Isolation and Antimicrobial Susceptibility of *Salmonella Typhimurium* and *Salmonella Enteritidis* in Fecal Samples from Animals

López-Martín JI¹, González-Acuña D², García CA³, and Carrasco LO⁴

¹Department of Pathology and Preventive Medicine, Faculty of Veterinary Sciences, University of Concepcion, Concepcion, Chile
²Department of Animal Science, Faculty of Veterinary Sciences, University of Concepcion, Concepcion, Chile
³Department of Microbiology, Faculty of Biological Sciences, University of Concepcion, Concepcion, Chile
⁴Department of Anatomy and Comparative Pathology, Faculty of Veterinary Medicine, Cordóba, Spain

**Abstract**

*Salmonella enterica* is a microorganism with high zoonotic potential, distributed worldwide, having more than 2,610 identified *serovars* and affecting different animal species, both production and wildlife animals. **Objective**: Prevention of human salmonellosis requires prior monitoring of *Salmonella* in animals. In this study, *Salmonella enterica* *serovars* from different animal origins were isolated and their antimicrobial susceptibility was determined. **Methods**: A total of 2193 samples from different origins (feces of cattle, sheep, horses, pigs, dog, chickens and seagulls and chicken eggs) were analyzed for bacterial typing and antimicrobial susceptibility was studied by the Kirby-Bauer method including 9 antibacterial agents (florfenicol, amoxicillin, cefotiofur, gentamicin, oxytetracycline, trimethoprim/sulfamethoxazole, enrofloxacin, ampicillin and cefoperazone) and MIC<sub>50</sub> and MIC<sub>90</sub> for 6 of them were determined. **Results**: A 4.38% serovar isolation (n = 96), including 6 *S. Typhimurium* (6.25%) from equine cattle and pig feces, 19 S. Enteritidis (19.79%) from seagulls and pigs and 71 other *serovars*, was obtained from the various animal origins. Serovar *S. Typhimurium* showed high resistance to oxytetracycline and gentamicin by the Kirby-Bauer method and a MIC<sub>90</sub> of 512 µg.mL<sup>-1</sup> for oxytetracycline and trimethoprim/sulfamethoxazole antibiotics. By the diffusion method, serovar *S. Enteritidis* was resistant to trimethoprim/sulfamethoxazole and its MIC<sub>50</sub> was 256 µg.mL<sup>-1</sup> for oxytetracycline. The 32% of *Salmonella* isolates showed multi-resistance, 2 strains, isolated from pigs (one *S. Typhimurium* and one *S. Enteritidis serovars*), showed resistance to 5 antimicrobials tested. **Conclusion**: The constant release of these *serovars* to the environment, reaching also animal food, is a permanent potential risk for public health, turning into a first priority the establishment of control and antibiotic therapy strategies.

**Keywords**: *Salmonella enterica*; *S. typhimurium*; *S. Enteritidis*; Antimicrobial; Salmonellosis; MIC<sub>50</sub>; MIC<sub>90</sub>

**Introduction**

Different *serovars* of *Salmonella enterica* subspecies *enterica* are potentially zoonotic pathogens. Different animal species, distributed throughout the world, have been detected as carriers of this pathogenic agent [1]. More than 2610 *S. enterica* *serovars* have been recognized worldwide, most of them being major causative agents of diseases in humans and animals, producing gastroenteritis and other acute infections [2]. In the United States, it is estimated that, in humans, approximately 44% of hospitalizations, 44% of deaths and 20% of illnesses are the consequence of foodborne pathogens [3], representing an incidence of 15.2 cases per 100,000 individuals [4].

*Salmonella* is a persistent pathogen in the environment, able to easily survive and proliferate [1]. The most commonly isolated *serovars* worldwide from various animal sources continue to be *S. Enteritidis* and *S. Typhimurium* which, besides producing gastroenteritis, are found in asymptomatic carriers in a wide variety of animal species [5-8]. Of these, *S. Enteritidis* it is the most prevalent one followed by *S. Typhimurium* (52.3% and 23.3% of the cases, respectively) [9].

