

Antimicrobial resistance patterns of *Salmonella enterica* subsp. *enterica* serovar Derby and Typhimurium isolated from pigs slaughtered in southern Brazil

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Abstract

Salmonella enterica subsp. *enterica* serovar (S.) Derby and *S. Typhimurium* were commonly isolated from slaughter pigs and pork. Thus, the monitoring of the resistance profile exhibited by strains of both serovars should be regularly conducted. This study aimed to assess the antimicrobial resistance pattern of strains isolated from pig carcasses and to investigate the genetic relatedness with isolates from intestinal content and lairage environment. Thirty-four *S. Derby* and seventeen *S. Typhimurium* strains isolated from carcasses (n=30), intestinal contents (n=16), and lairage environment (n=3) were tested. The antimicrobial resistance was determined by the agar disk diffusion test according to the document M31-A2 of the CLSI using twelve antimicrobials. Strains were also genotyped by pulsed-field gel electrophoresis, and the presence of class 1 integrons was investigated. The isolates were resistant to tetracycline (96%), sulfonamides (78.4%), streptomycin (76.5%), ampicillin (35.3%), gentamicin (29.4%), kanamycin (27.5%), nalidixic acid (23.5%), chloramphenicol (17.6%), and ceftazidime (1.9%). Only two isolates from intestinal content were susceptible at all antimicrobials. No resistance to cefotaxime and ciprofloxacin was detected. Five resistance patterns were found among *S. Derby* isolates, and the most prevalent (TE-S-S3) was detected in 26 strains (76.5%). Among strains of *S. Typhimurium*, eight patterns were found, and the most prevalent (K-TE-CN-AMP) was detected in four (23.5%) isolates. All *S. Derby* strains with the TE-S-S3 pattern belonged to a common pulsotype (De1) and harbored Class 1 integrons. Resistant strains of *S. Derby* and *S. Typhimurium* isolated from carcasses presented resistance patterns in common with strains from intestinal content and lairage environment. Results indicate that resistant clonal groups originated from pig feces and lairage environment are able to contaminate pig carcasses and enter the pork processing chain.

Introduction

Salmonella enterica is recognized as the most important cause of food-borne illness in southern Brazil. Although chicken and eggs are the foods most often involved in outbreaks, salmonellosis associated with pork consumption is also reported (COSTALUNGA and TONDO, 2002). Serovars Typhimurium and Derby figure among the most prevalent in pigs and pork in southern Brazil (BESSA et al., 2007; MÜRMANN et al., 2009). Multiresistant strains from both serovars have been frequently isolated from pigs at slaughter (MICHAEL et al., 2006b; BESSA et al., 2007), however the resistance pattern of strains originated from carcasses was not determined. The emergence of antimicrobial resistance in *Salmonella* strains isolated from food has been an increasing concern. Thus, this study aimed to assess the antimicrobial resistance pattern of *S. Derby* and *S. Typhimurium* strains isolated from pig carcasses and to investigate the genetic relatedness with isolates from intestinal content and lairage environment.

Material and Methods

Thirty *Salmonella* strains isolated from carcasses sampled in southern Brazil were included in this study. Moreover, strains isolated from intestinal content (n=18) of pigs belonging to the same slaughter batch as the carcasses, and strains (n=3) isolated from the holding environment were also tested. Among all tested strains, 34 belonged to serovar Derby and 17 to serovar Typhimurium.

The antimicrobial resistance was determined by the agar disk diffusion test according to the document M31-A2 of the CLSI using the following disks: thimetoprim (VW; 5 µg), kanamycin (K; 30 µg), tetracycline (TE; 30 µg), ceftazidime (CAZ; 30 µg), sulfonamides (S3; 300 µg), chloramphenicol (C; 30 µg), gentamicin (CN; 10 µg), streptomycin (S; 10 µg), nalidixic acid (NA; 30 µg), ampicillin (AMP; 10 µg), cefotaxime (CTX; 30 µg), ciprofloxacin (CIP; 5 µg). *Escherichia coli* ATCC 25922 was used as quality control of disk diffusion tests.

Salmonella isolates were genotyped by the macro-restriction of total DNA, followed by pulsed field gel electrophoresis (PFGE). The macro-restriction analysis was performed following the PulseNet protocol (<http://www.cdc.gov/PulseNet/protocols.htm>). Whole DNA was digested with XbaI (20U) and BlnI (10U). Moreover, all isolates were submitted to investigation of int1 integrase gene from class 1 integrons by PCR assays, as previously described (FRENCH et al., 2003).

