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Chapter 4

INVOLVEMENT IN FOODBORNE OUTBREAKS, RISK FACTORS AND OPTIONS TO CONTROL SALMONELLA ENTERITIDIS SE86: AN IMPORTANT FOOD PATHOGEN IN SOUTHERN BRAZIL

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ABSTRACT

A specific strain of *S.* Enteritidis (SE86) was responsible for more than 95 % of the investigated salmonellosis occurred in the State of Rio Grande do Sul (RS), Southern Brazil, from 1999 to 2013. The aim of this chapter is to demonstrate the involvement of this strain with the salmonellosis outbreaks and to discuss the factors that probably contributed to *S*. Enteritidis SE86 had become one of the most important food pathogens in Southern Brazil. During 1999 to 2006, 190 salmonellosis outbreaks were investigated in RS and one DNA banding profile was identified among *S*. Enteritidis isolated from foods, blood and feces of victims. The causative strain was named *S*. Enteritidis SE86. The main risk factors for the outbreaks caused by SE86 were 1) the consumption of homemade mayonnaise prepared with raw eggs; 2) holding foods in room temperature for more than 2 hours, 3) cross-contamination due to the contact of food with contaminated equipment and utensils. The growth of SE86 in homemade mayonnaise was modeled and results demonstrated that SE86 was able to grow faster than others *Salmonella* serovars, during the first six hours at environmental temperature; however SE86 did not grow in homemade mayonnaise at 10^{o} C, during 18 hours. Further studies have demonstrated that SE86 was able to form biofilms on stainless steel, stainless steel welds and polypropylene surfaces, and survived to 400 and 200 ppm sodium hypochlorite. After sodium hypochlorite exposure, SE86 expressed RpoS and Dps proteins, which are involved with oxidative stress. Due to exposure to sub-lethal pH, SE86 became acid-adapted and

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increased its thermal resistance. Acid-adapted SE86 was able to survive to simulated gastric fluid pH 1.5 and became more virulent than other *Salmonella* serovars for germ-free mice. Isolates of SE86 remain sensitive to several antibiotics, nevertheless resistance to ampicillin and nalidixic acid and multidrug resistance has increased during last decade.

INTRODUCTION

Foodborne Diseases in Southern Brazil

Currently, Brazil exports foods to approximately 160 countries and is a major exporter of poultry, beef, coffee, sugarcane, soybeans, corn, pork, and cocoa [1]. In order to attend the high standards demanded by exportation, high quality and safe foods are produced by Brazilian food industries. However, in a country with approximately 200 million people, several different realities can be found, including in food production. Similar to other countries, many establishments prepare foods without adequate conditions, resulting in food-borne outbreaks that, in most of the cases, are not notified or registered. The majority of the food-borne outbreak notifications came from the Southern and Southeast regions of Brazil, because the surveillance services are better structured in these regions. The occurrence of food-borne outbreaks in other regions is not clear [2]. The Southeast region of Brazil is composed by the States of Espírito Santo, Rio de Janeiro, Minas Gerais and São Paulo, while the Southern region grouped the States called Paraná, Santa Catarina, and Rio Grande do Sul. These regions are well developed, presents several industrialized areas and high standards of life quality. Rio Grande do Sul (RS) is the southernmost State of Brazil and has a population of 10.7-million-people, distributed in 496 cities [3].

In Brazil, *Salmonella* sp. was identified as the major cause of reported food-borne diseases, during the period from 2000 to 2013 [4]. Among the 3,857 etiologic agents identified in food-borne illnesses, *Salmonella* sp. was identified in 39.46 % of the outbreaks, followed by *Staphylococcus aureus* (19.68 %), *Escherichia coli* (12.32 %), and *Bacillus cereus* (7.65 %) [4]. Unfortunately in approximately 50 % of the investigated food-borne outbreaks, the etiological agent was not identified [5, 6]. Considering the whole country, mixed food preparations and eggs and egg-based products, respectively, were the most common food vehicles of food-borne disease outbreaks. According to the Brazilian official data, the majority of outbreaks occurred in private homes, followed by restaurants, bakeries and schools [4].