Non-typhoid salmonellosis in humans is mainly caused by contamination of foods from animal origin [8,10,11]. Human infections with *S. Enteritidis* originate mainly from eggs and egg products when consumed raw or undercooked, while *S. Typhimurium* infections originate mainly from pigs, cattle and chicken as well as environmental contamination from household pets or contaminated birds [1,11].

In general, approximately 95% of human cases of salmonellosis are associated to the consumption of contaminated products such as meat, chicken, eggs, milk, seafood and fresh produce [12]. In China, *Salmonella* *serovars* were isolated from 54% of chicken samples, 31% of pig samples, 17% of cattle samples and 20% of sheep samples [7]. In northern Vietnam, where only pig and chicken samples were obtained, the frequency of *Salmonella* *serovars* isolation was 39.6% and 42.9%, respectively [13]. With respect to eggs, 4.82% were positive for *Salmonella* [14]. On the other hand, 2 (1.33%) of 150 eggshell samples were determined as contaminated with *Salmonella*, including both *S. Enteritidis* and *S. Typhimurium* [15]. The *serovar* most commonly identified in chicken meat is the serovar *S. Enteritidis*, also reported as the most common in human cases of salmonellosis [7]; however, other authors reported serovar *S. Typhimurium* as the most common [14].

**Received** January 25, 2016; **Accepted** March 16, 2016; **Published** March 25, 2016


**Copyright**: © 2016 López-Martín JL et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
In the United States, during the years 1973-2011, 5% of outbreaks of salmonellosis were due to consumption of meat products [10].

In addition, in a review, was reported that a number of marine animals and fish eating birds are reservoirs of S. enterica serovars without showing any clinical manifestation of the disease [16]. In general, seagulls have been involved in major outbreaks of salmonellosis in animals and in humans [17].

However, the predominant serovars vary not only according to the species affected, but also among different geographical areas of a region [8].

It is known that intestinal salmonellosis can be a self-limited disease not requiring antibiotic therapy [5]. However, bacteremia may appear in young or old individuals in humans or various other animal species and also in immunocompromised individuals. Thus, transference of antibiotic resistance from environmental to human pathogenic bacteria is considered a major risk for public health. Therefore, the presence of antibiotic resistant bacteria, mainly in fecal contamination, plays an important role in the spread of these resistance by several mechanisms, including gene transfer [18]. In 2003, was reported that 22.5% of non-typhoid Salmonella in humans was resistant to at least 1 antimicrobial agent [12]. Recently, was indicated that in isolates from children in Kenya, 97% of S. Typhimurium and 92% of S. Enteritidis showed resistance to at least one antimicrobial agent [19]. Multidrug resistance (MDR) has been reported for S. Typhimurium isolated from bovines and porcines in Japan where 82% of the samples showed resistance to at least one antibiotic and 70% to three or more antibiotic agents [6]. Also, both healthy animal samples and clinical samples from different species showed that 44% of Salmonella isolates were resistant to at least one antibacterial agent, and 4.8% of these isolates showed the same resistance phenotype previously described for humans [12]. Moreover, 90.1% of S. Enteritidis resistant strains isolated from various human and animal origins were resistant to at least one antibacterial in Brazil [20] and nearly 80% of Salmonella isolates from chicken, pig, cattle and lamb meat were resistant to at least one antimicrobial while 53% of them were resistant to more than three antimicrobial agents [7]. Rodriguez-Rivera et al. isolated Salmonella from bovine feces and from the environment in farms showing a 23.6% resistance from 1 to 11 antimicrobial agents, representing 50 different antibacterial resistance patterns, and concluded that it represents a potential risk for public health [21].

The most common MDR was to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline [6,19,22]. The indiscriminate use of these compounds makes difficult the treatment of bacterial infections in animals and humans [23].

