Frequency, serotyping and antimicrobial resistance pattern of *Salmonella* from feces and lymph nodes of pigs¹

João B.P. Guerra Filho, Ricardo S. Yamatogi, Fábio S. Possebon, Sueli A. Fernandes, Monique R. Tiba-Casas, Gustavo H.B. Lara, Márcio G. Ribeiro and José P.A.N. Pinto

**ABSTRACT.**- Guerra Filho J.B.P., Yamatogi R.S., Possebon F.S., Fernandes S.A., Tiba-Casas M.R., Lara G.H.B., Ribeiro M.G. & Pinto J.P.A.N. 2016. Frequency, serotyping and antimicrobial resistance pattern of *Salmonella* from feces and lymph nodes of pigs. *Pesquisa Veterinária Brasileira* 36(12):1165-1170. Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia Universidade Estadual Paulista, Distrito de Rubião Júnior s/n, Botucatu, SP 18618-970, Brazil. E-mail: josepaes@fmvz.unesp.br

Salmonellosis is a foodborne disease caused by bacteria of the genus *Salmonella*, being pigs and pork-products potentially important for its occurrence. In recent decades, some serovars of *Salmonella* have shown increase of resistance to conventional antimicrobials used in human and animal therapy, with serious risks for public health. The aim of this study was to evaluate feces (n=50), mediastinal (n=50), mesenteric (n=50) and mandibular (n=50) lymph nodes obtained from slaughter houses for *Salmonella* spp. Positive samples were serotyped and subjected to an *in vitro* antimicrobial susceptibility test, including the extended-spectrum beta-lactamase (ESBL) production. *Salmonella* species were identified in 10% (20/200) of total samples. From these, 20% (10/50) were identified in the submandibular lymph nodes, 18% (9/50) in the mesenteric lymph nodes, 2% (1/50) in feces and 0% (0/50) in the mediastinal lymph nodes. The serotypes found were *Salmonella* Typhimurium (55%), *S. enterica* subsp. *enterica* 4,5,12: i: - (35%), *S*. Brandenburg and *S*. Derby with 5% (5% each). All strains showed resistance to at least one antimicrobial; 90% were resistant to four or more antimicrobials, and 15% were multidrug-resistant. Resistance to ciprofloxacin, tetracycline and nalidixic acid was particularly prevalent amongst the tested serovars. Here, we highlighted the impact of pigs in the epidemiological chain of salmonellosis in domestic animals and humans, as well as the high antimicrobial resistance rates of *Salmonella* strains, reinforcing the necessity for responsible use of antimicrobials for animals as an emergent One Health issue, and to keep these drugs for human therapy approaches.


---

¹ Received on November 20, 2015. Accepted for publication on June 16, 2016.

2 Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia Universidade Estadual Paulista (Unesp), Distrito de Rubião Júnior s/n, Botucatu, SP 18618-970, Brazil. *Corresponding author: josepaes@fmvz.unesp.br

3 Instituto Adolfo Lutz, Av. Doutor Arnaldo 351, São Paulo, SP 01246-902, Brazil.

---

**RESUMO.**- [Isolamento, sorotipagem e padrões de resistência a antimicrobianos de *Salmonella* em fezes e linfonodos de suínos.] Nas últimas décadas, o aumento de cepas circulante de *Salmonella* concomitantemente a resistência microbiana tem despertado a preocupação dos órgãos de Saúde Pública. Deste modo, o objetivo do presente trabalho foi pesquisar a presença de *Salmonella* a partir de fezes (n=50), linfonodos mediastinos (n=50), mesentéricos (n=50) e submandibulares (n=50) oriundos de um abatedouro suíno. As cepas isoladas foram sorotipadas e testadas quanto a resistência antimicrobiana. A presença de *Salmonella* isolada foram em 10% (20/200) do total de amostras, sendo 20% dos linfonodos submandibulares, 18% dos linfonodos mesentéricos e 2% das fezes. Os sorotipos encontrados foram *S*. Typhimurium (55%), *S. enterica* subsp. *enterica* 4,5,12: i: - (35%), *S*. Brandenburg (5%) e *S*.
Derivatives of fluoroquinolones are more severe, especially in immunocompromised by gastroenteritis. However, in some cases the manifestations lead to an underestimated notification (Santos et al. 2009, Duggan et al. 2010, Carrasco et al. 2012, Gomes-Diniz et al. 2013), even when the occurrence of mild symptoms leads to an underestimated notification (Santos et al. 2002).