Salmonellosis in Southern Brazil

During the 1990s, *Salmonella* spp. became the major cause of reported food-borne diseases in the State of RS [7], surpassing *S. aureus*, which was identified as the major agent of food-borne diseases of this State, from 1987 to 1993. According to the Sanitary Surveillance Service of RS (DVS/RS), the reason for that was the implementation of mandatory Good Manufacturing Practices (GMP) training courses for people responsible for food services of RS. The better control of food manipulation decreased the contamination originated by food handlers, reducing outbreaks caused by *S. aureus* [3].

Official data of DVS/RS about food-borne salmonellosis of RS were analyzed by periods (1997 to 1999, 2001 to 2002, and 2003 to 2004) and published elsewhere [8, 9, 10]. Data have demonstrated that more than 46,000 people were involved, and approximately 5,200 people were hospitalized, during all the periods. Most of salmonellosis outbreaks occurred in springtime, due to keep foods outside refrigeration. This inadequate procedure occurred because, at springtime, RS presents mild temperatures at mornings and people conclude that refrigeration of foods is not necessary. At afternoons, the temperature increases and microorganisms multiply on foods, causing the outbreaks. The incidence of outbreaks caused by other etiological agents also increases, confirming the negligence regarding control of temperature [8]. At summertime, the number of salmonellosis usually decreases because population is aware about food-borne diseases, storing foods in adequate refrigeration.

From 1997 to 2004, in RS, the most frequent food vehicle of *Salmonella* was potato salad mixed with homemade mayonnaise prepared with raw eggs [8, 9, 10]. A recent study reported that this preparation remained as the most important food vehicle of salmonellosis in RS [11], even though other food preparations were also identified, for example, pastry products (15.94 %), beef (12.32 %), processed meat (9.42 %), chicken meat (6.52 %), and pork (2.17 %) [11]. According to Vaz, et al. (2010) [12], in Brazil and in other parts of the world, *S.* Enteritidis has been associated with human foodborne infections caused by the ingestion of contaminated foods of animal origin, mainly undercooked poultry meat and eggs. Nunes, et al. (2003) [13], analyzed *Salmonella* isolated in 1995 to 1997 from Brazilian healthy and diseased chicken, food-borne outbreaks of human gastroenteritis related to the consumption of egg products, poultry meat, pipped embryos of broiler chicks, meat meals, and diverse food products, as cheese, mayonnaise, cake, and bacon and reported that phage type (PT) 4 was the most frequent type of *S*. Enteritidis found. The same authors stated that the results obtained were in accordance with the worldwide trends in distribution patterns for different *S*. Enteritidis phage types [13]. Pires, et al. (2014) [14] studied the source attribution of human salmonellosis worldwide and demonstrated illnesses and outbreaks were most commonly attributed to exposure to contaminated food, mainly eggs, broiler chickens, and pigs. The same authors also mentioned that exposure to raw vegetables was also an important source of salmonellosis.

Salmonella serovars responsible for food-borne outbreaks in RS have changed along the years. For example, in 1990, *S.* Typhimurium was the predominant *Salmonella* serovar (86 %) isolated from foods involved with salmonellosis of RS, while *S.* Enteritidis was not found in those samples [8]. However, in 1993, *S.* Enteritidis was isolated from 64 % of the suspected foods, surpassing *S.* Typhimurium, which was isolated from only 4 % of the food involved with salmonellosis [8]. During the 90s, this surpassing of *S.* Typhimurium by *S.* Enteritidis was also observed in the United States and other countries [15]. The rapid increase in the prevalence of *S.* Enteritidis in different countries was attributed to the formation of an ecological niche occurred due to the successful campaign to eradicate *S.* Gallinarum and *S.* Pullorum from chicken flocks, which are the causative agents of fowl typhoid and pullorum disease in chickens [16, 17].

