Streptococcus suis in employees and the environment of swine slaughterhouses in São Paulo, Brazil: Occurrence, risk factors, serotype distribution, and antimicrobial susceptibility

Taíssa Cook Siqueira Soares, Marcelo Gottschalk, Sonia Lacouture, Jane Megid, Paulo Eduardo Martins Ribolla, José Carlos de Figueiredo Pantoja, Antonio Carlos Paes

Abstract

Streptococcus suis is an important pathogen in the swine industry. This article is the first to report the occurrence, risk factors, serotype distribution, and antimicrobial susceptibility of *S. suis* recovered from employees and environmental samples of swine slaughterhouses in Brazil. Tonsillar swabs from all 139 pig-slaughtering employees and 261 environmental swabs were collected for detection of *S. suis* and serotyping by monoplex and multiplex polymerase chain reaction, respectively. Antimicrobial susceptibility was determined by the disk-diffusion method. Although *S. suis* was not detected in any of the tested employees, it was isolated from 25% of the environmental samples. Significant differences ($P < 0.05$) in the occurrence of *S. suis* were observed between slaughterhouses and between areas of low, medium, and high risk. The most frequent serotypes were 4 and 29, each accounting for 12% of the isolates, followed by 5, 12, 21, and 31, each accounting for 6%. High rates of susceptibility to the antimicrobials doxycycline (100%), ceftiofur (94%), ampicillin (81%), and cephalexin (75%) were observed. However, multidrug resistance was observed in all the isolates. Because *S. suis* is present in the environment of swine slaughterhouses, on carcasses and knives, as well as on the hands of employees in all areas, all employees are at risk of infection.

Résumé

Streptococcus suis *est un agent pathogène important dans l'industrie porcine. Cet article est le premier à rapporter la fréquence, les facteurs de risque, la distribution des sérotypes, et la sensibilité aux antimicrobiens d'isolats de* S. suis *provenant des employés et d'échantillons de l'environnement d'abattoirs de porcs au Brésil. Des échantillons des amygdales des 139 employés et 261 échantillons environnementaux furent prélevés pour détection de* S. suis *et sérotypage par réaction d'amplification en chaine monoplex et multiplex, respectivement. La sensibilité aux antimicrobiens a été déterminée par la méthode de diffusion en disque. Bien que* S. suis *ne fut isolé d'aucun des employés testés, la bactérie a été isolée de 25 % des échantillons environnementaux. Des différences significatives (*P $< 0,05$*) dans la fréquence de* S. suis *furent observées entre les abattoirs et entre les zones à risque faible, moyen, et élevé. Les sérotypes 4 et 29 étaient les plus fréquents, comptant chacun pour 12 % des isolats, suivi des sérotypes 5, 12, 21, et 31, chacun comptant pour 6 %. Des pourcentages élevés de sensibilité à la doxycycline (100 %), au ceftiofur (94 %), à l'ampicilline (81 %) et à la céphalexine (75 %) ont été notés. Toutefois, de la résistance multiple fut observée chez tous les isolats. Étant donné que* S. suis *est présent dans l'environnement des abattoirs de porc, sur les carcasses et les couteaux, ainsi que sur les mains des employés dans toutes les zones, tous les employés sont à risque de s'infecter.*

(Traduit par Docteur Serge Messier)

Introduction

Streptococcus suis is recognized worldwide as an important pathogen of intensive swine production and an important zoonotic agent. It has been associated with a variety of infections, such as meningitis, peritonitis, endocarditis, and septic shock, in individuals working in close contact with swine or pork products (1,2). In Vietnam and Thailand, respectively, *S. suis* was reported as the 1st and 2nd causes of bacterial meningitis in adults (2,3). More than 15 cases of *S. suis* infection in humans have also been described in South America, mainly in Argentina (4).