Brazil is considered the 4th largest producer of pork in the world, second only to China, European Union and United States, respectively, reaching an annual production over 3 million tons and exports over 600 thousand tons in 2013 (ABIPECS 2013). In this country, from 2000 to 2013, were reported 8,871 foodborne diseases outbreaks, being Salmonella the causative agent in 1,522 of them. Among these total outbreaks, 277 (4.26%) were associated with pork products.

Salmonella is closely related to the swine production chain, being isolated in several production steps, including primary production, transport of animals, the pre-slaughter and pre-evisceration steps, especially during scalding, opening of the abdomen and withdrawing of colon (Letellier et al. 2009, Duggan et al. 2010, Carrasco et al. 2012, Gomes-Neves et al. 2012). Salmonella infections in pigs occurs through oral infection, and later spreads to the lymphatic system, which acts as barrier at the first moment, but can become reservoirs posteriorly allowing environmental-elimination of the agent and its dissemination through other animals (Straw et al. 2012). Therefore, the isolation of Salmonella from pigs' lymph nodes indicates its carrier status and the analysis of intestinal contents is related to its excretory potential (Davies et al. 1998, Bahnson et al. 2006).

In recent decades, S. Enteritidis and S. Typhimurium serovars have caused great concern (Bollaerts et al. 2008). In most cases where humans have contracted one of these serovars, the pathogen infection is self-limiting, characterized by gastroenteritis. However, in some cases the manifestations are more severe, especially in immunocompromised patients (EFSA 2010). In these cases, the conventional treatment for salmonellosis is based on fluoroquinolones and quinolones antimicrobials for adults, third-generation cephalosporins for children, and chloramphenicol in patients with endocarditis or endovascular infection (Lesser & Miller 2005, EFSA 2010). Nevertheless, several studies have pointed out the isolation of multidrug-resistant Salmonella strains, including to the main drugs of choice in the therapeutic practices for veterinary and human protocols (EFSA 2010).

In the European Union is recorded occurrence of multidrug-resistant strains in 16 countries for pigs, and 14 countries for pork samples (EFSA 2010). In Spain, studies report Salmonella resistance to streptomycin (46% of tested serovars), tetracycline (30%), sulfonamides (25%) and ampicillin (23%), with 36% of serovars multidrug-resistant (Gomez-Laguna et al. 2011). In a similar study conducted in Vietnam, from poultry and pork meat strains, resistance of Salmonella to at least one antimicrobial was found in 78.4% of samples, with 23.2% MDR (Thai et al. 2012). In a study carried out in Brazil, different serotypes of Salmonella isolated from pigs showed resistance to sulfonamides (83.9%), tetracycline (37.4%), cotrimoxazole (25.2%), ampicillin (20.2%), chloramphenicol (16.1%), streptomycin (14.1%), and nalidixic acid (10.1%), and 24.2% were formally multidrug-resistant (Castagna et al. 2001).

It is well recognized that the Enterobacteriaceae family includes many species that produce enzymes that hydrolyze beta lactam antibiotics. One of the predominant enzymes, Extended Spectrum Beta Lactamase (ESBL), inactivates penicillins, cephalosporins and monobactams (Sousa Junior et al. 2004).

Here, we investigated the frequency of Salmonella spp. in feces, mesenteric, mediastinal and submandibular lymph nodes from pigs, as well as serotype characterization and in vitro resistance profile of strains to several antimicrobials, choosen based on Clinical Laboratory Standards Institute guidelines (CLSI 2013).

MATERIALS AND METHODS

Animals and sample collection. Two hundred specimens from pigs were sampled. Of these, 50 fecal samples were collected in the evisceration and inspection tables, using sterile plastic bags and 150 lymph nodes without apparent abnormalities (without lymphadenitis) of pigs were removed from carcasses and placed in sterile plastic bags (being 50 mediastinal, 50 mesenteric and 50 submandibular), taken randomly from different animals. The animals were slaughtered in the finishing phase (150-180 days) in slaughterhouses under Brazilian Federal Inspection Service. Sampled pigs came from up 20 piggeries of 10 different cities located in Sao Paulo and Santa Catarina States, Brazil. The animals were from medium scale farms housing between 350–950 animals kept in intensive indoors system, with concrete-floored, and fed exclusively with commercial feed. Immediately after collection, the samples were kept refrigerated (4-8°C) and stored at -20°C until the diagnostic procedures.