Geimba, et al. (2004) [16] analyzed 75 *Salmonella* spp. isolated from foods involved with food-borne outbreaks occurred from 1999 to 2000 in RS and found that 73 (97 %) of them were *S.* Enteritidis. Only one isolate was serotyped as *S.* Derby and other one as *S.* Typhimurium. Among the strains of *S.* Enteritidis, 83 % were positive for the presence of *spv*R gene (*Salmonella* Plasmid Virulence regulatory gene) and a positive correlation (*P*

<0.05) between *S.* Enteritidis and the presence of *spv*R was reported [16, 3]. In the period from 2001 to 2002, Oliveira, et al. (2007) [18] investigated 85 *Salmonella* isolated from foods responsible for salmonellosis occurred in RS and observed that 93 % were *S.* Enteritidis. Only six isolates were serotyped as *S.* Javiana (1), *S.* Infantis (1), *S.* Agona (1), *S.* Typhimurium (1), and *S. enterica* subspecies enterica 1, 4 [18].

Recently, Capalonga et al. (2014) [11] reported that 84.7 % of the 163 *Salmonella* isolated from foods involved with salmonellosis in RS, during 2007 to 2012, were *S.* Enteritidis. The second and third most frequent serovars were *S.* Schwarzengrund (5.5 %) and *S.* Typhimurium (3.7 %).

Molecular Characterization of *S.* Enteritidis in Southern Brazil

Oliveira, et al. (2007) [18] demonstrated that *S.* Enteritidis responsible for salmonellosis in RS during the years of 2001-2002 were grouped in only two PCR-ribotyping banding profiles (R1 and R2), being that profile R1 comprised 92.4 % of the strains. The same strains were analyzed by RAPD and demonstrated four banding patterns (A to D). Profile A grouped 81 % of the strains. Using PCR ribotyping, Pulsed Field Gel Electrophoresis (PFGE) and DNA sequencing, Oliveira, et al. (2009) [19] investigated the clonal relationship of 152 isolates of *S.* Enteritidis involved with food-borne outbreaks of RS, from 1999 to 2002, concluding that a specific strain of *S.* Enteritidis was found in the majority of the investigated outbreaks. After that, during 1999 to 2006, the Official Laboratory of RS (FEPPS/LACEN/RS) isolated 931 *S.* Enteritidis isolates in 190 outbreaks officially investigated. Among them, *S.* Enteritidis isolates from 80 different outbreaks were randomly chosen to be analyzed (the researchers picked ten isolates from each year of the period comprised from 1999 to 2006). The 80 *S.* Enteritidis samples were isolated from blood (n=12) and feces (n=68) of salmonellosis victims in RS, and were analyzed by means of PCR ribotyping and XbaI macro restriction PFGE. Results identified only three closely related PCR ribotyping patterns (R1, R2, and R3) and just one PFGE profile. The PCR ribotyping profile were the same profile previously found in *S.* Enteritidis isolated in suspected foods [18]. The PFGE profile grouped 97 % of the strains isolated from 1999 to 2006 [20]. These findings confirmed the occurrence of the same DNA banding pattern among *S.* Enteritidis isolated in foods, blood and feces of the victims of salmonellosis from 1999 to 2006 in RS, suggesting that a specific strain of *S.* Enteritidis was responsible for the majority of the cases of salmonellosis in this Brazilian State. This strain was named *S.* Enteritidis SE86 [3].

Lately, Capalonga, et al. (2014) [21] studied 163 *S.* Enteritidis isolated from foods involved in foodborne salmonellosis occurred in RS from 2007 to 2013 and found only one PCR ribotyping profile, which was compatible to the profile of SE86. Based on these results, the researchers concluded that SE86 have caused several salmonellosis outbreaks in RS, from 1999 to 2013.

Risk Factors for Salmonellosis in Southern Brazil

According to DVS/RS [8, 9, 10, 3], the risk factors for salmonellosis outbreaks in RS were: 1) the consumption of homemade mayonnaise prepared with raw eggs (use of eggs

without sanitary inspection), 2) holding foods in room temperature for more than 2 hours, 3) cross-contamination due to the contact of foods with surfaces of contaminated equipment and utensils, and 4) maintenance of food in improper cooling. These risk factors, as well as studies related to them are discussed below.

