51 Asia, with three large outbreaks in China reported in 1998, 2005 and 2016 (6). Clinical  
52 manifestations of *S. suis* infection include meningitis, septicaemia, endocarditis and arthritis  
53 (7). In France, human infections are scarce, the eighth French case since 1995 having been  
54 reported in 2016 (8) and the latest case corresponds to a woman hospitalised for meningitis after  
55 preparing wild boar meat, with *S. suis* identified as the causative agent of the disease (7).

56 Several families of antimicrobials are commonly used in veterinary medicine to treat bacterial  
57 infections. These include tetracyclines, beta-lactams, polymyxins, macrolides-lincosamides,  
58 aminoglycosides, sulphonamides or trimethoprim-sulphonamides (TMPSs) (9). The antibiotic  
59 families mostly prescribed for gastrointestinal pig infections worldwide are tetracyclines,  
60 lincosamides, pleuromutilins and macrolides (10-13). Chlortetracycline, tetracycline and  
61 amoxicillin are used for treating respiratory and infectious diseases (14, 15). In France,  
62 tetracycline, beta-lactams (penicillins), macrolides and TMPSs are generally used for  
63 respiratory infections, polypeptides and tetracycline for sepsis and gastrointestinal infections  
64 (12). beta-lactams (penicillins) are indicated for treating *S. suis* meningitis, septicaemia and/or  
65 arthritis (12). In France, the regulation of antibiotics in veterinary medicine must meet French  
66 and European standards. The use of these molecules is subject to marketing authorisations and  
67 be indicated in the "Summary of product characteristics" (SPC) sheet. Checks are made on their  
68 distribution, prescription and use in accordance with European laws. In 2010, the European

69 Union initiated a common European programme called “One Health” (16). This programme is  
70 based on the premise that there is a direct link between human health, animal health and more  
71 generally environmental health. In 2012, France implemented its first “EcoAntibio” plan in  
72 order to reduce the use of antibiotics nationwide by more than 25% in 5 years. This plan was a  
73 success, as the use of antibiotics dropped by 37% (9). Resapath (a French surveillance network  
74 for antimicrobial resistance in bacteria from diseased animals) is part of the antimicrobial  
75 resistance surveillance system for animals set up in France. The Resapath network collects and  
76 analyses data from routine antimicrobial susceptibility tests (disc diffusion method) performed  
77 by diagnostic laboratories in the network, and publishes an annual report. For *S. suis*, this report  
78 includes the proportions of susceptible isolates for 12 antibiotics (amoxicillin, oxacillin,  
79 erythromycin, tylosin, spiramycin, lincomycin, streptomycin, kanamycin, gentamicin,  
80 tetracycline, doxycycline and trimethoprim-sulphonamides) (17). Except from Resapath, few  
81 data on the resistance of *S. suis* in France are available in the literature (18, 19). Therefore, the  
82 aim of this study was to investigate resistance of a collection of *S. suis* strains isolated in France  
83 to a larger number of antimicrobials. This collection of strains was fully characterised regarding  
84 serotype, and included strains isolated from humans and wild boars. The statistical associations  
85 between resistance/susceptibility and the isolation period, the strain’s geographical origin,  
86 serotype, host and pathotype were analysed.

## 87 **2. Materials and Methods**

### 88 **2.1. Bacterial strains**

89 Two hundred isolates of *S. suis* were selected from the ANSES strain collection (n=1,619),  
90 resulting from different research programs, in order to get a set of strains representative of the  
91 diversity of serotypes, geographical origins and hosts available. All strains isolated from human  
92 cases or wild boar were selected. This set included 21 strains of serotype 1, 20 of serotype 1/2,  
93 32 of serotype 2, 30 of serotype 3, 30 of serotype 7, 33 of serotype 9, and 34 strains of other

