SALMONELLA CLINICAL ISOLATES FROM BRAZILIAN PIG HERDS: GENETIC RELATIONSHIP AND ANTIBIOTIC RESISTANCE PROFILING

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Abstract

In Brazil, since 2011 clinical cases of salmonellosis has been increasing substantially. Nevertheless, few information is available about the antimicrobial profile, distribution, serotypes and genetic relationship among the strains. The objectives of this study were: to identify the *Salmonella* serotypes, to characterize the *in vitro* antimicrobial resistance profiles and to determine the genetic relationship of clinical isolates in Brazil. During 2016, clinical isolates of *Salmonella* (111) from nine States were sent to Embrapa Swine and Poultry for complementary analysis. First, isolates were serotyped by Kauffmann White Scheme. In parallel, the strains were tested against fifteen antimicrobials by disk diffusion method and genotyping was performed by Pulsed Field Gel Electrophoresis (PFGE) using the *XbaI* restriction enzyme. As expected, the main serovars found were Typhimurium and Choleraesuis. Four strains showed resistance to only one antimicrobial and 76.5% (85/111) were considered multi-resistant. The highest level of resistance was found against to tetracycline. More than 80% of the strains were susceptible to fosfomycin, lincomycin/spectinomycin and norfloxacin. It was possible to identify one major Choleraesuis clonal group present in different Brazilian States. Further, several small clonal groups were obtained for Typhimurium. In conclusion, clinical salmonellosis caused by Typhimurium and Choleraesuis is endemic in pig production areas and the majority of the strains are multi-resistant.

Introduction

Enteric diseases are a big concern for pig production due the economic impact resulting of poor animal performance. Among all the bacteria that are involved in enteric disorders, *Salmonella* is always present and plays an important role as pathogen and reservoir of antimicrobial resistance genes. The major *Salmonella* serovars associated with clinical disease in pigs are related with pathogenic features. Septicemic salmonellosis is usually caused by host-restricted serovar Choleraesuis and severe enteritis due to the ubiquitous serovar Typhimurium (Mastroeni et al., 2006).

Until 2011 few clinical cases of salmonellosis were reported in Brazil, indicating that the problem used to occur less frequently than other countries. However, at beginning of 2013 the number of salmonellosis outbreaks has been increasing

substantially, mainly in the most important swine production states (Vanucci et al., 2014). Also, a study by Santos et al. (2016) has reported an occurrence of *S.* Choleraesuis in outbreaks with respiratory and circulatory disorders during 2013 to 2015 in Brazil.

In order to understand the epidemiology of this disease, the phenotypes and clonal relationships investigation between strains from different areas can provide valuable information (Zhao et al., 2007). Furthermore, to determine the antimicrobial resistance profile is important in the clinical perspective and helps to understand the epidemiological context. In Brazil, there are still a limited published data relative to *Salmonella* clinical isolates characterization. In view of that, the objectives of this study were: to identify the serotypes, to determine the antimicrobial resistance profiles and to relate the isolates by pulsed-field gel electrophoresis (PFGE).

Material and methods

During 2016, clinical isolates of *Salmonella* provided from three specialized diagnostic laboratories were sent to Embrapa Swine and Poultry for phenotypic and genotypic characterization. Initially the isolates were serotyped according Kauffmann White Scheme by slide agglutination. Strains without phase 2 flagellar antigen expression after two tries of phase inversion were considered monophasic.

Antimicrobial resistance profile was determined against fifteen antimicrobials by disk-diffusion method according to Clinical and Laboratory Standards Institute, document VET 01-S2 and M100 (CLSI, 2013, CLSI 2016). In particular, disc zone diameters determined for colistin (resistance ≤ 8mm and susceptibility ≥ 11mm) has been according with Maalej et al, 2011 and Rodríguez et al, 2004. The following antimicrobial were analyzed: ceftiofur (CEF) 30μg; ciprofloxacin (CIP) 5μg; colistin (COL) 10μg; doxycycline (DOX) 30μg; enrofloxacin (ENR) 5μg; streptomycin (STR) 10μg; florfenicol (FFC) 30μg; fosfomycin (FOS) 200μg; gentamicin (GEN) 10μg; lincomycin/spectinomycin (LSC) 109μg; marbofloxacin (MAR) 5μg; neomycin (NEO) 30μg; norfloxacin (NOR) 10μg; sulfamethoxazole-trimethoprim (SXT) 25μg; and tetracycline (TET) 30μg.