The asymptomatic carrier state in humans is poorly understood. Few epidemiologic studies have been done to establish the prevalence of *S. suis* among individuals in the main groups at risk (5,6). Of the 35 capsular serotypes currently identified, serotype 2 is considered the most virulent and the most frequently isolated from both swine

Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Veterinária e Zootecnia, Universidade Estadual Paulista Júlio de Mesquita Filho, Distrito de Rubião Júnior, s/no. Botucatu, São Paulo, Brasil CEP 18.618.970 (Soares, Megid, Pantoja, Paes); Centre de recherche en infectiologie porcine et avicole, Faculté de médecine vétérinaire, Université de Montréal, 3200, rue Sicotte, Saint-Hyacinthe, Québec J2S 2M2 (Gottschalk, Lacouture); Departamento de Parasitologia, Instituto de Biociências de Botucatu, Universidade Estadual Paulista Júlio de Mesquita Filho, Distrito de Rubião Júnior, s/no. Botucatu, São Paulo, Brasil CEP 18.618.970 (Ribolla).

Address all correspondence to Dr. Taíssa Cook Siqueira Soares; telephone: 51-14-38802101; e-mail: taissacook@hotmail.com

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Table I. Occurrence of *Streptococcus suis* according to slaughterhouse, sample source (hand or knife), and risk area

	Slaughterhouse; Number of positive samples/Number of collected samples (and %)				
Variable	A	B	C	D	All
Sample source					
Hand	7/22 (32)	7/35 (20)	4/32 (12)	17/43 (40)	35/132 (26)
Knife	3/15 (20)	2/17 (12)	4/20 (20)	7/23 (30)	16/75 (21)
P-value	0.42	0.46	0.46	0.46	0.40
Risk area[a]					
Low	2/8 (25)	3/11 (27)	1/15 (7)	0/7 (0)	6/41 (15)
Medium	7/14 (50)	3/29 (10)	4/23 (17)	14/37 (38)	28/103 (27)
High	1/15 (7)	3/12 (25)	3/14 (21)	10/22 (45)	17/63 (27)
P-value	0.03	0.32	0.51	0.08	0.25
Total	10/37 (27)	9/52 (17)	8/52 (15)	24/66 (36)	51/207 (25)
P-value					0.03

[a] Low risk: piggery, stunning, dehairing and stamping; medium risk: scalding and washing, dressing, and inspection of carcasses; high risk: bleeding, evisceration, and washing and inspection of viscera.

and humans. However, serotypes 1, 4, 5, 14, 16, 21, and 24, as well as an untypeable strain, have also been isolated from clinical cases in humans (7–13). Moreover, serotypes 20, 22, and 26 have recently been suggested as belonging to a new streptococcal species (14).

Currently available vaccines provide only partial and serotype-specific protection. Therefore, antimicrobial agents have become important in treating and controlling infections caused by *S. suis*. Differences in the level of resistance between countries and serotypes, and over time, have been demonstrated (15–17). In Brazil, which is the 4th most important pork producer and exporter in the world, few scientific papers have been written about this pathogen (18–20). The present study is the first that aimed to determine the occurrence, serotype distribution, and antimicrobial susceptibility, as well as the resistotypes, of *S. suis* recovered from employees and the environment of swine slaughterhouses in Brazil.

Materials and methods

Samples

During a period of 4 mo, from June to September 2013, 400 samples were collected from various sources at 4 slaughterhouses in 4 cities in the state of São Paulo, Brazil. In each slaughterhouse, tonsil samples were taken from all 139 pig-slaughtering employees in 3 risk areas: low risk (piggery, stunning, dehairing, and stamping), medium risk (scalding and washing, dressing, and inspection of carcasses), and high risk (bleeding, evisceration, and washing and inspection of viscera). During slaughter 261 environmental swabs were also collected: 132 and 75 from employee hands and knives, respectively; 42 from the cut surface of the thorax of the carcasses; 4 from scalding tanks; 4 from dehairing tables; and 4 from the floor of killing rooms. The swabs were conditioned and transported in sterile tubes containing Stuart transport medium (OXOID LTDA, Basingstoke, Hampshire, England) and processed 3 to 6 h after collection.

Detection and typing of *S. suis*

Each sample was analyzed by polymerase chain reaction (PCR) both without culture and after bacteriologic culture. Swabs were cultured on 5% sheep blood agar plates and introduced into microtubes containing 300 μL of the Chelex 100 resin (Bio-Rad Laboratories, Hercules, California, USA) used for DNA extraction. The plates were incubated at 37°C in aerobic conditions and inspected for growth after 24 and 48 h. All colonies of 1 to 2 mm in diameter showing α-hemolysis were considered potential *S. suis* (21). The *S. suis*-like strains and DNA extracted directly from the swabs were submitted to monoplex and multiplex PCR for identification and serotyping, respectively, as previously described (22,23).