94 serotypes (6 strains of serotype 4 and 1 or 2 strains of each of the following serotypes: 5, 8, 10,  
95 11, 12, 14, 15, 16, 18, 21, 24, 27, 28, 29 and 31) (Table S1). In France, more than 50% of  
96 national swine production is based in Brittany, so we chose to include 130 strains collected in  
97 Brittany and 70 from other regions of France. Most of the strains were isolated from pigs  
98 (n=178); the remaining 22 were isolated from humans (n=13) or wild boars (n=9). Strains were  
99 allocated to two groups according to the isolation period (88 strains for the “before 2010” group,  
100 and 112 for “after 2015”) to examine a potential impact of the French EcoAntibio plan. Finally,  
101 strains were classified into three pathotypes. The “systemic” group contained 127 strains  
102 isolated from samples of the nervous system, blood or joint when the causes of death were  
103 attributed to an infection by this pathogen. The “respiratory” group hosted 31 strains isolated  
104 from pulmonary samples obtained either from sick or clinically healthy animals on farms that  
105 had had outbreaks of *S. suis* infections. The strains in the “non-clinical” group (n=36) were  
106 from the upper respiratory tract with no sign of *S. suis* infection in animals and 6 strains were  
107 not classified due to a lack of information. More details can be found in the supplementary data  
108 (Table S1). All the strains used were confirmed by serotyping using the PCR method described  
109 by Okura et al. (20).

## 110 **2.2. Culture conditions and antimicrobial susceptibility testing**

111 All the strains were stored at -80°C in buffered peptone water solution with glycerol (20%).  
112 Each strain was sub-cultured on Columbia Agar with 5% Sheep Blood (BIO-RAD®, Marne la  
113 Coquette, France) and incubated overnight at 37°C under 5% CO<sub>2</sub>. Antimicrobial susceptibility  
114 was tested using the disc (agar) diffusion method, according to Clinical Laboratory Standards  
115 Institute (CLSI) standards (VET01-A and VET01-S) (21, 22). Briefly, an inoculum of 1.10<sup>8</sup>  
116 CFU per mL was prepared for each strain and used to inoculate a Mueller-Hinton plate  
117 containing 5% of sheep blood (120-mm square plates, BIO-RAD®) before the application of  
118 antimicrobial discs (BIO-RAD, Marne-La-Coquette, France or Mast Diagnostics, Amiens,

119 France). The plates were incubated 22 hours  $\pm$  1 hour at 35°C and, after bacterial growth,  
120 inhibition zone diameters were measured with a sliding calliper. The reference strain *S.*  
121 *pneumoniae* ATCC 49619 was used as a quality control according to CLSI recommendations.

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

### 127 **2.3. Strain categorisation**

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

### 139 **2.4. Statistical analyses**

140 The strains were classified according to different resistance profiles (Table S1) in proportion to  
141 their resistance/susceptibility to the antibiotics tested. In total, 24 resistance profiles were  
142 identified following the analyses carried out. The first (univariate) step entailed using a Chi-  
143 squared test and Fisher correction (required for limited expected counts) to explore for each

144 antibiotic possible links with other antimicrobial resistance rates together with links between  
145 each antimicrobial resistance profile and the isolation period, serotype, geographical origin,  
146 host and/or pathotype. The second (multivariate) step considered all the antimicrobial  
147 resistances. First, a multiple correspondence analysis (MCA) was performed to illustrate  
148 whether the antimicrobial resistance rates for the different antibiotics or the antimicrobial  
149 resistance profiles depend on the isolation period, serotype, geographical origin, host and/or  
150 pathotype (the latter variables being considered as supplementary in the analysis). Then, a  
151 multiple factor analysis (MFA) was used to determine whether there is a correlation between  
152 the antimicrobial resistance observed for the antibiotics of the same family and antibiotics of  
153 different families. For this purpose, each family was considered as a block of variables. In order  
154 to ensure robustness of the various tests and analyses, statistical analyses were performed only  
155 on groups with at least five representative strains. All the analyses were performed using the R  
156 software with the FactoMineR package (28). Only results with a p-value equal to or lower than  
157 0.05 were considered significant and subsequently interpreted.

### 158 **3. Results**

#### 159 **3.1. Determination of cut-off values using the NRI method**

160 The NRI method applied to the distributions of inhibition zone diameters can be used to define  
161 two groups corresponding to the two observed populations divided by the COWT (Table 1).  
162 Only one population was observed for gentamicin, chloramphenicol, florfenicol, linezolid,  
163 quinupristin/dalfopristin and vancomycin. Two were observed for ampicillin, penicillin,  
164 cefotaxime, ceftiofur, imipenem, clindamycin, lincomycin, erythromycin, tilmicosin, tylosin,  
165 enrofloxacin, levofloxacin, norfloxacin and trimethoprim-sulphamethoxazole. Finally, a  
166 complex pattern was observed for tiamulin and tetracycline. In the absence of CLSI clinical  
167 interpretative criteria, the COWT value was therefore determined manually for tiamulin and the  
168 CLSI's interpretive criteria for *Streptococcus* spp. were used for tetracycline.