Salmonella identification. For the isolation of Salmonella spp, the samples defrost under refrigeration for 24 hours. Feces samples were fractionated in 1g aliquots and packed in sterile plastic bags. Samples of lymph nodes were externally disinfected with alcohol 70% and then fractionated to obtain a 1g aliquot, which was also transferred to sterile plastic bag. For each bag was added 9mL of buffered peptone water 1% (BPW) (Oxoid®,
The serotyping of the 20 isolated strains identified the following serotypes: S. Typhimurium (n=11/20, 55%), S. enterica subsp. enterica 4,5,12:i - (n=7/20, 35%), and S. Brandenburg and S. Derby (n=1/20, 5%) each.

The highest antimicrobial resistance rates of isolates were found for ciprofloxacin (CIP) and tetracycline (TET) (n=18/20, 90% each), followed by nalidixic acid (NAL) (n=16/20, 80%).

S. Typhimurium strains showed resistance to several antimicrobials, especially in relation to tetracycline (n=11/11), followed by ciprofloxacin (n=10/11), nalidixic acid, sulfonamides and chloramphenicol (n=8/11), streptomycin (n=7/11), trimethoprim-sulfamethoxazole (n=6/11), ampicillin (n=4/11), and gentamicin and cefotaxime (n=1/11) (Fig.1).

A total of S. enterica subsp. enterica 4,5,12:i - strains (n=7/7) showed resistance to nalidixic acid, sulfamethoxazole trimethoprim-sulfonamides, tetracycline and streptomycin. For the other antimicrobials the resistant values of isolates were n=6/7 for ciprofloxacin, n=5/7 for chloramphenicol and n=1/7 to ampicillin, gentamicin and cefotaxime (Table 2).

Table 1. Number, frequency and serovars of Salmonella positive lymph nodes and feces from slaughtered pigs

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Positives (%)</th>
<th>Serovars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submandibular lymph nodes</td>
<td>50</td>
<td>10 (20%)</td>
<td>S. enterica subsp. enterica 4,5,12:i-</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>50</td>
<td>9 (18%)</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>Feces</td>
<td>50</td>
<td>1 (2%)</td>
<td>S. enterica subsp. enterica 4,5,12:i-</td>
</tr>
<tr>
<td>Mediastinal lymph nodes</td>
<td>50</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>200</td>
<td>20 (10%)</td>
<td></td>
</tr>
</tbody>
</table>

**Different letters indicate statistical difference (p<0.05) in the percentage of positives for each sample type.**

**RESULTS**

Salmonella strains were identified in 10% (n=20/200) of samples. The highest rates were found in the submandibular lymph nodes (n=10/50, 20%), followed by mesenteric lymph nodes (n=9/50, 18%). In contrast, the lowest prevalence were found in fecal samples (n=1/50, 2%). No strain of the pathogen was isolated in mediastinal lymph nodes. Mesenteric and submandibular lymph nodes showed statistical similarity (p>0.05) but differed when compared to samples of feces and mediastinal lymph nodes (p<0.05) (Table 1).
Table 2. Antimicrobial resistance profile of different serotypes of Salmonella isolated from slaughtered pigs

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td>CIP</td>
</tr>
<tr>
<td>S. Brandenburg</td>
<td>CIP, CRO</td>
</tr>
<tr>
<td>S. Typhimurum subsp. enterica 4.5,12:i-</td>
<td>NAL, CIP, CLO, TET</td>
</tr>
<tr>
<td>S. Typhimurum subsp. enterica 4.5,12:i-</td>
<td>NAL, CIP, CLO, EST, TET</td>
</tr>
<tr>
<td>S. Typhimurum</td>
<td>CIP, SOT, SUL, TET</td>
</tr>
<tr>
<td>S. Typhimurum subsp. enterica 4.5,12:i-</td>
<td>NAL, CIP, CLO, EST, TET</td>
</tr>
<tr>
<td>S. Typhimurum subsp. enterica 4.5,12:i-</td>
<td>NAL, AMP, CIP, SUL, TET</td>
</tr>
<tr>
<td>S. enterica subsp. enterica 4.5,12:i-</td>
<td>NAL, CIP, SUT, SUL, EST, TET</td>
</tr>
<tr>
<td>S. Typhimurium subsp. enterica 4.5,12:i-</td>
<td>CIP, TET</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>AMP, CLO, SUT, SUL, EST, TET</td>
</tr>
<tr>
<td>S. Typhimurium subsp. enterica 4.5,12:i-</td>
<td>AMP, CIP, CLO, EST, SUL, TET</td>
</tr>
<tr>
<td>S. Typhimurium subsp. enterica 4.5,12:i-</td>
<td>NAL, CIP, CLO, SUT, SUL, EST, TET</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>NAL, CIP, CLO, SUT, SUL, TET</td>
</tr>
<tr>
<td>S. Typhimurum subsp. enterica 4.5,12:i-</td>
<td>NAL, CIP, CLO, SUT, SUL, TET</td>
</tr>
<tr>
<td>S. Typhimurium subsp. enterica 4.5,12:i-</td>
<td>NAL, CIP, CLO, SUT, SUL, TET</td>
</tr>
<tr>
<td>S. Typhimurium subsp. enterica 4.5,12:i-</td>
<td>NAL, AMP, SUT, GEN, SUL, EST, TET</td>
</tr>
<tr>
<td>S. Typhimurium subsp. enterica 4.5,12:i-</td>
<td>AMP, CTX, CIP, CLO, SUT, SUL, EST, TET</td>
</tr>
</tbody>
</table>

