# Antimicrobial susceptibility of *Streptococcus suis* isolated from clinically healthy swine in Brazil

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## Abstract

*Streptococcus suis* is an important pathogen in the swine industry. This study is the first to report on the antimicrobial susceptibility of *S. suis* isolated from clinically healthy pigs in Brazil; the fourth major pork producer in the world. The antimicrobial susceptibility of 260 strains was determined by disc diffusion method. Strains were commonly susceptible to ceftiofur, cephalexin, chloramphenicol, and florfenicol, with more than 80% of the strains being susceptible to these antimicrobials. A high frequency of resistance to some of the antimicrobial agents was demonstrated, with resistance being most common to sulfa-trimethoprim (100%), tetracycline (97.69%), clindamycin (84.61%), norfloxacin (76.92%), and ciprofloxacin (61.15%). A high percentage of multidrug resistant strains (99.61%) were also found. The results of this study indicate that ceftiofur, cephalexin, and florfenicol are the antimicrobials of choice for empirical control of the infections caused by *S. suis.*

## Résumé

Streptococcus suis *est un pathogène important de l'industrie porcine. Cette étude est la première à rapporter la susceptibilité antimicrobienne de souches* S. suis *isolées de porcs cliniquement sains provenant du Brésil, le quatrième producteur de porc à l'échelle de la planète. La susceptibilité antimicrobienne de 260 souches fut examinée par la méthode de diffusion des disques imprégnés d'antibiotiques. Plusieurs souches étaient susceptibles au ceftiofur, céphalexine, chlorphénicol, et florfénicol, avec plus de 80 % des souches qui étaient susceptibles à ces antimicrobiens. Une haute fréquence de résistance à certains des antimicrobiens futs démontrée, principalement au sulfa-triméthoprime (100 %), tetracycline (97,69 %), clindamycine (84,61 %), norfloxacine (76,92 %), et ciprofloxacine (61,15 %). Un pourcentage élevé de souches multirésistantes (99,61 %) a également été observé. Les résultats de cette étude indiquent que le ceftiofur, la céphalexine et le florfénicol sont les antimicrobiens de choix pour le contrôle empirique des infections causées par* S. suis.

*(Traduit par les auteurs)*

*Streptococcus suis* is recognized worldwide as an important pathogen in intensive swine production and has been associated with meningitis, septicemia, endocarditis, arthritis, pneumonia, and, occasionally, endometritis, abortion, rhinitis, and vaginitis (1). Moreover, *S. suis* is a zoonotic agent and may cause a variety of infections in individuals working in close contact with swine or pork products (2). Strains of *S. suis* are divided into 35 serotypes, according to its polysaccharide capsular antigens (3–6). The natural habitat of *S. suis* is the upper respiratory tract, particularly the tonsils and nasal cavities, and the genital and alimentary tracts of pigs. The movement of healthy carrier pigs harboring the microorganism is the main route of transmission between herds (7).

Since current vaccines are bacterins and provide only partial serotype-specific protection, antimicrobial agents have become increasingly important in treating and controlling infection caused by *S. suis.* Previous studies have investigated the antimicrobial susceptibility of strains from diseased and healthy pigs in different countries (8–12). Differences in the level of resistance have been observed between different countries, serotypes, and over time (9,13,14). In Brazil, there is only one report showing the susceptibility profiles of strains of *S. suis* isolated from diseased pigs (15). Brazil is the fourth major pork producer in the world, producing, consuming, and exporting around 3300, 2700, and 600 million tonnes, respectively. The objective of this study was to determine the antimicrobial susceptibility, as well as the resistotypes, of Brazilian *S. suis* strains recovered from clinically healthy pigs.

A total of 260 *S. suis* field strains were included in this study. During a 4-month period from November 2009 to February 2010, palatine tonsils, nasal cavities, and vaginal samples were collected using cotton swabs from 240 clinically healthy pigs, including 24 sows, 96 suckling pigs, 60 nursery pigs, and 60 fattening pigs, from 3 different farms located in 3 different cities within the state of São Paulo, Brazil; one of the most important swine producing states in Brazil.