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the *S. suis* strains was determined by the disk-diffusion method with the use of Müeller–Hinton agar supplemented with 5% defibrinated sheep blood, according to the recommendations of the Clinical and Laboratory Standards Institute (24). The antimicrobial agents used in this study were amikacin (30 μg), ampicillin (10 μg), azithromycin (15 μg), ceftiofur (30 μg), cephalexin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), doxycycline (30 μg), enrofloxacin (5 μg), erythromycin (15 μg), florfenicol (30 μg), gentamicin (10 μg), levofloxacin (5 μg), norfloxacin (10 μg), penicillin (10 IU), streptomycin (10 μg), tetracycline (30 μg), and trimethoprim/sulfamethoxazole (TMP/SMX) (25 μg).

Statistical analysis

Statistical tests were done with PROC FREQ and PROC GLIMMIX of SAS/STAT software (SAS Institute, Cary, North Carolina, USA, version 9.3). Differences were considered significant when the *P*-value was less than 0.05.

Results

Among the 139 employees tested, no human carrier was identified. Table I shows the detection of *S. suis* in the environment according to slaughterhouse, source of sample (hand or knife), and risk area. Of the 207 hand and knife swabs, 51 (25%) were positive for *S. suis:* 35 (26%) of the hand swabs and 16 (21%) of the knife swabs. Of the hand and knife swabs collected from low-risk, medium-risk, and high-risk areas, *S. suis* was isolated from 15% (6/41), 27% (28/103), and 27% (17/63), respectively. Significant differences ($P < 0.05$) were observed in the occurrence of *S. suis* between slaughterhouses and between the areas of low, medium, and high risk, especially for slaughterhouses A and D. The likelihood of isolating *S. suis* from collected samples was 2.7 times greater at slaughterhouse D than at slaughterhouse B and 3.1 times greater at slaughterhouse D than at slaughterhouse C.

In addition, *S. suis* was isolated from the surface of the dehairing table of slaughterhouse A and from 6 carcasses: 3 sampled at slaughterhouse B and 3 at slaughterhouse C.

Among the 261 environmental swabs (of hands, knives, carcasses, scalding tanks, dehairing tables, and the floor of killing rooms), 58 (22%) were positive for *S. suis*, on average, when PCR was done without culture, whereas only 16 (6%) yielded positive results when cultured. All samples from which *S. suis* was isolated were PCR-positive. Therefore, only 16 strains could be submitted for typing PCR and antimicrobial susceptibility testing. Of those, 8 (50%) were untypeable. Of the remaining 8 strains, the most frequent serotypes were 4 and 29, each accounting for 12%, followed by 5, 12, 21, and 31, each accounting for 6%. However, serotypes 2 and 14, the serotypes most commonly affecting humans, were not found.

The results of the susceptibility testing of the 16 *S. suis* strains are shown in Table II. Among β-lactams, the most effective antimicrobial was ceftiofur, followed by ampicillin, cephalexin, and penicillin. Surprisingly, the prevalence of penicillin susceptibility was generally low. A high frequency of resistance to some of the antimicrobial agents was demonstrated, the most important being to streptomycin (94%), clindamycin (94%), tetracycline (88%), TMP/SMX (75%), erythromycin (75%), azithromycin (69%), norfloxacin (62%), and ciprofloxacin (62%). All 16 strains showed multidrug resistance (resistance to 3 or more antimicrobial agents): 15 (94%) were resistant to at least 7 antimicrobials and 13 (81%) to at least 10. None of the 16 were resistant to all 19 antimicrobials.

The antimicrobial susceptibility profiles (resistotypes) of the *S. suis* strains were constructed with the use of all 19 antimicrobial agents (Table III). A predominant resistotype could not be identified. The strains belonging to the serotypes already described as being potentially zoonotic were resistant to 15 or 16 (serotype 4) and 17 (serotype 5) antimicrobials.