The isolates relationship was investigated by macrorestriction analysis with *Xba*I. The technique was performed based on PulseNet protocol (www.cdc.gov/pulsenet/pdf/ ecoli-shigella-salmonella-pfge-protocol-508c.pdf). The DNA fragments were separated by pulsed-field gel electrophoresis (PFGE) using CHEF-DR III (BioRad). Whole cell DNA of *S.* Braenderup H9812 digested with *Xba*I served as size marker. Macrorestriction profiles (pulsotypes) were analyzed using BioNumerics software package, version 3.0. The similarities were determined by Dice correlation coefficient, with a maximal position tolerance of 1.7% (Carriço et al., 2005). Pulsotypes were clustered the unweighted pair group method with arithmetic averages (UPGMA).

Results and discussion

Studied clinical cases were diagnosticated in nine States of Brazil from 2011 to 2016 as follow: Santa Catarina/n=37, Minas Gerais/n=35, Rio Grande do Sul/n=11, São Paulo/n=10, Paraná/n=8, Mato Grosso/n=3, Mato Grosso do Sul/n=1, Goiás/n=1 and Distrito Federal/n=1. Out of 111 strains, 64 were from septicemic and 45 from enteric cases (two strains lacking information). The serotypes distribution were: Typhimurium 60/111 (monophasic 34/60; Typhimurium 26/60); Choleraesuis 40/111; Rissen 5/111;

and a single isolate of Heidelberg, Panama, Derby, Grupo D, Anatum, and Bovins-morbificans.

From 111 tested *Salmonella* sp. only four strains showed resistance to one antimicrobial. In the other hand, 85 (76.5%) isolates were resistant to three or more antimicrobial classes and classified as multi resistant according to Schwarz et al. (2010). The highest frequency of resistant isolates was found against tetracycline (90,99%), followed by gentamicin (77,47%), doxycycline (76.57%) and florfenicol (74.77%) as summarized in Figure 1. In contrast, more than 80% of the strains were susceptible to fosfomycin and lincomycin/spectinomycin. Comparable results were found by Vannucci et al (2014) and Santos et al. (2016) in *Salmonella* Choleraesuis isolates from Brazilian clinical outbreaks.

Specifically for Choleraesuis and Typhimurium confirmed serovars, it was possible to get PFGE pulsotypes from 90 isolates. The results had shown one major Choleraesuis clonal group (pulsotype C1- Figure 2) in 35/38 isolates, conversely for Typhimurium (including the monophasic ones) it was found 16 pulsotypes. Choleraesuis pulsotype C1 was wide distributed in the pig production area encompassing six States: MG, SP, PR, GO, RS and SC. Besides that, 14 pulsotype C1 isolates showed the same resistance profile [DoxStrpFfcGenTet] and more 10 isolates contained the same basic profile with others additional antibiotics.

As expected the serovar Typhimurium has presented a large genotype and phenotype variability. It was obtained several small clonal groups from 52 isolates, the biggest one with 13 isolates and a single profile in 8 isolates. Likewise, 43 resistance profiles were determined for 60 Typhimurium isolates, the major clonal group presented 9 profile in 13 isolates. The serovar Typhimurium is considered ubiquitous and wide spread in Brazilian pig farms (Kich et al. 2011), in view of that is logical to found more genetic differences among the isolates. Also, the heterogeneity resistance profiles for Typhimurium may be attributed to the different antimicrobial exposures that the microorganisms are submitted to animal husbandry environments (Mathew et al., 2007).