For all the above, there is no doubt that prevention of human salmonellosis requires prior monitoring of Salmonella from animal origin. Therefore, the aims of this study were to isolate, identify, serotyping and to determine the antimicrobial susceptibility of S. enterica strains isolated from fecal samples of different animal species to obtain data to contribute to the control and to determine the dissemination of Salmonella serovars. Results reported here include, in part, the genotypic and phenotypic characterization of Salmonella isolates from different animals origin.

**Materials and Methods**

**Samples**

The Veterinary Microbiology Diagnostic Laboratory, Department of Pathology and Preventive Medicine, Faculty of Veterinary Sciences, University of Concepción, Chile (MedVet-UdeC Laboratory) received fecal swab samples from horses (47), pigs (326) and cattle (72) with gastroenteritis. In addition, fecal samples of dogs from the Veterinary Clinical Hospital, Faculty of Veterinary Sciences, University of Concepción and from stray dogs (258) plus sheep samples (131) were analyzed. Research projects also provided egg samples (444), fecal swabs of backyard chickens (669) and fecal swabs of seagulls from the port of Talcahuano, Chile (306), collected by statistical random system. In total, 2193 samples of diverse animal sources were obtained and analyzed searching for the presence of Salmonella enterica serovars.

**Isolation and identification of Salmonella enterica**

The conventional bacteriological method of the MedVet-UdeC Laboratory was used. Fecal swab samples were subjected to an initial pre-enrichment in buffered peptone water (Merck) and incubated for 24 hours at 37°C and 0.5 mL were transferred to 10 mL Tetrathionate Broth (Merck) and incubated at 42°C for 24 h. Finally, a loopful was transferred to dishes containing XLD agar (Merck), a Salmonella selective medium, in triplicate, and incubated for 18 h at 37°C. Presumptive Salmonella colonies were phenotypically confirmed by biochemical properties in differential agars, such as TSI, LIA, Simmons citrate and SIM (Merck) and incubated at 37°C for 24-48 hrs. Colonies biochemically confirmed as S. enterica were subjected to serological confirmation by agglutination with polyvalent antisera O (Denka Senken Co. ltda). S. Typhimurium and S. Enteritidis, ATCC 14028 and 13076, respectively, were used as positive controls. After biochemistry and serology tests confirmed the identification of S. enterica, the strains were sent to the National Reference Laboratory, Public Health Institute (ISP), Chile for serotyping. The serovars isolates were stored to 20°C and constitute part of the culture collection of the MedVet-UdeC Laboratory.

**Antimicrobial susceptibility**

The qualitative agar diffusion method (Kirby-Bauer method) was used employing Mueller Hinton agar. Nine antibacterial agents Florfenicol (FLO) 30 μg, amoxicillin (AMX) 20 μg, cefotiofur (CLR) 30 μg, gentamicin (GEN) 10 μg, oxytetracycline (TET) 30 μg, trimethoprim/sulfamethoxazole (SXT) 25 μg, enrofloxacin (EEE) 30 μg, ampicillin (AMP) 10 μg and Cefoperazone (CEF) 75 μg were used. The antimicrobial susceptibility was determined according to the standard diameter of inhibition (mm) for each antibiotic used [24]. Minimum Inhibitory Concentrations (MIC) were determined by the serial dilution method in Mueller Hinton broth for 6 of the 9 antibiotics used: AMP, TET, SXT, FLO, EEE and CLR. A 1024 μg mL-1 stock solution was obtained for each antibiotic [24]. Incubation was carried out at 37°C for 24 hours. Control strains were E. coli ATCC 25922 and S. enterica ATCC® 12172. The MIC (MIC<sub>50</sub> and MIC<sub>90</sub>) was determined for each of the antibiotics used and the sensitivity or resistance was determined according to the protocol described [24].

