

Article

Diversity and antimicrobial resistance of *Salmonella* isolated from swines feces and carcasses

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ABSTRACT

The aim of this study was to determine the most frequent *Salmonella* serovars in swine feces in the farm, piggery's waiting slaughterhouse and after in their carcasses during slaughter, as well as their antimicrobial resistance profiles. Eighty six strains of *Salmonella* spp. were used, they were previously isolated from three different lots in different collections. The identification of serovars was done by serotyping and the resistance to antimicrobial agents was determined by disc diffusion. A variety of serovars was observed, and the 86 strains were serologically identified as: 28 (32.55%) *S. Typhimurium*, 20 (23.26%) *S. Agona*, 17 (19.77%) *S. Infantis*, 6 (6.98%) *S. Panama*, and 15 (17.44%) *S. Minnesota*. The diversity of serovars indicated that different factors influence the infection of finishing pigs and the persistence of microorganisms in the carcass after slaughter of these animals. More than 50% of the isolates were resistant to nine of the 11 tested antibiotics. The drugs that microorganisms had the highest percentages of sensitivity were sulphazotrim and norfloxacin, 22.1% and 14%, respectively. *S. Typhimurium* serovar was the most isolated, including in the carcasses, and also showed the largest difference in antimicrobial resistance when compared to other serovars. The profile of multidrug resistance observed in this study highlights to the necessity of a judicious observation of antimicrobial resistance in zoonotic foodborne bacteria.

Keywords: Salmonellosis, Antibiogram, Serotyping, Multiresistance.

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Diversidade e resistência aos antimicrobianos de *Salmonella* isoladas de fezes e carcaças de suínos

RESUMO

Objetivou-se determinar os sorovares de *Salmonella* mais freqüentes em fezes de suínos na granja de terminação, pocilga de espera do frigorífico e em suas carcaças durante o abate, assim como, seus perfis de resistência aos antimicrobianos. Utilizou-se 86 cepas de *Salmonella* spp previamente isoladas de três lotes diferentes em coletas distintas. A identificação dos sorovares foi realizada por meio de sorotipificação e a resistência aos antimicrobianos foi determinada pela técnica de difusão de discos. Foi observada uma multiplicidade de sorovares, sendo as 86 cepas identificadas sorologicamente como: 28 (32,55%) *S. Typhimurium*, 20 (23,26%), *S. Agona*, 17 (19,77%) *S. Infantis*, 6 (6,98%) *S. Panama*, e 15 (17,44%) *S. Minnesota*. A diversidade de sorovares indicou que diferentes fatores influenciam na infecção de suínos em terminação e na persistência do microrganismo na carcaça destes animais após o abate. Mais de 50% dos isolados apresentaram resistência a nove dos 11 antibióticos testados. As drogas as quais os microrganismos apresentaram os maiores percentuais de sensibilidade foram o sulfazotrim e a norfloxacina, 22,1% e 14%, respectivamente. *S. Typhimurium* foi o sorovar mais isolado, inclusive nas carcaças, e também o que demonstrou maior diferença quanto à resistência aos antimicrobianos, quando comparado aos outros sorovares. O perfil de multirresistência observado neste estudo alerta para a necessidade de vigilância e monitoramento sistemático da resistência aos antimicrobianos em bactérias zoonóticas transmitidas pelos alimentos.

Palavras-chave: Salmonelose, antibiograma, sorotipagem, multirresistência.

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1 Introduction

Salmonellosis is one of the leading causes of foodborne gastroenteritis in humans worldwide, and is considered to be an important public health problem in both, industrialized and developing countries (EFSA, 2010; LOUREIRO et al., 2010).

The diversification of the industrial production of pork and the commercial exchange of animals and their derivatives for human consumption may be important disseminators of *Salmonella* spp. in the food chain. Thus, the presence of this microorganism in the production process

represents an important sanitary barrier to trade food of animal origin and their by-products (SEIXAS et al., 2009).

Bacteria of the genus *Salmonella* are isolated worldwide, however, in areas of intensive breeding, especially of swine and birds, reports are even more frequent (OIE, 2004). The increase of the density and the indiscriminate use of antibiotics, inserted in the process of producing foods of animal origin, can act as selection pressure for some serovars and for their resistance to antimicrobials.