Conclusion

Clinical salmonellosis is endemic in pig production areas, the main involved serovars are Typhimurium and Choleraesuis. One big clonal group of Choleraesuis and several small groups of Typhimurium are widely distributed in Brazil. The *Salmonella* strains involved in these cases present high level of antimicrobial multi resistance.
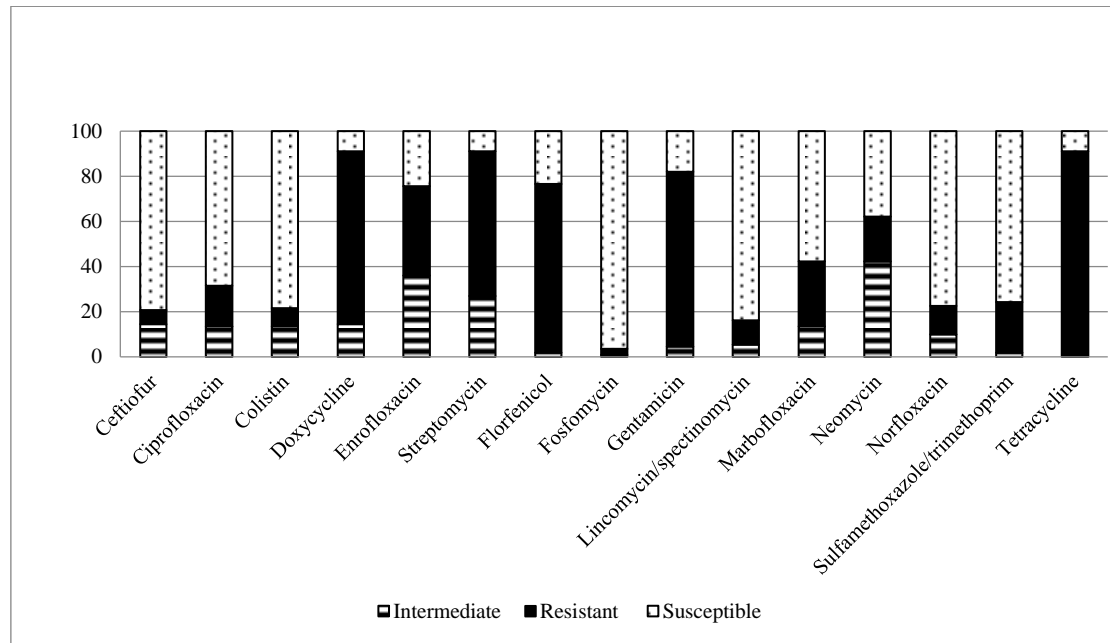
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Figure 1. Percentage of *in vitro* antimicrobial resistance of 111 *Salmonella* clinical isolates.
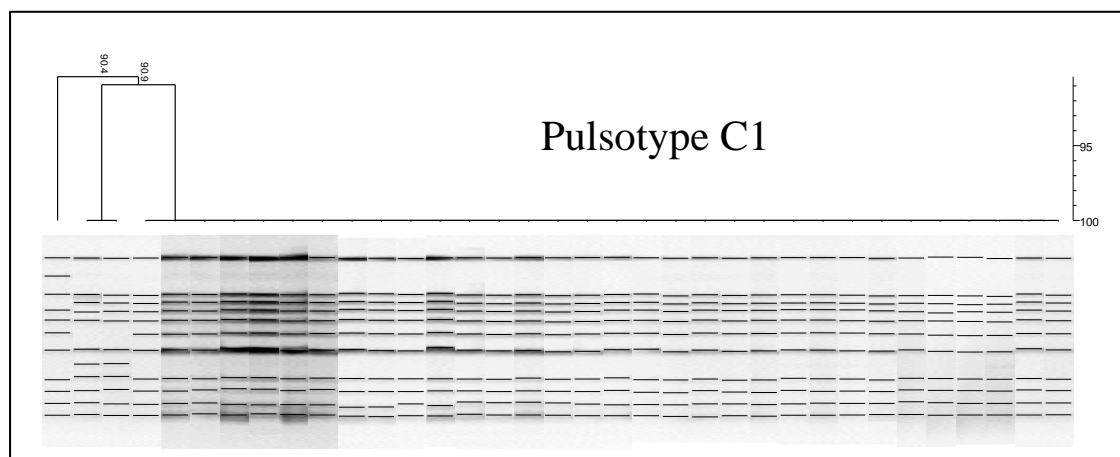

Figure 2. PFGE dendogram of *S.* Choleraesuis isolates from Brazilian clinical septicemic cases

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