169

170

171 Table 1: Normalised Resistance Interpretation cut-off value obtained with the disc diffusion  
 172 method for 200 *S. suis* strains.

Class	Antimicrobial agent	Disc load (µg)	Distribution pattern	NRI cut-off value (mm)*	CLSI (mm)**	Wild-type population (mm)	Non-wild-type population (mm)
<i>Aminoglycosides</i>	Gentamicin	500	Unimodal	23		23-39	NA <sup>2</sup>
<i>β-Lactams</i>	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 <sup>2</sup>
	Ceftiofur	30	Multi-modal	24	≥21 (S) <i>S. suis</i>	24-36	NA <sup>2</sup>
	Imipenem	10	Multi-modal	30		32-44	NA <sup>2</sup>
<i>Phenicols</i>	Chloramphenicol	30	Unimodal	19	≥21 (H) <i>Streptococcus spp.</i>	19-30	NA <sup>2</sup>
	Florfenicol	30	Unimodal	21	≥22 (S) <i>S. suis</i>	21-34	NA <sup>2</sup>
<i>Lincosamides</i>	Clindamycin	2	Multi-modal	20	≥19 (H) <i>Streptococci</i>	20-29	6-19
<i>Macrolides</i>	Lincomycin	15	Multi-modal	22		22-32	6-21
	Erythromycin	15	Multi-modal	22	≥21 (H) <i>Streptococci</i>	32-24	6-17
	Tilmicosin	15	Multi-modal	10		10-21	6
<i>Fluoroquinolones</i>	Tylosin	30	Multi-modal	18		18-27	6-16
	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
<i>Pleuromutilins</i>	Tiamulin	30	Multi-modal	NA <sup>1</sup>		14-29	6-13
<i>Oxazolidinones</i>	Linezolid	30	Unimodal	22		24-37	NA <sup>2</sup>
<i>Streptogramins</i>	Quinupristin/Dalfopristin	15	Unimodal	15		15-27	NA <sup>2</sup>
<i>Tetracycline</i>	Tetracycline	30	Multi-modal	NA <sup>1</sup>	≥23 (H) <i>Streptococcus spp.</i>	23-33	6-22
<i>Diaminopyrimidines-sulphonamides</i>	Trimethoprim-Sulphamethoxazole	25	Multi-modal	12		12-31	6-11
<i>Glycopeptides</i>	Vancomycin	30	Unimodal	18	≥17 (H) <i>Streptococcus spp.</i>	20-32	NA <sup>2</sup>

173 \*NRI cut-off calculated for 22 antibiotics. \*\*Interpretive criteria provided by the CLSI (22) for  
 174 Swine (S) or Human (H). NA<sup>1</sup>, no diameter cut-off available. NA<sup>2</sup>, no values for the non-wild-  
 175 type clusters. The CLSI Breakpoint and NRI cut-off are both expressed in millimetres, as are  
 176 the values obtained for the wild-type and non-wild-type clusters.