Table II. Antimicrobial susceptibility of 16 *S. suis* strains recovered from slaughterhouse employees' hands and knives

	Number (and %) of strains		
Antimicrobial	Susceptible	Intermediate	Resistant
Beta-lactams			
Ampicillin	13 (81)	2 (12)	1 (6)
Penicillin	5 (31)	8 (50)	3 (19)
Cephalexin	12 (75)	1 (6)	3 (19)
Ceftiofur	15 (94)	1 (6)	0 (0)
Aminoglycosides			
Amikacin	3 (19)	7 (44)	6 (38)
Streptomycin	1 (6)	0 (0)	15 (94)
Gentamicin	7 (44)	4 (25)	5 (31)
Macrolides			
Azithromycin	4 (25)	1 (6)	11 (69)
Erythromycin	2 (12)	2 (12)	12 (75)
Quinolones			
Ciprofloxacin	3 (19)	3 (19)	10 (62)
Enrofloxacin	5 (31)	5 (31)	6 (38)
Levofloxacin	6 (38)	2 (12)	8 (50)
Norfloxacin	4 (25)	2 (12)	10 (62)
Lincosamides			
Clindamycin	1 (6)	0 (0)	15 (94)
Tetracyclines			
Doxycycline	16 (100)	0 (0)	0 (0)
Tetracycline	1 (6)	1 (6)	14 (88)
Sulfonamides			
Trimethoprim/ sulfamethoxazole	3 (19)	1 (6)	12 (75)
Chloramphenicol	9 (56)	6 (38)	1 (6)
Florfenicol	8 (50)	5 (31)	3 (19)

Discussion

Carriers are individuals who harbor a potentially virulent infectious agent without showing clinical signs of infection. In this study, no healthy carrier employees were detected. However, human carrier status has previously been demonstrated in slaughterhouse workers (5,6).

Significant differences in the occurrence of *S. suis* between slaughterhouse areas have already been demonstrated. Breton et al (25) showed that the likelihood of isolating *S. suis* from an eviscerator's hand was 4 times greater than the likelihood of isolation from a butcher's hand and that the likelihood of isolating *S. suis* from an eviscerator's knife was 7 times greater than the likelihood of isolation from a butcher's knife. Evisceration is considered a high-risk area owing to the risks associated with accidents involving sharp objects and exposure to the viscera of pigs. In contrast, Rojas et al (5) found a higher frequency of *S. suis* in medium-risk areas. In the present study, there were significant differences in the occurrence of *S. suis* between areas at slaughterhouses A and D, indicating that all employees were at risk of infection.

As suggested by Marois et al (26), the PCR test was more sensitive than bacteriologic culture. In their study, 57% of biopsy specimens and 72% of swabs of swine tonsil yielded *S. suis* after culture, whereas the proportions of positive samples were significantly greater, at 71% and 81%, respectively, when PCR was carried out

Table III. Resistotypes of the 16 strains

Strain no.	Resistotype
1	AMI-AMP-AZI-CEP-CIP-CLI-CHL-ENR-ERY-STR-FLO-GEN-LEV-NOR-PEN-SUL-TET
2	AMI-AMP-CIP-CHL-ENR-ERY-STR-FLO-GEN-PEN-SUL-TET
3	AMI-AZI-CEP-CIP-CLI-CHL-ENR-ERY-STR-FLO-GEN-LEV-NOR-PEN-SUL-TET
4	AMI-AZI-CEP-CIP-CLI-ENR-ERY-STR-FLO-GEN-LEV-NOR-PEN-SUL-TET
5	AMI-AZI-CEFT-CIP-CLI-CHL-ENR-ERY-STR-FLO-GEN-LEV-NOR-PEN-SUL-TET
6	AMI-AZI-CIP-CLI-CHL-ENR-ERY-STR-FLO-LEV-NOR-PEN-SUL-TET
7	AMI-AZI-CIP-CLI-CHL-ERY-STR-NOR-PEN-SUL-TET
8	AMI-AZI-CIP-CLI-ENR-ERY-STR-LEV-NOR-SUL-TET
9	AMI-AZI-CIP-CLI-ERY-STR-TET
10	AMI-AZI-CLI-ERY-STR-GEN-NOR-PEN-SUL-TET
11	AMI-AZI-CLI-ERY-STR-GEN-TET
12	AMI-CIP-CLI-ENR-STR-FLO-GEN-LEV-NOR-SUL-TET
13	AMI-CLI-ERY
14	AMP-CEP-CIP-CLI-ENR-STR-GEN-LEV-NOR-PEN-SUL-TET
15	AZI-CIP-CLI-ENR-ERY-STR-LEV-NOR-SUL-TET
16	AZI-CIP-CLI-CHL-ENR-ERY-STR-FLO-LEV-NOR-SUL-TET

