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Initial Identification and Sensitivity to Antimicrobial Agents of *Salmonella* sp. Isolated from Poultry Products in the State of Ceara, Brazil

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ABSTRACT

The objective of this research was to isolate and to verify the sensitivity to antimicrobial agents of strains of *Salmonella sp.* isolated from poultry products in the state of Ceara, Brazil. A total number of 114 samples was collected from 63 broiler carcasses derived from two processing plants and two supermarkets, and 51 excreta samples were collected in broiler farms located in the state of Ceara, which used three live production stages. Each excreta sample consisted of a fresh excreta pool from 100 birds. Samples were submitted to microbiological analyses, and the isolated *Salmonella* strains were tested for antimicrobial sensitivity. No *Salmonella* was isolated from excreta samples, while broiler carcass samples showed a high contamination rate of11.8%. Three serotypes were identified: *Salmonella enterica* serovar Enteritidis, 50%; *Salmonella enterica* serovar Panama 33%, and *Salmonella enterica* serovar Newport, 17%. As to the susceptibility tests to antimicrobial agents, 100% of the isolated *Salmonella* strains showed resistance to Ampicillin and Tetracycline, and sensitivity to Gentamycin, Netilmycin, Carbenicillin, Chloramphenicol.

INTRODUCTION

Brazil is one of the largest broiler producers in the world. In order to maintain this annual productivity, rational management emphasizing the quality of poultry products is necessary. For this purpose, the government has implemented health programs aiming at controlling diseases that cause economic and health problems. General prophylactic procedures and biological security practices applied in every stage of broiler production (broiler grand-parent farms, broiler parent farms, commercial broiler chick farms, and hatcheries) decrease, but do not prevent the presence of bacteria. Among the pathogenic microorganisms in industrial poultry production, the genus *Salmonella* is of paramount importance.

In terms of public health, there are *Salmonella* strains that may cause paratyphoid infection, as well as *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella infantis,* and *Salmonella agona,* which are important sources of food-borne illnesses. These bacteria are not related to specific diseases, and are capable of infecting indistinctly several animal species, including humans (Lax *et al.*, 1995). The importance of *Salmonella* in the public health is very significant; for instance, it accounted for nearly 84% of food-borne human illnesses in Scotland from 1980 to 1989 (Oboegbulem *et al.*, 1993), and 81% in Italy from 1991 to 1994 (Scuderi *et al.*, 1996). According to the Centers for Disease Control and Prevention in United States, *Salmonella* is responsible for 1.34 million of cases of disease, 16.430 hospitalizations, and 582 deaths each year (Mead *et al.*, 1999). The total annual cost resulting from food-

borne *Salmonella* infections in United States is estimated in about US$3.5 billion (Pathogen, 1995).

The epidemiology of *Salmonella* infections in birds is very complex (Hinton, 1988), and it is difficult to determine how a flock was infected, or how the dissemination occurred in the flock. Hong'ombe *et al*. (1999) identified *S. enteritidis* in broiler carcasses ready for market in several different situations. Many authors identified poultry products as sources of infection of *Salmonella* that causes enteritis in humans (Dhillon *et al*., 2001). Approximately 10% of salmonellosis cases are caused by poultry meat, and in the United States, there are between 15 and 20 cases of salmonellosis for each 100,000 people (Bryan & Doyle, 1995). According to Skov *et al.* (2002), the number of diagnosed cases of salmonellosis in humans in Denmark increased during the last decade, and the serotypes *S. enteritidis* and *S. typhimurium* were the most commonly isolated strains. Major outbreaks of human salmonellosis were caused by the consumption of incorrectly manipulated foods in restaurants and institutional kitchens. Undercooked food, slow freezing, and keeping foods for many hours under no refrigeration are considered as contributing factors for the emergence of this disease in humans (Costa, 1996).

In spite of the programs of Brazilian Agriculture Ministry to control poultry pathogens, there are reports of outbreaks of salmonellosis, and later detection of the pathogen in broiler carcasses and foods containing eggs in Brazil. The main factors of this contamination are improper handling of these products in some companies, the epidemiologic complexity of *Salmonella,* and deficient inspection during production, processing, and trade of animal products. However, studies on the contamination of broilers with Salmonella are practically null in our region.

Due to the need to constantly monitor of the health quality of birds for human consumption, this study aimed at isolating and identifying *Salmonella* serotypes present during live production, and trading of chicken meat, as well as the behavior of *Salmonella* spp. strains relative to common antimicrobial drugs.

MATERIALS AND METHODS

Collection of excreta samples during live production

A total number of 63 excreta samples were collected from 21 broiler flocks in three management stages: starter (1 day of age), grower (20 days of age), and finisher (45 days of age), from broiler companies located in the state of Ceara. Each sample consisted of a fresh excreta pool from 100 birds, randomly collected in commercial broiler houses. The collected samples were placed in sterile plastic bags, and were submitted to the laboratory, following the criteria recommended by the National Poultry Health Program – PNSA (Brazil/MAARA, 2002).