Consumption of Homemade Mayonnaise

Homemade mayonnaise (HM) was identified as the food vehicle mostly involved with salmonellosis in Southern Brazil [22]. It is well known that eggs and egg products are among the most important food vehicles of *S.* Enteritidis, because the outer shell egg surfaces or the internal egg contents can be contaminated [23]. In general, the external contamination is due to chicken feces during or after oviposition, on the other hand the internal infection can be the result of penetration through the eggshell or by direct contamination of egg contents, before oviposition, originating from reproductive organs infection [24]. Mayonnaise, often prepared with raw eggs, is widely consumed and probably the most used sauce around the world [25]. It is a food preparation frequently involved with foodborne outbreaks worldwide [26, 27, 28, 29]. In the State of RS, HM prepared with raw eggs and mixed with cooked potato is the principal side dish of *churrasco*, a typical meat meal similar to barbecue, highly consumed in this region of Brazil [22, 30].

In order to allow the development of a quantitative microbial risk assessment for *S.* Enteritidis SE86 on HM, a study was performed in order to modeling the growth of SE86 on HM at different temperatures [22]. Using predictive microbiology programs, growing curves were built by fitting data to the Baranyi's DMFit, and generated R^2 values greater than 0.98 for primary models. Secondary model was fitted with Ratkowsky equation, generating R^2 and RMSE values of 0.99 and 0.016, respectively. Experimental data showed that SE86 did not grow on HM at 7°C, for 30 days. At 10°C, no growth was observed until approximately 18 h and the infective dose (considered 10^6 CFU) was reached after 8.1 days. However, 10^6 CFU of SE86 were attained after 6 h at 37°C. Experimental data demonstrated smaller *lag* phases than those generated by ComBase Predictive Models, suggesting that SE86 is very well adapted for growing on HM. Corroborating this finding, Malheiros, et al. (2007) [31] demonstrated that SE86 was able to grow faster than others *Salmonella* serovars (Bredeney and Typhimurium) during the first six hours on HM stored at environmental temperature. The frequent association of *S.* Enteritidis with eggs and this faster growing can be factors that contribute to the frequent involvement of SE86 with salmonellosis caused by the consumption of HM. Elias, et al. (2014) [22] reported that a safe HM can be prepared if non-contaminated eggs (or cooked yolks) are used and HM are stored below 7° C.

Cross-Contamination by Contaminated Equipment and Utensils

According to DVS/RS, cross-contamination was the third most important risk factor for salmonellosis in RS [8, 9, 10, 3]. Based on this, the biofilm formation capability of SE86 was investigated on different material surfaces. For example, Tondo, et al. (2010) [32] immersed **c**oupons of stainless steel and polyethylene in bacterial suspensions of SE86, *S.* Typhimurium, and *S.* Bredeney, during 15, 30, and 60 minutes. The three serovars showed

similar counts of adhered cell to both materials (5.0 to 6.5 log CFU. cm^{-2}), and the time of exposure did not influence the counts of adhered cells on both surfaces; however, Scanning Electron Microscopy revealed larger clusters of SE86 on both materials, which was not found for the other serovars [32].

Casarin, et al. (2014) [33] investigated the adhesion of *S.* Enteritidis SE86 and *Listeria monocytogenes* on stainless steels (types 304 and 316) after 0, 1, 2, 4, 6, and 8 h of contact time, at room temperature. The study also evaluated the influence of the material topography and the hydrophobicity of cells and material surfaces in the attachment process. Results demonstrated that both bacteria were able to adhere on both types of stainless steels. However, at the beginning of contact time between cells and surfaces, higher numbers of adhered cells of *S.* Enteritidis SE86 were observed. S. Enteritidis SE86 also demonstrated a higher negative total energy of adhesion than *L. monocytogenes*, meaning that the adhesion of SE86 was thermodynamically more favorable than the adhesion of *L. monocytogenes* to both types of stainless steels [33]. The hydrophobicity of the investigated surfaces was also determined based on contact angle measurements. According to the results, both bacteria were hydrophilic and stainless steel surfaces were hydrophobic. Despite of the different levels of hydrophobicity/hydrophilicity, there was no correlation between adhesion and the surface hydrophobicity [33].