The only one S. Brandenburg strain shows resistance exclusively to ciprofloxacin and ceftriaxone. The other single isolate of S. Derby was resistant against ampicillin, trimethoprim-sulfamethoxazole, sulfonamides, streptomycin, tetracycline, chloramphenicol and ciprofloxacin (Table 2).

Among the serovars identified, all were resistant to at least one of the antimicrobials tested, with 90% of them (n=18) resistant to at least four antimicrobial simultaneously. Considering the standards determined by CLSI for MDR samples, simultaneously resistant to ampicillin, chloramphenicol, streptomycin, trimethoprim-sulfamethoxazole and tetracycline, 15% (n=3) of the samples showed this pattern (Table 2). There was no strain positive for the ESBL enzyme production.

**DISCUSSION**

The present study revealed 10% frequency of Salmonella isolated from fecal and lymph nodes of pigs. This result is relatively lower to similar studies described worldwide. In Portugal, Vietnam and European Union were reported respectively 17.6%, 34.8% and 33% of positive identification of Salmonella in pigs (EFSA 2010, Ellerbroek et al. 2010, Gomes-Neves et al. 2012). Particularly in Brazil, different studies carried out in different regions revealed 16.6% and 67% of Salmonella from slaughtered pigs (Bessa et al. 2004, Sanchez et al. 2007, Silva et al. 2009, Kich et al. 2011).

Salmonella was most prevalent in the submandibular and mesenteric lymph nodes, then feces, and then mediastinal lymph nodes. It is known that the amount of feces sampled is related to the chances of the pathogen isolation (Davies et al. 1998), so the lower isolation rate in feces can be related to the sample aliquot used (1g). Other factor which can be associated to the lower frequency of Salmonella in the fecal samples is the intermittent excretion of the pathogen. On the other hand, Salmonella is present intracellularly in the lymph nodes, which acts as reservoirs of the pathogen (Bahnson et al. 2006), being less exposed to other factors that would difficult the pathogen detection by the isolation technique, being the presence of Salmonella in the lymph nodes an indicator of the carrier status of the animal.

The differences observed in the isolation of Salmonella from specific lymph nodes relates to the anatomical position of them. The most prevalent occurrence of the pathogen was observed in the mesenteric and submandibular lymph nodes. The high prevalence of Salmonella in mesenteric and submandibular lymph nodes is associated with the proximity of them, respectively, to the gastrointestinal fecal contents, taking these nodes as an initial barrier to the pathogen. In many cases, the submandibular infection does not develop enteric signs, turning the animals into reservoirs. In fact, the oral-fecal cycle of Salmonella infections in pigs was investigated in other studies, showing that 70% of the isolates were identified in the tonsils and submandibular lymph nodes (Bahnson et al. 2006, Straw et al. 2012).

The high occurrence of S. Typhimurium identified in pigs sampled, agreed with increased prevalence of this serotype in the global scenery (Carrasco et al. 2012). In 2011, S. Typhimurium was also the predominant serovar reported in the United States, followed by S. Enteritidis (CDC 2013). The high prevalence of this serovar has been reported in European Union (EFSA 2010) and Brazil (Kich et al. 2011) as well.