177

### 178 3.2. Antibiotic susceptibility of the strains

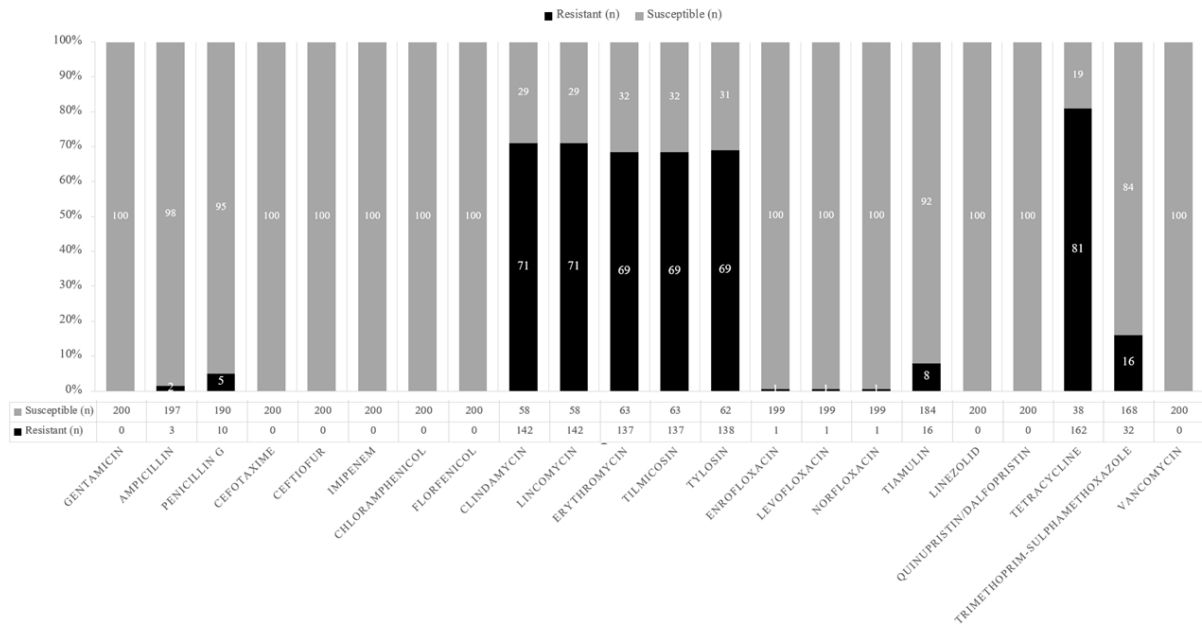
179 No resistance was observed for the following antibiotics: Gentamicin (aminoglycosides) (to  
180 high concentration levels), linezolid (oxazolidinones), quinupristin/dalfopristin  
181 (streptogramins) and vancomycin (glycopeptides) (Table 1). For phenicols (chloramphenicol  
182 and florfenicol),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

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

201

202

203



204

205 Figure 1: Resistance rates observed for 200 *S. suis* strains. *The percentage of strains without or*  
 206 *with resistance mechanisms (in light grey or black respectively) were represented for each*  
 207 *antibiotic tested. The antibiotics are classified according to the antimicrobial family to which*  
 208 *they belong.*

209

210

211 In total, 86% of the strains tested were resistant to at least one antibiotic (n=171). Among these  
 212 isolates, 138 can be classified as multi-resistant as they are resistant to three families of  
 213 antibiotics. Thirty-five, eight and three strains were resistant to respectively four, five or six  
 214 families of antibiotics. The resistance patterns are included in the supplementary data (Table  
 215 S2). The most common multi-resistance patterns observed in this study are for macrolides  
 216 (erythromycin, tilmicosin, tylosin) associated with lincosamides (clindamycin, lincomycin) and  
 217 tetracycline (97 strains, profile P11), followed by macrolides-lincosamides, tetracycline and  
 218 trimethoprim-sulphamethoxazole (20 strains, profile P15).

219 **3.3. Prevalence of antimicrobial resistance according to the isolation period, serotype,**  
220 **geographical origin, host and pathotype**

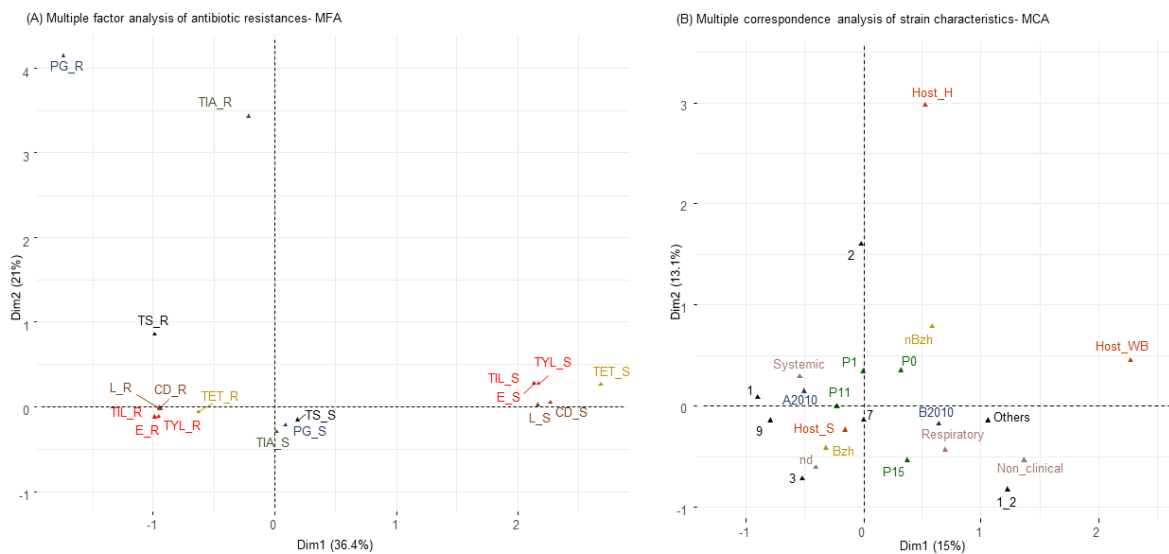
221 A significant decrease in resistance to TMPS occurred between the two periods, with a  
222 proportion of resistant strains dropping from 23.5% (20/85) before 2010 to 10.4% (12/115) after  
223 2015 ( $p < 0.035$ ).