In another study Casarin, et al. (2014) [34] investigated the adhesion of *S.* Enteritidis SE86 and *L. monocytogenes* on the surface of metal inert gas (MIG), and tungsten inert gas (TIG) welds. Results demonstrated that *S.* Enteritidis SE86 initially adhered significantly more than *L. monocytogenes* on both types of welds, and there was a significant difference ($P<0.05$) in the adhesion of SE86 when 0h and 1h of contact time were compared. According to the data obtained, bacteria were hydrophilic, while weld surfaces were hydrophobic [34].

Resistance to Differents Sanitizers

Machado, et al. (2010) [35] evaluated the resistance of *S.* Enteritidis SE86, *S.* Typhimurium, and *S.* Bredeney to sanitizers, using the suspension test recommended by the Brazilian Ministry of Agriculture. Three commonly used sanitizers in food industries were tested: peracetic acid, quaternary ammonium and sodium hypochlorite. The results demonstrated that SE86 was resistant to 400 ppm sodium hypochlorite and survived for up to 15 minutes of exposure to 200 ppm of this sanitizer, a fact which was not demonstrated by the other strains [35]. In other study, Tondo, et al. (2010) [32] tested the same microorganisms to biofilm formation capability on surfaces of material commonly used in food industries and food services. Coupons of stainless steel and polyethylene were immersed in cultures of SE86, *S.* Typhimurium, and *S.* Bredeney during 15, 30 and 60 minutes. After that, coupons were exposed to different concentrations of peracetic acid, sodium hypochlorite, and quaternary ammonium [32]. The sanitizers did not inactivate all the microorganisms adhered on both materials, being that least 1 log CFU. cm^{-2} of the microorganisms remained viable [32]. The study demonstrated that *S.* Typhimurium and SE86 were more resistant to quaternary ammonium than *S.* Bredeney on polyethylene surface, and the reduction of SE86 was smaller than the other serovars after sodium hypochlorite treatments [32].

The resistance to sodium hypochlorite demonstrated by SE86 was compared to the resistance of other pathogenic strains of *S.* Enteritidis isolated from Albania, Zimbabwe,

Morocco, and Pakistan [32, 35]. The strains were exposed to 200 ppm sodium hypochlorite for 5, 10, 15, and 20 minutes, according recommendations of the Brazilian Ministry of Agriculture. Results demonstrated that none of the *S.* Enteritidis were totally inactivated after 20 minutes of exposure and the reduction rates were similar. Only the strain isolated in Albania demonstrated to be significantly more sensitive [36].

The rule of *rpo*S and *dps* genes in the resistance to 200 ppm sodium hypochlorite was investigated in SE86. Mutants of SE86 were constructed using the method described by Uzzau, et al. (2001) [37]. The survival of the Wild Type (WT) strain, as well as of the attenuated strains, was determined by bacterial counts. Tagged proteins (Dps and RpoS) were detected by means of SDS-PAGE and also immunoblotting with anti-FLAG antibodies. SE86 lacking *dps* demonstrated greater sensitivity compared to the WT SE86 exposed to sodium hypochlorite. The RpoS and Dps proteins were actively expressed under the conditions investigated, suggesting that these SE86 genes are related to oxidative stress caused by sodium hypochlorite [36].

Acid and Thermal Resistance of *S*. Enteritidis SE86

Malheiros, et al. (2008) [38] cultivated SE86, *S.* Typhimurium, and *S.* Bredeney in Nutrient Broth (NB), as well as in Nutrient Broth supplemented with 1 % glucose (NBG). The latter was used in order to induce acid adaptation of cells exposed to sub-lethal pH generated by the degradation of glucose. The acid-adapted and non acid-adapted microorganisms were exposed to different pH (3.5, 4.0, and 4.5) and temperatures (52, 56, and 60 ºC), and survival curves were created [38]. Results showed that *S*. Bredeney demonstrated higher resistance to pH 3.5 and 4.0; nevertheless, SE86 demonstrated a better capacity for acid adaptation (7.5 times greater) than other *Salmonella* serovars. At pH 4.5, all serovars demonstrated a similar acid resistance, remaining at the same levels of viable cells for 300 minutes. At 52 ºC, acid adaptation was able to protect only SE86. At 56 ºC and 60 ºC, non-adapted and acid-adapted SE86 strains were more thermally resistant than other serovars tested, demonstrating that SE86 was more thermal resistant than other strains. SDS-PAGE analysis demonstrated differences in the protein profiles of non-adapted and acid-adapted cells of all serovars [38].