**Results**

**Bacterial strains**

Ninety-six strains of S. enterica were obtained, representing a 4.38% isolation rate. Of these, 66 corresponded to S. Typhimurium (6.25%), 19 to S. Enteritidis (19.79%), and the remaining 71 strains corresponded to other serovars (Table 1). S. Typhimurium serovars were obtained from clinical samples from pigs, ruminants and equines. With respect to S. Enteritidis serovars, 17 were obtained from cloacal samples of seagulls.
From the 258 canine fecal samples and 609 chicken cloacal swabs from chickens (609) no S. enterica isolates were obtained. The 17.65% of S. enterica isolates were obtained from cloacal samples of seagulls, followed by porcine fecal samples (9.51%).

### Antimicrobial susceptibility

According to the Kirby-Bauer method for antibiotic susceptibility, S. Typhimurium and S. Enteritidis serovars isolated from different sources showed a high resistance to ampicillin, followed by trimethoprim/sulfamethoxazole and gentamicin and oxytetracycline (Table 2). S. Typhimurium isolates (n = 6) showed a high resistance to oxytetracycline and gentamicin (50%); however, a higher resistance to trimethoprim/sulfamethoxazole (31.6%) was observed in S. Enteritidis serovars (Table 2).

Table 3 shows the results of resistance of the S. Typhimurium and S. Enteritidis serovars according to the MIC<sub>90</sub> and MIC<sub>50</sub> values. The highest resistance was obtained for oxytetracycline and trimethoprim/sulfamethoxazole. S. Enteritidis serovars were mainly resistant to tetracyclines but S. Typhimurium serovars showed a high MDR to tetracycline, trimethoprim/sulfamethoxazole, amoxicillin and florfenicol. In general, Salmonella isolates showed high sensitivity to cephalosporins and fluoroquinolones.

Table 4 shows the patterns of resistance. A 32% of the serovars isolated showed MDR. Among them, 6 S. Typhimurium serovars and the 2 S. Enteritidis serovars were obtained from pig clinical samples. Strains showing MDR to 5 antibiotics were both isolated from pigs, one being S. Typhimurium and other S Enteritidis.

### Discussion

In the present study, the highest percentage of Salmonella spp. isolates were identified as S. infantis serovar and they were collected from pigs and seagulls and it was followed by S. Enteritidis and S. senftenberg serovars, also obtained from seagulls. The diversity of serovars isolated from the samples of different animal species confirms the different source of contamination, making it even more interesting to study the different possible sources of contamination considering the diversity of carrier individuals. In general, there is a high consistency between the literature reports and the findings of the present work. Thus, researchers of both human health and animal health should take appropriate precautions when working with Salmonella due to its different zoonotic potential and its role in public health, particularly when dealing with wildlife. A previous work had already reported the role of seagulls residing in the Chilean coast as potential vectors of pathogens multiresistant to antimicrobials with the ensuing risk for public and animal health [25].
Table 3: Percentage distribution of resistance and MIC<sub>50</sub> and MIC<sub>90</sub> (μg/mL) of S Typhimurium and S Enteritidis serovars.

<table>
<thead>
<tr>
<th>Serovars</th>
<th>Typhimurium and Enteritidis</th>
<th>%R</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (μg/mL)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEE</td>
<td>17 4 1 2 1 32 64 128 256 512 1024 &gt;1024</td>
<td>6.7</td>
<td>&lt;0.5 0.5 1 2 4 8 16 32 64 128 256 512 1024 &gt;1024</td>
<td>6.7 0.5 1 2 4 8 16 32 64 128 256 512 1024 &gt;1024</td>
</tr>
<tr>
<td>TET</td>
<td>2 7 5 3 3 1 2 2</td>
<td>6.7</td>
<td>15.8 8 256</td>
<td>6.7 15.8 8 256</td>
</tr>
<tr>
<td>SXT</td>
<td>1 3 4 4 5 2 5 1</td>
<td>6.7</td>
<td>10.5 32 64</td>
<td>6.7 10.5 32 64</td>
</tr>
<tr>
<td>CLR</td>
<td>5 2 18</td>
<td>6.7</td>
<td>0 2 2</td>
<td>6.7 0 2 2</td>
</tr>
<tr>
<td>AMP</td>
<td>14 5 3</td>
<td>6.7</td>
<td>15.8 1 8</td>
<td>6.7 15.8 1 8</td>
</tr>
<tr>
<td>FLO</td>
<td>6 2 5</td>
<td>6.7</td>
<td>5.3 4 16</td>
<td>6.7 5.3 4 16</td>
</tr>
</tbody>
</table>