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**Table I. Specific breakpoints for each of the antibiotics used**

| Antimicrobials | Susceptible | Intermediate | Resistant |
|---|---|---|---|
| Ampicillin | ≥ 26 | 19–25 | ≤ 18 |
| Azithromycin | ≥ 18 | 14–17 | ≤ 13 |
| Cephalexin | ≥ 18 | 15–17 | ≤ 14 |
| Ceftiofur | ≥ 21 | 18–20 | ≤ 17 |
| Ciprofloxacin | ≥ 21 | 16–20 | ≤ 15 |
| Clindamycin | ≥ 19 | 16–18 | ≤ 15 |
| Chloramphenicol | ≥ 21 | 18–20 | ≤ 17 |
| Doxycycline | ≥ 16 | 13–15 | ≤ 12 |
| Erythromycin | ≥ 21 | 16–20 | ≤ 15 |
| Enrofloxacin | ≥ 23 | 17–22 | ≤ 16 |
| Florfenicol | ≥ 22 | 19–21 | ≤ 18 |
| Levofloxacin | ≥ 17 | 14–16 | ≤ 13 |
| Norfloxacin | ≥ 17 | 13–16 | ≤ 12 |
| Penicillin | ≥ 28 | 22–27 | ≤ 21 |
| Sulfa + Trimethoprim | ≥ 16 | 11–15 | ≤ 10 |
| Tetracycline | ≥ 23 | 19–22 | ≤ 18 |

**Table II. Antimicrobial susceptibility of 260 *Streptococcus suis* strains isolated from clinically normal pigs in Brazil**

| Antimicrobials | Susceptible strains (%) | Intermediate strains (%) | Resistant strains (%) |
|---|---|---|---|
| Ampicillin | 174 (66.92) | 69 (26.54) | 17 (6.54) |
| Azithromycin | 189 (72.69) | 22 (8.46) | 49 (18.85) |
| Cephalexin | 219 (84.23) | 4 (1.54) | 37 (14.23) |
| Ceftiofur | 248 (95.39) | 9 (3.46) | 3 (1.15) |
| Ciprofloxacin | 64 (24.62) | 37 (14.23) | 159 (61.15) |
| Clindamycin | 6 (2.31) | 34 (13.08) | 220 (84.61) |
| Chloramphenicol | 213 (81.92) | 25 (9.62) | 22 (8.46) |
| Doxycycline | 162 (62.31) | 70 (26.92) | 28 (10.77) |
| Erythromycin | 124 (47.69) | 15 (5.77) | 121 (46.54) |
| Enrofloxacin | 115 (44.23) | 31 (11.92) | 114 (43.85) |
| Florfenicol | 212 (81.54) | 10 (3.85) | 38 (14.61) |
| Levofloxacin | 162 (62.31) | 16 (6.15) | 82 (31.54) |
| Norfloxacin | 46 (17.69) | 14 (5.39) | 200 (76.92) |
| Penicillin | 127 (48.85) | 86 (33.08) | 47 (18.07) |
| Sulfa + Trimethoprim | 0 (0.00) | 0 (0.00) | 260 (100.00) |
| Tetracycline | 6 (2.31) | 0 (0.00) | 254 (97.69) |

Swabs were cultured on a 5% sheep blood agar plate. The plates were incubated aerobically at 37°C and inspected for growth after 24 and 48 h. Three colonies 1 to 2 mm in diameter, showing alpha-hemolysis, were suspected as potential *S. suis* (16). The *S. suis*-like strains were identified using polymerase chain reaction (PCR). The DNA extraction was done using the Chelating Ion Exchange Resin (Chelex-100 resin; Bio-Rad Laboratories, California, USA) and the amplification was done as described by Okwumabua et al (17). The PCR positive strains were serotyped by coagglutination technique with sera against the 34 described serotypes (18).

The antimicrobial susceptibility of *S. suis* strains was determined by using the disc diffusion method in Mueller-Hinton agar supplemented with 5% defibrinated sheep blood, according to the recommendation by CLSI (19), following specific breakpoints indicated by the manufacturer (Cefar Diagnóstica Ltda, São Paulo, Brazil) as shown in Table I. The antimicrobial agents used in this study were: ampicillin (10 µg); ceftiofur (30 µg); ciprofloxacin (5 µg); florfenicol (30 µg); chloramphenicol (30 µg); norfloxacin (10 µg); enrofloxacin (5 µg); sulfa-trimethoprim (25 µg); tetracycline (30 µg); levofloxacin (5 µg); doxycycline (30 µg); and penicillin (10 UI), erythromycin (15 µg), cephalexin (30 µg), azithromycin (15 µg), and clindamycin (2 µg). The chi-squared test was used to estimate the relationship among herd, animal category, and antimicrobial susceptibility. Differences were considered significant when $P < 0.05$.