Perez, et al. (2012) [39] evaluated the survival and the capability of intestinal invasion of SE86 and *S.* Typhimurium (ST99), after acid adaptation. Both strains were cultivated in Nutrient Broth supplemented with 1 % glucose (NBG) and were exposed to simulated gastric fluid (FGS) pH 1.5. In a second step, approximately 8 log of both strains (either acid-adapted or non-adapted) were orally inoculated in germ-free adult male *Wistar* mice. Animals were observed at aseptic conditions for twelve days [39]. Animal feces and portions of the gastrointestinal tract were analyzed microbiologically, and the appearance of intestinal morphological abnormalities was investigated [39]. Animals were submitted to the mortality curve. The results indicated that acid-adapted SE86 had a significant higher survival rate ($p < 0.05$) than non-adapted SE86, non-adapted ST99 and also than acid-adapted ST99 after exposure to FGS. The *in vivo* experiments demonstrated that acid-adapted SE86 and acid-adapted ST99 were able to cause intestinal morphological abnormalities [39]. Acid-adapted SE86 showed higher counts in the ileum-cecal junction than the other strains, suggesting that acid adaptation influenced the virulence of this strain [39, 3]. All strains were able to rapidly

multiply in germ-free mice; however mortality caused by acid-adapted SE86 was more intense [39]. Histopathological analyses revealed greater severity of the infection caused by SE86, a fact which was confirmed by the death of animals starting at the fourth day of infection. ST99 did not cause the death of animals until twelve days after inoculation [39].

The ability of *Lactobacillus acidophilus* LA10 to colonize and exert antagonistic effects against SE86 in the gastrointestinal tract of conventional mice was analyzed [40]. Doses of 10^8 viable cells of SE86 and *L. acidophilus* LA10 were administered by gavage to mice. The experiment used 4 groups of mice. Group 1 was administered only sterile saline solution and was considered the negative control. Group 2 was administered only SE86. Group 3 was first administered SE86 and, after 10 days, treated with *L. acidophilus* LA10. Group 4 was first administered *L. acidophilus* LA10 and, after 10 days, challenged with SE86. The results demonstrated that a significant number of SE86 cells were able to colonize the gastrointestinal tract of mice, specifically in the colon and ileum. *L. acidophilus* LA10 demonstrated antagonistic effect against SE86, with better results observed for Group 3 over Group 4. Thus, *L. acidophilus* LA10 demonstrated potential antagonistic effects against *S.* Enteritidis SE86, especially if administered after infection [40].

Antimicrobial Resistance of *S.* Enteritidis SE86

Even though the salmonellosis caused by *S.* Enteritidis usually is limited to gastrointestinal tract and the treatment does not involve antibiotics, the use of these drugs is recommended when salmonellosis affects immunocompromised patients or when the symptoms of salmonellosis are more severe such as the presence of blood in stools and fever [11]. The concern is that if resistant strains cause food poisoning in these patients they can be difficult to treat, making the probability of human death more likely. In the last decades, the resistance of *Salmonella* sp. to antibiotics has increased rapidly throughout the world, mainly due to indiscriminate and incorrect use of antibiotics [41, 3]. In spite of *S.* Enteritidis strains present low antimicrobial resistance when compared with some isolates of *S.* Typhimurium [42], attention should be given to the frequent isolation of multidrug-resistant *S.* Enteritidis [43]. Examples of that were reported in Tondo and Ritter (2012) [3].