224 Serotype 2 was over-represented among lincomycin-susceptible strains, with 20 strains out of  
225 61, i.e. 33% ( $p = 0.001$ ). Interestingly, penicillin resistance was associated ( $p < 0.001$ ) with less  
226 common serotypes (two strains of serotypes 11 and 12, and one strain of serotypes 1, 5, 9, 15,  
227 27 and 29). A similar result was observed for tiamulin-resistant strains, there being an over-  
228 representation of the serotypes classified under “others“, with seven strains out of sixteen from  
229 serotypes 1 or 9 (three and four strains respectively) compared with nine strains from serotypes  
230 5, 12, 15, 16, 27, 29 and 31 (1, 2, 1, 1, 1, 1 and 2 strains respectively) ( $p < 0.001$ ). Strains with  
231 these serotypes are less frequent and thus less studied than the others. Finally, among the thirty-  
232 two TMPS-resistant strains, there was an over-representation ( $p \leq 0.01$ ) of serotypes “1/2”  
233 (nine strains), “others” (seven strains) and serotype 3 (six strains).

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

238 Furthermore, strains isolated from humans or wild boars appear to have fewer acquired  
239 resistance mechanisms to macrolides (erythromycin  $p < 0.001$ , tilmicosin  $p < 0.001$ , tylosin  $p$   
240  $< 0.001$ ), lincosamides (clindamycin  $p = 0.002$ , lincomycin  $p = 0.001$ ) and tetracycline ( $p =$   
241  $0.003$ ) than strains isolated from pigs. However, these results must be taken with caution since  
242 in the collection the number of strains isolated from humans or wild boar ( $n=22$ ) is much lower  
243 than the number of strains isolated from pigs ( $n=178$ ).

244 Finally, regarding the correlations between resistance profiles and pathotypes, penicillin and  
 245 TMPS resistances are more frequently found in “non-clinical” or “respiratory” isolates ( $p <$   
 246  $0.001$ ,  $p = 0.011$  respectively) than in systemic isolates.  
 247 The multiple factor analysis (MFA), confirmed by Chi-squared test, revealed an association  
 248 between antimicrobial resistances to tetracycline, macrolides (tylosin, erythromycin,  
 249 tilmicosin) and lincosamides (lincomycin and clindamycin) ( $p < 0.001$ ) and between resistances  
 250 to penicillin and tiamulin ( $p < 0.001$ ) (Figure 2 (A)). An association between TMPS resistance  
 251 and lincosamides (lincomycin and clindamycin) resistance was also observed but to a lesser  
 252 extent ( $p = 0.042$ ).  
 253



254  
 255 Figure 2: Comparative analysis of the resistance profiles of 200 strains of *S. suis* isolated in  
 256 France against antibiotics belonging to different families. (A) *Multiple factor analysis of*  
 257 *antibiotic resistances: the different antibiotics belonging to the same family are represented*  
 258 *using the same colour code. Yellow was attributed to tetracycline, brown to lincosamides, red*  
 259 *to macrolides and blue, grey and black to penicillin, tiamulin and trimethoprim-*  
 260 *sulphamethoxazole respectively. Resistances are noted '\_R' and susceptibilities '\_S'.*