Another relevant finding in the current study is 35% (n=7/20) of positive isolation of S. enterica subsp. enterica 4.5,12:i-, a serotype considered similar to S. Typhimurium, characterized by minor differences in the flagellar phase. Currently, S. enterica subsp. enterica 4.5,12:i- is referred as one of the main serotypes isolated from pigs worldwide, particularly in Europe (EFSA 2010) and the United States, since it notifications increased 351% between 2001 and 2011 (CDC 2013). This monophasic Typhimurium-like strains are considered an emerging pathogen, being associated to several outbreaks with high antibiotic resistance rates, however Brazilian data about this serovar isolation are still scarce.

The others serotypes also identified in the present study, as S. Brandenburg and S. Derby, only one sample each, have similar results to those observed in other countries (Kich et al. 2011, Carrasco et al. 2012, Thai et al. 2012).

In the current study, up 80% of strains were found to be resistant to ciprofloxacin, tetracycline and nalidixic acid. Similar studies in Brazil have also found a prevalence of resistance by Salmonella isolated from pigs to tetracycline (96.5%) and nalidixic acid (95.5%) (Castagna et al. 2001), as well as multidrug resistant strains particularly to sulfonamides (97.8%) and streptomycin (82.6%) (Weiss et al. 2002). The high resistance rates of our isolates for ciprofloxacin are similar to those found in pigs from Spain (97.1%) (EFSA 2010). In contrast, studies in other countries revealed minor occurrence of resistant Salmonella strains against tetracycline, sulfonamides and nalidixic acid.
The high prevalence antibiotic-resistant strains of *Salmonella* encountered in our study is similar to the reported in other studies, such as investigations in pigs from Vietnam, Estonia, Ireland and Brazil. These studies reported multidrug resistance frequencies of 23.2%, 13.6%, 59.4% and 24.2%, respectively (EFSA 2010, Thai et al. 2012).

Multidrug resistant bacteria, including *Salmonella* species from animal and human origin is an emergent public health concern (Giguère et al. 2010). The high antimicrobial resistance rates in pig production can be attributed, among other reasons, to the improper use of antimicrobials for treatment of diseases or growth promotion in pork production (Gebreyes et al. 2004, Kich et al. 2011, Gomes-Neves et al. 2012). However, some resistance mechanisms to some antimicrobials are easily transferred between species, and their prevalence may be in part due to environmental contamination. Another important resistance acquisition route are the genes that promotes simultaneous resistance to different compounds. In the light of this, ubiquitous and opportunist pathogenic bacteria as *Salmonella* with resistance to conventional antimicrobials can survive in environment of farms, and are able to transmit drug resistance to other bacteria that infect wildlife and domestic animals, including pigs (Thakur et al. 2007). Indeed, despite the prohibition of the use of chloramphenicol in livestock 25 years ago in United States (Thakur et al. 2007) and 18 years ago in Brazil (Brasil 2003), a diverse range of bacteria remain resistant to these drugs even today.

In our study were not detected *Salmonella* strains ESBL-enzyme positive. However, in Europe ESBL production was referred in 0.6% of the strains isolated from pigs, when tested for cefotaxime and 0.5% for ceftazidime (EFSA 2014). Despite absence of *Salmonella* strains positive for ESBL-production in pigs sampled, the emergence of this pathogenic mechanism of antimicrobial resistance of *Salmonella*, requires continuous epidemiological vigilance studies.

In this study, we found that *Salmonella* sp. isolated from pigs exhibit high levels of resistance to antimicrobials. These antibiotic-resistant strains may potentially cause foodborne outbreaks of antibiotic-resistant salmonella in humans. *S. Typhimurium* was the most common serovar, even with the adoption of control measures implemented over several decades in pig farms. In addition, there is an increasing occurrence of *S. enterica* subsp. *enterica* 4,5,12: i:-, considered an emergent human pathogen and with few Brazilian data about this serovar available. The high resistance rates found in our study reveals that despite regional differences for resistance to some antimicrobials, the inappropriate use of antimicrobials increases the selection rate of multidrug resistant bacteria, including *Salmonella*. Thus, the selection of first-choice antimicrobial treatment should be based on local *in vitro* resistance patterns. Indeed, the responsible use of antimicrobials for animals is an emergent One Health concern, to conserve these drugs for human therapy approaches.

**Acknowledgements**. To Adolfo Lutz Institute for conducting the serotyping of strains and Cefar Diagnostic for providing the antibiogram discs.

**Conflict of Interest**. No conflict of interest.

**REFERENCES**


CSLI 2013. Performance standards for antimicrobial susceptibility testing M100-S23. Clinical Laboratory Standards Institute, Wayne, PA.