The antibiotic resistance of *S.* Enteritidis isolated from foods involved with salmonellosis in the State of RS State has been investigated by several studies. Most of the isolates demonstrated to be sensitive for the majority of the drugs evaluated, however resistance to streptomycin, gentamicin, and nalidixic acid were observed among microorganisms isolated in 1999 to 2000 and in 2001 to 2002. Few isolates exhibited multidrug resistance [3]. Oliveira, et al. (2012) [44] studied 80 *S.* Enteritidis isolated from salmonellosis in RS from 1999 to 2006, and observed that only three isolates (4 %) were fully susceptible to all the antibiotics tested. The major resistances were observed for ampicillin (81 %), streptomycin (19 %), and nalidixic acid (25%) [44, 3]. In the period of 2003 to 2006, Paula, et al. (2011) [45] evaluated 130 *S.* Enteritidis isolates responsible for salmonellosis occurred in RS and reported that the higher percentages of resistance were demonstrated for ampicillin (100 %) and nalidixic acid (48 %). When compared to previous studies [44, 46], results demonstrated that multi-resistance increased expressively, because 63 % of the isolates showed multi-resistance [45].

Among the 138 *S.* Enteritidis isolated from salmonellosis in the period from 2007 to 2012, the highest resistance percentages observed were for nitrofurantoin (94.2 %) and

nalidixic acid (89.1 %). Only two isolates were resistant to tetracycline, two to ampicillin, and two to cefoxitin. Three isolates were resistant to ceftazidime, ciprofloxacin, and trimethoprim/sulfamethoxazole, separately, while no isolate showed resistance to chloramphenicol, streptomycin, imipenem, and gentamicin [11]. Comparing the results of Geimba, et al. (2005) [46], Oliveira, et al. (2012) [44], De Paula, et al. (2011) [45], and Capalonga, et al. (2014) [11], it is possible to observe that the percentage of *S.* Enteritidis resistant to ampicillin and nalidixic acid is increasing over the years. For example, Geimba, et al. (2005) [46] observed that in 1999 12.8 % of the *S*. Enteritidis isolates were resistant to nalidixic acid, and in 2000 this percentage increased to 14.7 %. Oliveira, et al. (2012) [44] showed an increase in the resistance to the same antibiotic from 19.0 % to 24.3 %, between strains isolated from 2001 and 2002. De Paula, et al. (2011) [45] showed an increase from 40.7 % to 66.7 %, during the period of 2003 to 2006. Finally, Capalonga, et al. (2014) [11] demonstrated that, in 2007, 89.1 % of the isolates were resistant to nalidixic acid, increasing to 100 %, in 2012.

The raising resistance for nalidixic acid is issue of concern, because several studies have reported increasing numbers of *Salmonella* sp. resistant to quinolones in Germany [48], England and Wales [49], and Spain [50]. Emphasizing this concern, the CLSI added a guideline recommending clinical laboratories to routinely test for nalidixic acid resistance in extra intestinal *Salmonella* isolates in order to alert physicians about the emerging resistance [51]. Quinolones are widely used in food animal production and are able to select quinolone-resistant *Salmonella* sp. in animals. At the same time, quinolones are one of the few available therapies for serious *Salmonella* infections, particularly in adults.

CONCLUSION

S. Enteritidis SE86 has been involved with several food-borne salmonellosis occurred in the State of RS, during 1999 to 2013. The most important risk factors for salmonellosis caused by this strain were the consumption of homemade mayonnaise prepared with raw eggs, holding foods in room temperature for more than 2 hours; and cross-contamination due to the contact of foods with surfaces of contaminated equipment and utensils. SE86 was able to form biofilms on stainless steel AISI 316 and AISI 304, polyethylene, MIG and TIG weld surfaces, and survived to 400 and 200 ppm sodium hypochlorite. After being exposed to sub-lethal pH, SE86 became acid-adapted. The acid-adapted SE86 became more resistant to acid and thermal exposures and was more virulent to mice. In homemade mayonnaise, SE86 has grown faster than others *Salmonella* serovars (Bredeney and Typhimurium), during the first six hours at environmental temperature, however was not able to grow at 7° C. During the last decade, SE86 has shown increasing rates of resistance to ampicillin and nalidixic acid, but still sensitive to antibiotics like chloramphenicol, streptomycin, imipenem, and gentamicin.

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