According to Wray; Sojka (1977) it is widely accepted that animals infected with *Salmonella* are the major sources of infection for other animals and humans. Thus, constant monitoring with identification of serovars and establishment of the epidemiological relationship of these along the production chain is of great importance.

Thus, we aimed to know which serovars of *Salmonella* spp. are infecting the pigs at the termination after transport to the slaughterhouse and in their carcasses after slaughter, as well as establish the antimicrobial resistance profile of these strains in order to contribute to a better understanding of the epidemiology of these microorganisms and provide information that may assist in the implementation of measures for the production of safe food.

2 Material and methods

A total of 86 strains of *Salmonella* spp. isolated from swine feces housed in the finishing farm, in the holding pens and from their carcasses during slaughter were used. The collection in the carcasses (after depilation, evisceration and in mesenteric lymph nodes) were performed in a non-exporter slaughterhouse under municipal inspection in the state of Minas Gerais.

The strains were previously isolated by (PACHECO, 2009) and serotyping was carried out in the Setor de Enterobactérias da Fundação Osvaldo Cruz (FIOCRUZ).

The technique used for the antimicrobial resistance was the disk diffusion test according to the protocol described by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The antibiotics used for the test were: amoxicillin 10µg, norfloxacin 10µg, gentamicin 10µg, erythromycin 15µg, nalidixic acid 30µg, tetracycline 30µg and sulfazotrim 25µg (Laborclin®); ceftiofur 10µg, enrofloxacin 5 µg and lincomycin 2 µg (Sensifar®). The strain *E. coli* ATCC 25922 was used as a control. The plates were incubated at 37 ° C for 18 to 20 hours, and then the sensitivity halos (in millimeters) were measured using a pachymeter and subsequent classification of the microorganism as sensitive, intermediate or resistant to the tested antimicrobial.

The antimicrobial resistance profile determined for the different serovars was compared using the Chi-square test with 95% of confidence. The calculations were performed using the Biostat 5.0 program (AYRES, 2007).

3 Results and discussion

The 86 strains of *Salmonella* spp. were identified serologically as: 28 (32.55%) *S. Typhimurium*, 20 (23.26%) *S. Agona*, 17 (19.77%) *S. Infantis*, 6 (6.98%) *S. Panama* and 15 (17.44%) *S. Minnesota*.

The diversity of serovars identified in feces and swine carcasses, with a higher incidence of *S. Typhimurium* was also observed in another study. Bessa et al. (2004), in Rio Grande do Sul, identified 26 different serovars in 226 *Salmonella* isolates from 300 pigs slaughtered in three slaughterhouses. The most prevalent serovars were Typhimurium (24.3%), Agona (19.9%), Derby (13.2%) and Bredeney (12%). *S. Panama* and *S. Infantis* were also identified, but less frequently, 5.75% and 0.88%, respectively.

S. Typhimurium, *S. Agona* and *S. Infantis* were the most isolated serotypes in this study. According to Loureiro et al. (2010), these serovars are associated with cases of human salmonellosis in Brazil. Serovar *S. Typhimurium* was also the most isolated in swine samples in São Paulo and Rio de Janeiro by Filho (2014) and Cabral et al. (2017), respectively. In the

United States, the three serotypes most frequently isolated in humans with salmonellosis are Typhimurium, Enteritidis and Newport, denoting the potential risk of these agents (CDC, 2006).

S. Infantis and *S. Agona* are also recognized for their pathogenic potential, which, in addition to the gastroenteric condition, may cause septicemia in young animals and humans, especially in cases of severe infections in children (TESSMANN et al., 2008).

All serovars were isolated both in the farm and in the slaughterhouse, except for *S. Panama*, which was not found in fecal samples collected at the termination farm, as shown in Table 1. It is possible that the source of contamination of carcasses by this serovar is equipment or instruments used at slaughter and is not related to prior infection of the animals.