AMI — amikacin; AMP — ampicillin; AZI — azithromycin; CEP — cephalexin; CIP — ciprofloxacin; CLI — clindamycin; CHL — chloramphenicol; ENR — enrofloxacin; ERY — erythromycin; STR — streptomycin; FLO — florfenicol; GEN — gentamicin; LEV — levofloxacin; NOR — norfloxacin; PEN — penicillin; SUL — trimethoprim/sulfamethoxazole; TET — tetracycline; CEFT — ceftiofur.

without prior culture. However, although widely used, the *gdh*PCR used in that study can provide false-positive results (13).

Half of the *S. suis* strains in the present study were untypeable, a common finding for strains not isolated from clinical samples (M.G., *S. suis* Serotyping Reference Laboratory: unpublished data). All serotypes identified in this study had already been described as causes of disease in pigs (27), and some serotypes, such as 4, 5, and 21, had occasionally been isolated from humans (2,12,28).

The levels of susceptibility against β-lactam antimicrobials were, in general, similar to those obtained in other studies (15–17,29,30). Resistance to these agents has previously been described (30). A high frequency of resistance to tetracyclines, sulfonamides, macrolides (erythromycin and azithromycin), and lincosamides (clindamycin), as shown in the present study, has been described for strains isolated from diseased or healthy pigs (16,27,31,32). In Brazil, the use of antibiotics as growth promoters has been severely restricted. The use of certain antibiotics is prohibited in the swine industry. Others may be used for therapeutic or preventive treatment but only if the time required before slaughter is respected. In practice, though, the use of antibiotics in the swine industry in Brazil is mainly indiscriminate, without regard to antimicrobial resistance and accumulation of residues in animal meat and soil. This may explain the high frequency of resistance of the *S. suis* strains to several antimicrobials in the present study.

The frequency of resistance to florfenicol (19%) was 3 times greater than the frequency of resistance to chloramphenicol (6%). Similar results were obtained by others (20,33). Studies have shown an increase in the rate of resistance to florfenicol since the use of chloramphenicol was banned in animals intended for human consumption.

Penicillin and ampicillin have been, over the years, the antimicrobial agents of choice for treatment of *S. suis* infections. Other antimicrobials commonly used against bacterial infections in the swine industry are amoxicillin, cephalosporins, florfenicol, tetracycline, and TMP/SMX. The high rates of intermediate resistance of the strains in this study to penicillin, ampicillin, and florfenicol suggest an increase in resistance to these first-line agents. A good response to these antibiotics, which are generally used for empirical treatment of *S. suis* disease, should not be taken for granted.

Finally, the proportion of multidrug-resistant strains obtained in this study, 100%, is the highest reported for *S. suis,* including from studies involving clinical isolates from pigs (29,34).

In conclusion, *S. suis* is present in the environment of swine slaughterhouses, including on carcasses, knives, and the hands of employees in all areas, which suggests that all employees are at risk of infection. Owing to the presence of a high number of contaminants, the use of molecular biologic techniques is essential for the detection of *S. suis* in nonclinical samples. Information regarding the distribution of *S. suis* in microenvironments (farms, slaughterhouses, cities, states, and countries) is important for the implementation of effective control measures. Although β-lactams are still the antimicrobials most effective against *S. suis,* there is a need for continual surveillance of the antimicrobial resistance of this pathogen. The high rate of multidrug resistance among serotypes related to zoonotic disease may have an important impact on the treatment of infections caused by this pathogen in humans. Because *S. suis* is rarely studied, it is underrecognized in Brazil. The few studies that have been carried out were conducted by veterinarians. Ignorance of this pathogen and how to identify it correctly in human microbiology laboratories may explain the fact that no cases in humans have been described in Brazil so far.

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