Table 4: Resistance patterns obtained of the serovars S Typhimurium and S Enteritidis isolated from different animal sources.

<table>
<thead>
<tr>
<th>Resistance pattern</th>
<th>N</th>
<th>Porcine</th>
<th>Equine</th>
<th>Bovine</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S. Typhimurium y S. Enteritidis</td>
<td>EEE-TET-SXT-AMX- FLO</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

TET: Oxytetracycline; SXT: Trimethoprim/sulfamethoxazole; FLO: Florfenicol; AMX: Amoxicillin; EEE: Enrofloxacin.

31 Salmonella strains were isolated in cattle belonging to 12 different types of serovars, and the transduction of microbial resistance from S Heidelberg to S. Typhimurium in swine was demonstrated, conferring resistance to multiple beta-lactam antibiotics and tetracycline blaCMY-2, tet (A) and tet (B), respectively [18]. Later on, a 58% resistance to trimethoprim/sulfamethoxazole and 56% to tetracycline, followed by ampicillin and amoxicillin was reported [7]. In Chile, in a preliminary study, 20.5% of Salmonella strains isolated, mainly from pigs, showed MDR, being oxytetracycline the agent showing the highest resistance (69.1%) [31]. The results reported here show that serovars isolated from swine reach a high multi-resistance.

It is worth mentioning that the main Salmonella serovars showing multidrug resistance were obtained from clinical samples. It is also important to mention that many of the serovars isolated from seagulls showed an intermediate sensitivity according to the Kirby-Bauer qualitative method of susceptibility, but susceptibility to them will depend on which species will be infected by these serovars. It was reported that 9% of seagulls (Larus occidentalis) studied, in 2008, in California were carriers of Salmonella but only from one of those seagulls an antibiotic resistant Salmonella was isolated [32]. Subsequently, was reported a 24% (n = 216) isolation of Salmonella from young black-headed gulls (Larus ridibundus) being S. Enteritidis (PT 8 and 4) the most prevalent showing a 28% of resistance to antibiotics [33].

As mentioned above, the effect of antimicrobials in the equine species, with respect to the acquisition of bacterial resistance in humans, has not been reported; however the frequency pattern is repeated for this species. It makes sense, because horses can be infected, by direct or indirect contact, by Salmonella infected feces of several other animal species. Salmonella serovars isolated from horses (n = 232) were predominantly S. Typhimurium and the main antibacterial resistance was found for tetracycline and for ampicillin [27]. Most isolates were susceptible to cefiofur and enrofloxacin. It must be emphasized the diversity of resistance patterns shown in this work (n = 16) including antimicrobial susceptibility tests to 9 antimicrobials. Resistance
patterns included from only tetracycline to different combinations of antimicrobials and it can be concluded that S. Typhimurium phage type in horses, 506 (DT 104), corresponds to those found in humans, pigs and cattle for the same time period of this study.

In relation to meat of animal origin intended for human consumption, a high percentage of isolation of various *Salmonella* serovars from meat samples of pigs and chickens, including only 0.1% S. Typhimurium and only 1.2% *S. enteridis*, and a high frequency of antibacterial resistance to at least one antibiotic (78.4% of the isolates) was reported in North Vietnam [13]. In China, samples obtained from slaughtered chickens and pigs showed 45.2% and 29.2% of *Salmonella* isolation, respectively [34]. The predominant serovar in chickens was *S. Enteritidis* but pigs showed mainly *S. Typhimurium* plus *S. Derby* and *S. Enteritidis*. Highly multidrug resistant *Salmonella* was also reported in chicken eggs for human consumption [14].