Considering only the fecal samples, *S. Agona* was the most identified, but in the samples from animals slaughtered there was a higher incidence of *S. Typhimurium*. This serovar is one of the most involved in sporadic cases and outbreaks of gastroenteritis in Brazil (FREITAS, 2011). The number of isolates of *S. Typhimurium* in animals still housed on the farm after transport to the slaughterhouse and on their carcasses during slaughter shows that there is an amplification in the number of isolates during slaughter.

Table 1. Salmonella serovars isolated in finishing farm, holding pens and during the slaughtering stages of swines

Serovar	Finishing farm	Holding pens (slaughterhouse)	Slaughtering stages (slaughterhouse)	TOTAL
	N (%)	N (%)	N (%)	N (%)
<i>S. Typhimurium</i>	8 (9.30)	5 (5.81)	15 (17.44)	28 (32.56)
<i>S. Agona</i>	9 (10.46)	6 (6.98)	5 (5.81)	20 (23.25)
<i>S. Infantis</i>	4 (4.65)	5 (5.81)	8 (9.30)	17 (19.77)
<i>S. Minnesota</i>	6 (6.98)	6 (6.98)	3 (3.49)	15 (17.44)
<i>S. Panama</i>	--	1 (1.16)	5 (5.81)	6 (6.98)
TOTAL	27 (31.40)	23 (26.74)	36 (41.86)	86 (100)

N (%) - number of positive samples of each serovar and percentage of total samples identified as *Salmonella* spp.

The increase in the number of isolates in the carcasses was observed for *S. Typhimurium* and *S. Infantis* serovars. It is likely that strains of these serovars are more adapted to adverse environmental conditions, such as those occurring during slaughter. O'Connor et al. (2009) verified that *S. Typhimurium* may possess a gene involved in the coding of a Mg^{2+} carrier protein, which acts to protect the cell membrane integrity exposed to high temperatures, conferring greater thermotolerance.

The increase in the number of isolates in *S. Typhimurium* and *S. Infantis* during slaughter allows us to infer that probably animals with faecal positivity contribute to a contaminated final product. It also demonstrates the need for control measures in the field to reduce the presence of these pathogens in the intestinal contents of the animals and consequent decrease of the same during the stages of slaughter.

Although *S. Agona* was isolated from both, live animals and carcasses, it was not identified after evisceration (data not shown). Post-slaughter processing conditions are likely to have limited their survival and multiplication, and this partly explains their absence in carcasses after sawing.

S. Minnesota showed a reduction in the number of isolates during the slaughtering stages. Probably this serovar suffers injury during the processing of slaughter, disappearing or remaining in numbers not detectable in the technique of traditional isolation.

The presence of only one isolate of *S. Panama* in live animal feces as opposed to the five carcass isolates demonstrates that carcass contamination during slaughter stages is probably not related to the previous infection of the animals.

The multiresistance of antibiotics in the *Salmonella* genus is a worldwide trend and is in line with the present study, since most isolates have demonstrated this profile. Of the studied isolates, more than 50% presented resistance to nine of the 11 tested antibiotics. The drugs with the

highest sensitivity percentages were sulfazotrim and norfloxacin, 22.1% and 14%, respectively. However, even for these antimicrobials, the intermediate sensitivity indices observed were considered high, which may indicate the development of resistance in the medium term.

In the PREBAF report (BRASIL, 2008) on the monitoring of antimicrobial resistance of *Salmonella* and *Enterococcus* isolates from chicken carcasses, the treatment of enteropathogen infections using fluoroquinolones and third-generation cephalosporins has "a bleak outlook". The resistance profile observed in this study confirms that this is a reality also for infection caused by *Salmonella* isolated from swine. No strain was sensitive to ceftiofur, which is a third generation cephalosporin, and only four (4.7%) of the isolates were sensitive to enrofloxacin (fluorquinolone). Norfloxacin (second generation fluorquinolone), although the second most effective drug in this study, had only 12 (14.0%) of the isolates classified as sensitive.

Several authors and organizations have reported a significant increase in the identification of microorganisms isolated from animals and foods that are resistant to antimicrobials (HOPKINS et al., 2010). Many authors report that this occurrence is particularly associated with the use of therapeutic and subtherapeutic doses of antibiotics in animals and the indiscriminate use of antimicrobials in human medicine, both in hospitals and in the community (BEGUM et al., 2010).