Results from serotyping showed that most isolates (67%) were untypable with only 86 isolates being serotyped. The fact that most strains isolated from clinically healthy animals are untypable is a common finding (M. Gottschalk, reference laboratory for *S. suis* serotyping, unpublished data). Among the typable isolates, serotypes 22 (16 isolates), 30 (13 isolates), 21 (10 isolates), and 27 (9 isolates) were the most commonly identified. Serotypes 5, 7, 11, 14, 15, 16, 18, 19, 20, 24, 28, 29, and 34 were also identified with less than 3 isolates/serotypes. All of these serotypes have already been described as causes of disease in pigs (20) and, some of them (serotypes 5, 14, 16, and 24), in humans (2,21). A total of 16 isolates reacted with 2 or more serotypes, which is highly common within isolates from clinically healthy animals (unpublished observations).

The results of the susceptibility testing of the *S. suis* strains are shown in Table II. No significant differences were observed when comparing isolates recovered from different animal categories or regions of the country (result not shown). More than 80% of the strains were susceptible to ceftiofur (95.39%), cephalexin (84.23%), chloramphenicol (81.92%), and florfenicol (81.54%). A high frequency of resistance to some of the antimicrobial agents was demonstrated, with resistance being most common to sulfa-trimethoprim (100%), tetracycline (97.69%), clindamycin (84.61%), norfloxacin (76.92%), and ciprofloxacin (61.15%).

The antimicrobial susceptibility profiles (resistotypes) of the *S. suis* strains studied were constructed using all 16 antimicrobials agents. The overall distribution of resistotypes is shown in Table III. None of the 260 *S. suis* strains was susceptible to the 16 antimicrobials. Multidrug resistant strains (≥ 3 antimicrobial agents) were observed in 99.61% (259/260) of all isolates. Two hundred and fifty-three strains (97.30%) were resistant to at least 4 antimicrobials, 221 strains (85.00%) were resistant to at least 6 antimicrobials, and 9 strains were resistant to all 16 drugs. Of the 59 resistotypes, resistotype 19 (ampicillin, ciprofloxacin, clindamycin, doxycycline, norfloxacin, penicillin, sulfa-trimethoprim, and tetracycline), with 28 strains (10.76%), was the most frequently isolated. The strains belonging to serotype 5 were resistant to 11 different antimicrobials; those of serotype 7 were resistant to 9 or 10 antimicrobials; those of serotype 19 were resistant to 9 different antimicrobials; those of serotype 21 were resistant to 7, 8, 9, or 10 (7 to 10) different antimicrobials; and those of serotype 11 were resistant to 4, 5, 6, or 7 (4 to 7) different antimicrobials. The strains that were important to public health were resistant to 7 (serotype 14), 10 (serotype 16), and 13 to 14 (serotype 24) antimicrobials.

A high frequency of resistance to tetracyclines (tetracycline/97.69%), sulphonamides (sulfa-trimethoprim/100%), macrolides