261 *Abbreviations: TET: tetracycline, L: lincomycin, CD: clindamycin, E: erythromycin, TIL:*  
262 *tilmicosin, TYL: tylosin, PG: penicillin G, TIA: tiamulin, TS: trimethoprim-sulphamethoxazole.*  
263 *(B) Multiple correspondence analysis of all variables: In blue, black, yellow, red and pink:*  
264 *active variables (which were used to build the MCA): isolation periods (A2010, B2010),*  
265 *serotypes (1, 1/2, 2, 3, 7, 9, “Others”) geographical origins (Bzh, nBzh), hosts (swine, wild*  
266 *boars and Humans) and pathotypes (systemic, respiratory and non-clinical) respectively. In*  
267 *green: additional variables (resistance profiles) (the correspondence table of the resistance*  
268 *profiles is available as additional data, Table S2).*

269

270 The multiple correspondence analysis (MCA) (Figure 2 (B)) and Chi-squared test revealed a  
271 statistically significant link between a systemic pathotype and an antimicrobial resistance to  
272 macrolides and/or lincosamides and/or tetracyclines. A significant link was also found between  
273 the serotype “others”, the geographical origin “Brittany”, the pathotypes “non-clinical” or  
274 “respiratory” and resistance to beta-lactams and pleuromutilin. The Chi-squared test also  
275 showed the influence of serotypes and pathotypes on the resistance profiles observed in this  
276 study ( $p < 0.001$  and  $p < 0.001$  respectively).

277

#### 278 **4. Discussion**

279 The strains with an acquired resistance mechanism were differentiated from those without (non-  
280 wild type versus wild type) by calculating the COWT values of 22 antibiotics using the NRI  
281 method. When the COWT calculation was not possible, the strain was categorised using the  
282 threshold diameters described in the literature (25-27) and observations of the measured  
283 diffusion diameters. Five antibiotics are documented with MIC for *S. suis* in the CLSI’s  
284 VET01S (22) (penicillin G, ceftiofur, enrofloxacin, florfenicol and tetracycline) but  
285 interpretation diameters are given for only two of them (ceftiofur and florfenicol). The COWT

286 values obtained with the NRI method for ceftiofur and florfenicol in this work do not differ  
287 more than two millimetres from the interpretation diameters indicated in the CLSI's VET01S  
288 for *S. suis* (22). Likewise, the interpretation diameters indicated for *Streptococcus* spp only  
289 differ by two millimetres at most from the COWT values obtained with the NRI method for  
290 chloramphenicol and vancomycin.

291 No strain appeared resistant to linezolid (oxazolidinone), quinupristin-dalfopristin  
292 (streptogramins A+B), vancomycin (glycopeptides) and gentamicin (to high concentration  
293 level). Oxazolidinone (linezolid) appears to be very efficient against most Gram-positive  
294 bacteria, including pathogens of importance for human health (29). To our knowledge, *S. suis*  
295 resistance to linezolid and more broadly to the oxazolidinone family has only been reported in  
296 Asia in recent years (30-34). Two genes conferring two types of resistance mechanisms are  
297 known in the literature, the *cfr* gene (coding an rRNA methyltransferase) and the *optrA* gene  
298 (coding an ABC transporter) (35). The absence of resistance to linezolid in our collection of  
299 strains confirms that this resistance is not present or very rare in *S. suis* in France.  
300 Streptogramins are used against numerous highly-resistant pathogens and are therefore  
301 classified as antibiotics for last-resort human therapy (36, 37). Streptogramin A resistance in *S.*  
302 *suis* conferred by the *cfr* gene and *lsa(E)* gene (coding an ABC transporter) has only been  
303 described in a few genomics studies (29, 30, 34, 38, 39). These genes are combined with an  
304 *erm(B)* gene in order to provide resistance to the quinupristin-dalfopristin combination tested  
305 in our work. Vancomycin is among the last-resort antimicrobial agents for the treatment of  
306 multidrug-resistant Gram-positive bacterial infections (40). Resistance to vancomycin has been  
307 reported in China but not yet in Europe and more specifically in France, to our knowledge (32,  
308 40). This can be explained by the difference in use specific to each country and the nature of  
309 this resistance, as resistance is due to the presence of the VanG-type resistance operon (40). *S.*  
310 *suis* has a naturally low level of resistance to aminoglycosides. This family of antibiotics is

311 currently used in combination with beta-lactams in veterinary practice and is among the  
312 critically important antimicrobials (37). High-level resistance to aminoglycosides is due to  
313 several genes encoding three types of target modification enzymes leading to their inactivation,  
314 and are encoded by the *aac*, *aph* and *ant* genes (35). The Resapath network indicated the  
315 presence in France of a low rate (<2%) of high-level resistance in *S. suis* isolated from diseased  
316 pigs in 2019 (2% for streptomycin, 4% for kanamycin, 0% for gentamicin) (17). Our results are  
317 therefore in accordance with Resapath findings.