The resistance determined for each of the serovars showed that there was variation of antimicrobial resistance between serovars when analyzed by the chi-square test.

Table 2. Antimicrobial resistance of *Salmonella* isolated from swine feces and carcasses

Antimicrobial	Serovars (N=86)				
	Typhimurium	Agona	Infantis	Panama	Minnesota
	N=28	N=20	N=17	N=6	N=15
Amoxicillin	27 (96.4)	10 (50.0)	15 (88.2)	5 (83.3)	13 (86.7)
Gentamicin	27 (96.4)	14 (70.0)	15 (88.2)	6 (100.0)	13 (86.7)
Norfloxacin	20 (71.4)	7 (35.0)	11 (64.7)	6 (100.0)	4 (26.7)
Neomycin	28 (100.0)	14 (70.0)	15 (88.2)	6 (100.0)	14 (93.3)
Lincomycin	28 (100.0)	20 (100.0)	17 (100.0)	6 (100.0)	15 (100.0)
Ceftiofur	28 (100.0)	20 (100.0)	17 (100.0)	6 (100.0)	13 (86.7)
Tetracycline	27 (96.4)	16 (80.0)	17 (100.0)	6 (100.0)	15 (100.0)
Sulfazotrim	0	2 (10.0)	4 (23.5)	0	8 (53.3)
Erythromycin	26 (92.9)	15 (75.0)	17 (100.0)	6 (100.0)	15 (100.0)
Nalidixic Acid	18 (64.3)	17 (85.0)	15 (88.2)	6 (100.0)	13 (86.7)
Enrofloxacin	28 (100.0)	14 (70.0)	12 (70.6)	6 (100.0)	5 (33.33)

N (%) - number of samples and percentage in relation to the specified serovar.

S. Typhimurium was the serovar that showed the most difference in relation to the others studied, showing a higher resistance to amoxicillin, neomycin and enrofloxacin when compared to *S. Agona* ($P < 0.01$); to enrofloxacin compared to Minnesota serovars ($P < 0.01$) and *Infantis* ($P < 0.05$); and norfloxacin compared to Minnesota ($P < 0.05$).

S. Typhimurium was classified as multidrug resistant because of the resistance to three or more antimicrobial classes. All isolates of this serovar demonstrated resistance to neomycin (aminoglycoside), lincomycin (lincosamide), ceftiofur (cephalosporin) and enrofloxacin (fluorquinolone) and more than 96% showed resistance to amoxicillin (betalactam), gentamicin (aminoglycoside) and tetracycline. However, all were sensitive to sulfazotrim. In this study, the resistance of serovar *Typhimurium* to sulfazotrim was significantly lower than that observed for *Infantis* ($P < 0.05$) and Minnesota ($P < 0.01$) serovars.

The resistance to tetracycline in 96.4% of *S. Typhimurium* isolates was higher than that observed in other studies carried out in Brazil on swine samples. Resistance to this antimicrobial was also observed in 41.67% of strains isolated from fresh sausage by Spricigo (2008). Studies in Europe with porcine isolates marketed in the member countries showed lower

resistance than those of this study for *S. Typhimurium*, with indices varying from 36.05% to 59% (EFSA, 2006; EFSA, 2008). The increase in resistance levels to ampicillin, tetracycline and chloramphenicol since 1996 in *Salmonella* isolated from humans, pigs and chickens characterizes the emergence of multi-resistant *S. Typhimurium* (BEGUM et al., 2010). However, this author reports that isolates with these characteristics are also resistant to the sulfa and trimethoprim association (sulfazotrim), which was not observed in this study.

There was no difference between the resistance determined in the serovar Agona compared to the other antimicrobial isolates: gentamicin, lincomycin, ceftiofur, tetracycline, erythromycin, nalidixic acid and enrofloxacin. However, this serovar showed a lower number of isolates resistant to amoxicillin and neomycin compared to serovar Typhimurium ($P < 0.01$), norfloxacin compared to Panama ($P < 0.01$); and sulfazotrim compared to Minnesota ($P < 0.05$).