**Table III. Resistotypes of 260 *Streptococcus suis* strains isolated from clinically normal pigs in Brazil**

| Number of antimicrobials | Number of strains | Resistotype |
|---|---|---|
| 2 | 1 | CLI-SULT |
| 3 | 3 | CLI-SULT-TET |
| 3 | 3 | ENR-SULT-TET |
| 4 | 12 | CLI-ERI-SULT-TET |
| 5 | 1 | AZI-CIP-ERI-ENR-SULT |
| 5 | 1 | AZI-CLI-ERI-SULT-TET |
| 5 | 2 | AZI-CLI-DOX-SULT-TET |
| 5 | 14 | CLI-ERI-NOR-SULT-TET |
| 5 | 2 | ENR-LEV-NOR-SULT-TET |
| 6 | 6 | AZI-CLI-ERI-NOR-SULT-TET |
| 6 | 4 | AZI-CLI-ERI-PEN-SULT-TET |
| 7 | 4 | AMP-CIP-CLI-NOR-PEN-SULT-TET |
| 7 | 5 | AZI-CIP-CLI-ERI-ENR-SULT-TET |
| 7 | 3 | AZI-CIP-CLI-ERI-NOR-SULT-TET |
| 7 | 2 | AZI-CIP-CLI-ERI-PEN-SULT-TET |
| 7 | 1 | AZI-CLI-ERI-NOR-PEN-SULT-TET |
| 7 | 1 | AZI-CLI-ERI-ENR-NOR-SULT-TET |
| 7 | 7 | CIP-CLI-ERI-ENR-NOR-SULT-TET |
| 8 | 28 | AMP-CIP-CLI-DOX-NOR-PEN-SULT-TET |
| 8 | 11 | AZI-CIP-CLI-CLO-ERI-NOR-SULT-TET |
| 8 | 5 | AZI-CIP-CLI-ERI-ENR-NOR-SULT-TET |
| 8 | 2 | AZI-CIP-CLI-ERI-NOR-PEN-SULT-TET |
| 8 | 3 | AZI-CIP-CLI-DOX-ERI-NOR-SULT-TET |
| 8 | 5 | CIP-CLI-ERI-ENR-LEV-NOR-SULT-TET |
| 9 | 3 | AMP-AZI-CIP-CLI-ERI-ENR-NOR-PEN-SULT |
| 9 | 6 | AMP-CIP-CLI-DOX-ENR-NOR-PEN-SULT-TET |
| 9 | 2 | AZI-CEFA-CIP-CLI-ERI-ENR-NOR-SULT-TET |
| 9 | 13 | AZI-CIP-CLI-ERI-ENR-LEV-NOR-SULT-TET |
| 9 | 2 | AZI-CIP-CLI-ERI-ENR-NOR-PEN-SULT-TET |
| 9 | 4 | AZI-CIP-CLI-DOX-ERI-ENR-NOR-SULT-TET |
| 10 | 12 | AMP-AZI-CEFA-CLI-CLO-ERI-FLO-PEN-SULT-TET |
| 10 | 2 | AMP-AZI-CIP-CLI-ERI-ENR-NOR-PEN-SULT-TET |
| 10 | 3 | AZI-CEFA-CIP-CLI-DOX-ERI-ENR-NOR-SULT-TET |
| 10 | 3 | AZI-CIP-CLI-CLO-DOX-ERI-FLO-NOR-SULT-TET |
| 10 | 11 | AZI-CIP-CLI-ERI-ENR-LEV-NOR-PEN-SULT-TET |
| 10 | 11 | AZI-CIP-CLI-DOX-ERI-ENR-LEV-NOR-SULT-TET |
| 11 | 1 | AMP-AZI-CIP-CLI-DOX-ERI-ENR-LEV-NOR-SULT-TET |
| 11 | 4 | AMP-AZI-CIP-CLI-ERI-ENR-LEV-NOR-PEN-SULT-TET |
| 11 | 2 | AZI-CEFA-CIP-CLI-ERI-ENR-LEV-NOR-PEN-SULT-TET |
| 11 | 5 | AZI-CIP-CLI-ERI-ENR-FLO-LEV-NOR-PEN-SULT-TET |
| 11 | 4 | AZI-CIP-CLI-DOX-ERI-ENR-FLO-LEV-NOR-SULT-TET |
| 11 | 5 | AZI-CIP-CLI-DOX-ERI-ENR-LEV-NOR-PEN-SULT-TET |
| 12 | 1 | AMP-AZI-CEFA-CIP-CLI-DOX-ERI-ENR-NOR-PEN-SULT-TET |
| 12 | 1 | AMP-AZI-CEFA-CIP-CLI-ERI-ENR-LEV-NOR-PEN-SULT-TET |
| 12 | 3 | AMP-AZI-CIP-CLI-DOX-ERI-ENR-FLO-NOR-PEN-SULT-TET |
| 12 | 2 | AMP-AZI-CIP-CLI-DOX-ERI-ENR-LEV-NOR-PEN-SULT-TET |
| 12 | 2 | AMP-AZI-CIP-CLI-ERI-ENR-FLO-LEV-NOR-PEN-SULT-TET |
| 12 | 4 | AZI-CIP-CLI-CLO-DOX-ERI-ENR-LEV-NOR-PEN-SULT-TET |
| 12 | 2 | AZI-CIP-CLI-CLO-DOX-ERI-ENR-FLO-LEV-NOR-SULT-TET |
| 12 | 1 | AZI-CIP-CLI-DOX-ERI-ENR-FLO-LEV-NOR-PEN-SULT-TET |
| 13 | 2 | AMP-AZI-CEFA-CEFT-CIP-CLI-ERI-ENR-LEV-NOR-PEN-SULT-TET |
| 13 | 2 | AMP-AZI-CEFA-CIP-CLI-DOX-ERI-ENR-LEV-NOR-PEN-SULT-TET |