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

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



335 Among the 200 strains tested, 5% were resistant to at least one antibiotic of the beta-lactam  
336 family. In France, the Resapath network registered a resistance rate to beta-lactams lower than  
337 5% in 2019 (17). In Asia, the resistance rate to ampicillin (0.6-23% before 2010) is higher than  
338 both North America (2.3-3% before 2010 and 0.6% after 2010), and South America (0.5%) (25,  
339 27, 44). Resistance to ampicillin was reported to be lower than resistance to penicillin or  
340 ceftiofur (35). This is the case in our study for penicillin, with three strains resistant to ampicillin  
341 and ten strains resistant to penicillin G. A decrease in penicillin G resistance has been observed  
342 in North America (14-14.5% before 2010 dropping to 6.9% after 2010) whereas the resistance  
343 rate remains stable in Asia and Europe (respectively 0.9-27% and 13.5% in Asia and 0-13%  
344 and 2.6-5% in Europe before and after 2010) (25, 43). In this study, no difference between the  
345 two time periods were observed, in accordance with the literature. Overall, the resistance rate  
346 measured in this study is similar to the rate reported in Europe (Denmark, England and Sweden)  
347 but lower than the rate measured in North America and Asia (25-27, 41, 43). Penicillins — and  
348 especially amoxicillin — is the molecule used for first-line treatment of *S. suis* infections. Thus,  
349 investigations were pursued on the ten *S. suis* strains resistant to beta-lactams. Determination  
350 of the MIC indicated that ten strains were resistant to penicillin. All the strains resistant to  
351 penicillin were isolated from pigs in Brittany, and were categorised as “non-clinical” for five  
352 strains, “respiratory” for one strain and “systemic” for one strain, while the pathotype of three  
353 other strains was unknown. The resistant strains belong to various serotypes and were isolated  
354 at different periods of time: serotypes 1 (1 strain), 5 (1 strain), 9 (1 strain), 11 (2 strains), 12 (2  
355 strains), 27 (2 strains), and 29 (1 strain). In this study, none of the strains tested was resistant to  
356 cephalosporins. This is in accordance with the much lower level of resistance to cephalosporin  
357 described in Europe (0.7% before 2010 and 0% after 2010) than in Asia and North America  
358 (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 is 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 of serotype 9, and 9 of serotype “others” (one strain of serotype 5, two of serotype 12, one each of serotypes 15, 16, 27 and 29, and two 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 *cfp* gene and ABC-transporter *Isa(E)* (33). Trimethoprim-sulphamethoxazole 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-sulphamethoxazole 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

384 by Resapath but in the range described in Europe (up to 75% for erythromycin and 8% to 87.4%  
385 for clindamycin) (25). The erythromycin resistance rate increased in Asia after 2010 (96.8% in  
386 China) (43) but appears to have decreased in some European countries (48% in Denmark and  
387 46% in England) (26, 41). Variations in the rate of resistance to macrolides-lincosamides are  
388 observed worldwide, possibly explained by the usage of macrolides in pig production (12). In  
389 our study, differences in resistance were observed according to the geographical origin of the  
390 strains and the host, with a significant concentration of resistance in porcine isolates from  
391 Brittany.

392 A high proportion of resistance was also observed for tetracycline (81%) in accordance with  
393 the data from the Resapath network (17), which reported a resistance rate of 79% to tetracycline  
394 for *S. suis* isolated from pigs in 2019. In North America and Asia, resistance rates measured for  
395 tetracycline (87-100%, 86.9-96% respectively) were higher for strains isolated before 2010. In  
396 Europe, the resistance rate before 2010 was relatively heterogeneous, ranging from 48% in the  
397 Netherlands to 95.4% in Spain (25). After 2010, tetracycline resistance rates in Asia (97.8% in  
398 China) and Europe (75% in Denmark and 91% in England) were still high (26, 41, 43). In North  
399 America, resistance to oxytetracycline was also high (95.4%) (27). These observations are  
400 explained by the worldwide use of tetracyclines in all pig production stages (1st class of  
401 antibiotics used) (12). In our study, no change was observed in the rate of tetracycline resistance  
402 between the two time periods studied. As for macrolides-lincosamides, resistance to  
403 tetracyclines was higher in pigs than in humans or wild boars. The mechanisms of tetracycline  
404 resistance have been well studied. They include efflux pumps and ribosome protection proteins  
405 (35). The existence of numerous resistance genes may explain the diversity of the inhibition  
406 diameters observed.