Despite being frequently isolated from food and environment and in some clinical samples, there are few reports on resistance or multidrug resistance in *S. Agona*. In this study, this serovar showed the lowest percentages of isolates resistant to the drugs tested.

S. Panama presented a profile similar to that observed for serovar Typhimurium, with 100% of resistance to nine of the antimicrobials tested and 83.3% of isolates resistant to amoxicillin, but 100% sensitive to sulfazotrim. Regarding the other serovars, *S. Panama* showed to be more resistant to enrofloxacin when compared to *S. Minnesota* ($P < 0.05$) and norfloxacin in relation to serovar Agona ($P < 0.01$). There was less resistance to sulfazotrim by the isolates when compared to those identified as *S. Minnesota* ($P < 0.05$).

The resistance profile determined for the serovar Panama in this study is different from that observed by Castagna et al., (2004) in isolates of

feces and mesenteric lymph nodes of pigs at slaughter in three slaughterhouses in southern Brazil.

Isolates identified as *S. Infantis* presented a lower percentage of resistance to enrofloxacin compared to serovar Typhimurium, but a higher number of isolates resistant to sulfazotrim compared to this same serovar ($P < 0.05$). All isolates were resistant to lincomycin, ceftiofur, tetracycline and erythromycin. The antimicrobial resistance of *S. Infantis* in this study was higher than that observed by Tessmann et al. (2008). These authors determined sensitivity of 100% and 43% for nalidixic acid and tetracycline, respectively, in *S. Infantis* isolated from pork cuts at Pelotas, RS.

The isolates identified as *S. Minnesota* were less resistant to norfloxacin compared to *S. Typhimurium* and enrofloxacin compared to *S. Panama* ($P < 0.05$), but presented higher resistance to sulfazotrim compared to serovar Typhimurium ($P < 0.01$). *S. Minnesota* is considered an emerging etiologic agent of human salmonellosis (EFSA, 2008).

In Brazil, the use of antimicrobials avilamycin, bacitracin, chlorhexidine, flavomycin, chlorohydroxyquinoline, tylosin, lincomycin and erythromycin as feed additives in finishing pigs is permitted (BRASIL, 1998). Of these, the last two were tested in this study. With the exception of 8.1% of the isolates of *S. Typhimurium* and 25% of *S. Agona*, which presented a profile classified as intermediate to erythromycin, the other isolates were 100% resistant to these drugs.

The results of this study show that, independent of the differences identified among serovars for some antimicrobials, the isolates presented a multiresistance profile, especially for those most used in clinical treatment in farms such as enrofloxacin, lincomycin, amoxicillin, tetracycline, erythromycin, norfloxacin, ceftiofur and sulfazotrim, and the observed differences are likely to be more associated to the series than to the serovar. Resistance is worrying and demonstrates the need to reduce the use of antimicrobials in animal production as a way to reduce the selection

pressure to which these microorganisms are subjected. It also warns of the difficulties that may occur in the treatment of extraintestinal infections and in groups at risk, where antibiotic therapy and the need to monitor antimicrobial resistance in foodborne zoonotic bacteria are required.

Initiatives such as the monitoring of *Salmonella* resistance isolated from frozen chicken carcasses throughout the country carried out by ANVISA in the PREBAF project (BRASIL, 2008) provide important data. The information generated allows the adequate visualization of the problem to promote the implementation of effective control measures.

4 Conclusion

The most identified serovars were *S. Typhimurium*, *S. Agona* and *S. Infantis*. The multiplicity of *Salmonella* serovars identified at the analyzed points of the productive process indicates that different factors influence the infection of finishing pigs and their presence and persistence in the carcass of these animals after slaughter. This reinforces the importance of a rigorous monitoring that allows to subsidize actions that promote the adequate control of these pathogens from the farm to the steps of slaughter and guarantee the health of the consumer.

The strains showed higher resistance to lincomycin and ceftiofur. The pattern of multidrug resistance determined for different serovars alerts to the need for implementation of systematic monitoring of the antimicrobial response in foodborne zoonotic bacteria.

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