Results

Salmonella isolates were resistant to tetracycline (96%), sulfonamides (78.4%), streptomycin (76.5%), ampicillin (35.3%), gentamicin (29.4%), kanamycin (27.5%), nalidixic acid (23.5%), chloramphenicol (17.6%), and ceftazidime (1.9%). Only two strains (*S. Derby* and *S. Typhimurium*), obtained from intestinal contents, were susceptible at all antimicrobials. No resistance to thrimetoprim, cefotaxime and ciprofloxacin was detected. Five resistance patterns were found in *S. Derby* isolates, and TE-S-S3 was the most prevalent (76.5%), as depicted in Table 1. The most common pulsotype (De1) encompassed 26 strains isolated from carcass (20), feces (4) and lairage (2). Most strain of pulsotype De1 showed the resistance pattern TE-S-S3, and this pattern was found in isolates from carcasses, feces and lairage. Class 1 integrons were detected in 32 (94.1%) *S. Derby* strains.

Among the six *S. Typhimurium* strains isolated from carcass, one was resistant only to tetracycline. The most frequent multi-resistance pattern among carcass isolates was K-TE-CN-AMP, detected in four isolates. The pattern K-TE-CN-NA-AMP was detected in one carcass strain and in one isolate from the lairage. Ten strains isolated from feces exhibited a high diversity of resistance profiles. The pattern TE-S-C-CN-S3-AMP was the most common, followed by K-TE-S-C-CN-S3-AMP, TE-S3-NA-AMP, K-TE-S-C-S3-AMP and K-TE-S-CN-NA-AMP. Strains from carcass, feces and lairage presented no common PFGE profile, and in only seven (41.8%) of them were class 1 integrons detected.

Discussion

In the present study, most strains showed resistance patterns that included at least tetracycline, streptomycin or sulfonamide. While *S. Typhimurium* strains presented a great diversity in resistance profiles and unrelated pulsotype patterns, *S. Derby* showed mostly common phenotypic and genotypic patterns. The most frequent resistance phenotype (TE-S-S3) in our study was previously reported as the most prevalent in *S. Derby* strains originated from the same region (Michael et al., 2006b). This study demonstrated that strains exhibiting the TE-S-S3 pattern also belonged to a common pulsotype and carried class 1 integrons. In our study, strains of *S. Derby* isolated seven years later in the same region exhibited the same common genotypic and phenotypic profile. Thus, clonal groups with the TE-S-S3 pattern seem to circulate over time in pig herds of the region and can contaminate carcasses, as observed in our study. The resistance to antimicrobials has been associated with their use for extended time periods in farm animals (WEGENER, 2003). However, streptomycin and sulfonamide are not administered to pigs anymore, and the use of tetracycline has been steadily declining in the last years. In spite of that, resistance determinants are likely to persist in the *Salmonella* population.

Class 1 integrons carry frequently the *aadA* cassette gene, which is associated to streptomycin/ spectinomycin resistance (MICHAEL et al., 2006a). In our study class 1 integrons were present in all strains of pulsotype De1 that presented de TE-S-S3 profile. Moreover, carcass strains presenting this profile with additional markers, such as nalidixic acid resistance, also harbored class 1 integrons. It may indicate that other gene cassettes have been integrated in the variable region of the integrons. This may be an additional concern, since nalidixic acid resistance determinants can be responsible for diminished susceptibility to fluoroquinolone (GORMAN and ADLEY, 2004).

In spite of the low number of strains investigated in our study, common resistance profiles were observed in *S. Typhimurium* and *S. Derby* isolated from carcass, feces and lairage environment. It highlights that multi-resistant *Salmonella* strains, which colonize the pig gut or are found in the lairage environment, are able to enter the pork processing chain.

Conclusion

Salmonella Derby clonal groups originated from pig feces and lairage environment contaminate pig carcasses and enter the pork processing chain. Multi-resistance profiles and class 1 integrons are observed in all strains of the most prevalent clonal groups.

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Table 1. Antimicrobial resistance patterns and pulsed-field electrophoresis (PFGE) profiles of *S. Derby* strains isolated from carcass, intestinal content and lairage environment.

Antimicrobial resistance pattern	Number of <i>S. Derby</i> isolates (PFGE pulsotype)			Total
	Lairage	Intestinal content	Carcass	
TE-S-S3	2 (De1)	5 (De1; De4)	19 (De1; De2; De4)	26
TE-S-S3-NA		1 (De1)	3 (De1)	4
TE-CAZ-S-C-CN-S3-NA-AMP			1 (De1)	1
K-TE-C-CN-S3-AMP		1 (De3)		1
K-TE-S-C-S3-AMP			1 (De4)	1
Total	2	7	24	33

PFGE, pulsed-field gel electrophoresis; TE, tetracycline; S, streptomycin; S3, sulfonamides; NA, nalidixic acid; CAZ, ceftazidime; C, chloramphenicol; CN, gentamicin; AMP, ampicillin.