It is important to consider that some studies from around the world have reported ciprofloxacin resistant *Salmonella* [7], particularly in less frequent *serovars* [23]. Since this antibiotic is widely used in human salmonellosis it should be included in future epidemiological studies to monitor the development and spread of resistance to this antimicrobial in different countries and regions worldwide. Other studies reported 8.6% and 10% ciprofloxacin resistant *Salmonella Indiana* serovar strains in chickens and pigs, respectively [34].

It is necessary, therefore, to characterize the genotypic resistance of the isolates obtained in this work. For this purpose, the strains are kept in the culture collection of the MedVet-UdeC Laboratory at -20°C for further studies including patterns of genetic resistance to be determined by molecular techniques. Since it is known that the genetic transmission occurs easily among different bacterial species in the gastrointestinal environment [18], it is not unthinkable that this transmission might occur at a high frequency not only within the same animal species, but also between different species, not only production animals but also including wildlife, through the release of the feces of infected animals. It would be also important to maintain a database with the results obtained at different regions of the world in order to monitor or support control programs and food safety in countries not having sufficient information, as compared to the countries of the European Community [8]. It would be also advisable to include the phylogenetic study of *Salmonella* isolates to know their origins and relationship between different circulating *serovars*.

Undoubtedly, also it is necessary to determine the prevalence of the genes determining antibiotic resistance in the *serovars* isolated to assess variations within or between them. *Salmonella* possess various virulence and antimicrobial resistance mechanisms able to challenge public health strategies. As the understanding of the pathogenicity mechanisms and factors leading to antimicrobial resistance development improves, there is a hope to limit the load of these pathogens to the environment.

It is an important challenge for the anti-*Salmonella* therapy, especially in humans, the diversity of treatments. Veterinary medicine has a similar challenge because, for example, the treatment for certain animal species involves the use of cephalosporins which are contraindicated for other animal species, finally complicating the treatment. Non-typhoid *Salmonella* can cause serious septicemic diseases in adults, particularly in immunocompromised individuals having viral diseases making them more susceptible to these infections, which also carry resistance to several antimicrobials and also in very young individuals with viral diseases producing a decrease in the immune response, especially in human medicine in children under 5 years of age.

However, it has been detected in humans a decrease in the frequency of *S. Typhimurium* and *S. Enteritidis* with a concomitant increase of other *serovars* [12]. Moreover, it is interesting the association, made in Ethiopia, of *Salmonella* infections in humans with the consumption of raw vegetables, whose contamination may result from animal feces [35]. Since infection outbreaks will vary depending on the *serovar* involved and its antimicrobial resistance pattern, information of *serovars* of *S. enterica* circulating in a given geographical area and their antibiotic susceptibility is necessary to design appropriate interventions to control these outbreaks. Thus, the phylogenetic study of the *serovars* found in the different sources analyzed in the present work becomes relevant, as it is also the case of the genetic study of the predominant *serovars* in a region.

In conclusion, the control and monitoring of *Salmonella* *serovars* from different animal sources is of great importance for human health. The permanent potential risk of these *serovars* present in feces contaminating the environment and, thus, animal food, constitute the first priority to control infections and to establish antibiotic therapy strategies.

**Funding**

Laboratory of Veterinary Microbiology, Department of Pathology and Preventive Medicine, Faculty of Veterinary Sciences, University of Concepcion, Chile.

**Acknowledgements**

Authors wish to thank the very special collaboration of Mr. Fredy Riquelme, laboratory technician at the MedVet-UdeC Laboratory for his assistance.

**References**