407 In our study, 138 strains were classified as multi-resistant. Among them, the most common  
408 resistance pattern observed was the macrolides-lincosamides-tetracycline pattern (97 strains).

409 This agrees with the association between resistance to tetracycline and macrolides-lincosamides  
410 detected by MFA. This resistance pattern is the most frequently described in the literature and  
411 generally results from a co-localisation of a tetracycline resistance gene with an *erm(B)* gene  
412 on mobile genetic elements (25-27, 35, 41, 42, 44, 47). Macrolides and tetracyclines are among  
413 the most frequently used antibiotics in pig production worldwide (12). The World Health  
414 Organization has also defined them, among others, as critically (macrolides) or highly  
415 (lincosamides and tetracyclines) important antimicrobials for human medicine (36). In our  
416 study, antibiotics used exclusively in veterinary or human medicine as well as antibiotics used  
417 in both spheres were tested against strains isolated from swine, wild boars, and humans. There  
418 do not appear to be differences in the resistance profiles of these strains in terms of the type of  
419 medical field in which these antibiotics are used. The strain collection used for our study  
420 includes a small number of human (n = 13) and wild boar (n = 9) isolates and it would be  
421 beneficial to test more strains in order to confirm these results. However, this shows the  
422 presence of these resistance genes as well as their persistence given the lack of selection  
423 pressure for some molecules. Among all the strains tested (200), three showed resistance to six  
424 antimicrobial families: beta-lactams, macrolides, lincosamides, pleuromutilins, tetracyclines  
425 and trimethoprim-sulphamethoxazole. These strains belong to the least represented serotypes  
426 (5, 27 and 29) in our collection and were of non-determined pathotype “not determined”, “non-  
427 clinical” and “respiratory” isolates respectively.

428 In order to observe a potential impact of the French EcoAntibio plan intended to reduce the  
429 prescription, sale and consumption of antibiotics, we analysed strains isolated before 2010 and  
430 strains isolated after 2015. A significant change ( $p = 0.035$ ) in the TMPS resistance rate was  
431 observed (63% of resistant strains before 2010 and 38% after 2010) in our collection. Between  
432 2011 and 2019, the exposure of pigs to this antimicrobial fell by 60.6% in France (17), which  
433 could have a positive impact on the reduction of TMPS resistance in *S. suis*. In France, more

434 than half of national pig production is concentrated in Brittany. Overall, strains from this region  
435 appear significantly more resistant than those from other French regions. In the literature, the  
436 impact of the close proximity of pigs on the spread of resistance has already been studied at the  
437 scale of a few herds (48) or in a country, and the link between the intensity of animal production  
438 and the spread of resistance has been proven (27, 39, 41).

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

456

457 **Supplementary Materials**

458 All the data obtain with the Anses strains and use in this paper are presented in Tables S1 and  
459 S2.

460 Table S1: Antibiotic susceptibility of *S. suis* strains (n = 200) collected in France

461 Table S2: Antibiotic susceptibility/resistance profiles of *S. suis* strains (n = 200) collected in  
462 France

### 463 **Author Contributions**

464 MDT, EJ, CM, VL, SP: funding acquisition, study design, conceptualization. MDT, CM, VL,  
465 SP, MHBA: strains selections and supply. MDT, EJ, CM, VL, SP, SB: investigation, data  
466 curation. SB, CC, MDT: Statistical analyses. MDT formal analysis, writing the original draft.  
467 CM, VL, SP, EJ, SB, CC, MHBA: validation. MDT, CM, VL, SP, EJ, SB, CC, MHBA  
468 writing—reviewing and editing. All authors have read and agreed to the published version of  
469 the manuscript.

### 470 **Funding**

471 This research received no external funding, MDT was supported by ANSES and INRAE.

### 472 **Acknowledgments**

473 The automatic and manual excel programs used for the cut-off values were made available  
474 through the courtesy of P. Smith, W. Finnegan, and G. Kronvall.

### 475 **Conflicts of Interest**

476 The authors declare no conflict of interest.

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