**Table III. (continued)**

| | | |
|---|---|---|
| 13 | 3 | AMP-AZI-CIP-CLI-DOX-ERI-ENR-FLO-LEV-NOR-PEN-SULT-TET |
| 13 | 3 | AZI-CEFA-CIP-CLI-DOX-ERI-ENR-FLO-LEV-NOR-PEN-SULT-TET |
| 13 | 1 | AZI-CIP-CLI-CLO-DOX-ERI-ENR-FLO-LEV-NOR-PEN-SULT-TET |
| 14 | 1 | AMP-AZI-CEFT-CIP-CLI-CLO-DOX-ERI-ENR-FLO-NOR-PEN-SULT-TET |
| 14 | 4 | AZI-CEFA-CIP-CLI-CLO-DOX-ERI-ENR-FLO-LEV-NOR-PEN-SULT-TET |
| 16 | 6 | AMP-AZI-CEFA-CEFT-CIP-CLI-CLO-DOX-ERI-ENR-FLO-LEV-NOR-PEN-SULT-TET |
| 16 | 3 | AMP-AZI-CEFA-CEFT-CIP-CLI-CLO-DOX-ERI-ENR-FLO-LEV-NOR-PEN-SULT-TET |

AMP — Ampicillin; AZI — Azithromycin; CEFA — Cephalexin; CEFT — Ceftiofur; CIP — Ciprofloxacin; CLI — Clindamycin; CLO — Chloramphenicol; DOX — Doxycycline; ERI — Erythromycin; ENR — Enrofloxacin; FLO — Florfenicol; LEV — Levofloxacin; NOR — Norfloxacin; PEN — Penicillin; SULT — Sulfa + Trimethoprim; TET — Tetracycline.

(erythromycin/46.54%), and lincosamides (clindamycin/84.61%) was found within the Brazilian strains. *Streptococcus suis* strains isolated from diseased or clinically healthy pigs with high levels of resistance to these classes of antimicrobials have already been described in the literature for other countries (9–11,22) and could be related with the wide use of these antimicrobials in veterinary medicine.

The antimicrobial agents representing the class of quinolones that showed high levels of resistance: norfloxacin (76.92%), ciprofloxacin (61.15%), enrofloxacin (43.85%), and levofloxacin (31.54%). The resistance to enrofloxacin (43.85%) demonstrated in this study was higher than the results of strains from diseased pigs in Spain (2%) (10) and other European countries (0%) (11).

The result of this study showed a higher rate of resistance to florfenicol (14.61%) than to chloramphenicol (8.46%). Similarly, a higher rate of resistance to florfenicol (21.1%) was demonstrated by Zhang et al (12). Since chloramphenicol has been banned in food animals and replaced by florfenicol, antimicrobial susceptibility studies have been showing an increase in the rate of resistance to florfenicol.

Among beta-lactams, the most effective antimicrobial was ceftiofur (95.39%), followed by cephalexin (84.23%), ampicillin (66.92%), and penicillin (48.85%). These results are in accordance with other studies that have described *S. suis* strains as mainly susceptible to these antimicrobial agents (9,10,13,14). However, a large number of strains were classified as intermediate to ampicillin (26.54%) and to penicillin (33.08%). This indicates that there is increased antimicrobial resistance to this first line class of antimicrobial agents.

The most prevalent resistotypes demonstrated by Vela et al (10) and Zhang et al (12) were formed by 4 and 5 antimicrobials, respectively, whereas the most prevalent resistotype found in this study consisted of 8 antimicrobials. The percentage of multidrug resistant strains showed in this study (99.61%) was higher than that demonstrated by Zhang, et al (12), far beyond those described in studies involving clinical strains (10,23), the highest already reported for *S. suis* strains. The differences observed between countries might be explained by different usage of antimicrobial agents or due to methodological differences between studies. Standardization of susceptibility testing methods at an international level is necessary to facilitate comparisons.

In conclusion, this is the first study not only in Brazil, but also in the American continent, of antibiotic resistance from isolates recovered from clinically healthy pigs. It is important to mention that results presented in this study represent isolates recovered in recent years in one important swine producing state in Brazil. Further studies with isolates recovered over several years are necessary to evaluate the progression of resistant patterns within Brazilian strains. Beta-lactams are still the most active antimicrobials against *S. suis* strains. The results of this study show that ceftiofur, cephalexin, and florfenicol are the antimicrobials of choice for empirical control of the infections caused by *S. suis* in Brazil. The presence of resistant and intermediate strains to ampicillin and penicillin also suggests the need for a continuous surveillance of the susceptibility pattern of this pathogen. The high percentage of multidrug resistant strains belonging to serotypes previously described as causing clinical disease in pigs and humans, may lead to complications in the control and treatment of the infections by *S. suis*.

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