Collection of samples from broilers carcasses at retail

Broiler carcasses were collected from sales points and processing plants, and were divided into the following categories: fresh carcasses (14 samples), collected in two processing plants with no federal sanitary inspection (7 carcasses in each plant); refrigerated carcasses (18 samples), ten in one supermarket and eight in another; and frozen carcasses (19 samples), ten samples collected in one supermarket, and nine from another.

Microbiological analyses

The excreta samples (live production phase) and the carcasses were randomly collected during typical management, slaughter, and sales days.

Feces samples weighing 25 g were diluted in 225 mL of buffered peptone water at 0.1%, and carcass samples were submitted to the "carcass wash" method, through the addition of 300 mL of buffered peptone water at 0.1%, as described by Cox *et al*. (1978), and then placed in Erlenmeyer flasks.

Bacterial culture followed the procedures established by National Poultry Health Program – PNSA (Brasil/MAARA, 2002) with some modifications. The protocol started with pre-enrichment: the solutions resulting from the process "carcass wash" were incubated at 37 ºC for 24 hours. Aliquots from pre-enrichment were inoculated into selective enrichment liquid media at a ratio of 1/100 in Rappaport-Vassiliadis broth and at1/10 in Selenite-Cysteine broth. A loopful of each broth was streaked on plates of Brilliant Green agar, MacConkey agar, and *Salmonella-Shigella* agar. The temperature and the period of incubation were standardized at 37 ºC for 24 hours, respectively, for both feces and carcass samples. Two or three suspected colonies of *Salmonella* from each plate were collected for presumptive identification by biochemistry tests. The media utilized for presumptive identification were: Triple Sugar Iron slant agar (TSI); Lysine-Iron slant agar (LIA); Sulfur Indol Motility agar (SIM), and the Citrate test. Tubes were incubated at 37 ºC for 24 hours. Colonies with biochemistry profile of *Salmonella*

were submitted to serologic tests by the use of polyvalent serum against O and H *Salmonella* antigens. The colonies that agglutinated during the period of one to two minutes were considered as positive for *Salmonella*, and were preserved in Nutrient agar. Isolates were submitted to Adolfo Lutz Institute in São Paulo, Brazil, for complete identification and serotyping.

Antimicrobial sensitivity test

The behavior of all isolated of *Salmonella* sp. strains was checked as to the action of antimicrobial drugs. Strains were submitted to sensitivity tests according to the BAUER-KIRBY method (Bauer *et al.*, 1966). Each strain was inoculated in BHI (brain and heart infusion) broth, and after 24 hours of incubation at 37 ºC they were streaked using sterile swabs on Mueller-Hinton agar plates. Plates were kept in environmental temperature for a period of five minutes, and then diffusion disks with antimicrobial drugs were distributed on the plates and incubated for 24 hours at 37 ºC. The antimicrobial drugs were: Ampicillin (10 mg); Amoxycillin (10 mg); Amikacin (30 mg); Norfloxacin (10 mg); Tetracycline (30 mg); Chloramphenicol (30 mg); Netilmycin (30 mg); Gentamycin (10 mg); Sufonamide (300 mg), and Carbenicillin (100 mg). Results were interpreted by measuring inhibition zones with the use of a millimeter scale rule. The results were presented as resistant or sensitive according to *National Committee for Clinical Laboratory Standards* (NCCLS, 2000).

Statistical Analysis

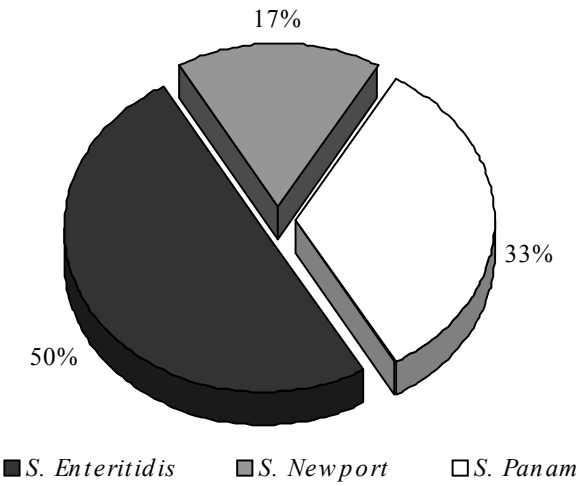
Companies and categories of carcasses were compared using the Chi-square (x^2) non-parametric test for qualitative analysis at a probability of 5% ($p< 0.05$).

RESULTS

The presence of *Salmonella spp.* was investigated in 63 excreta samples of 21 flocks in three different live production stages. No *Salmonella* isolated from any of the samples, thereby characterizing the analyzed flocks as free from *Salmonella* infection.

Salmonella was isolated in all categories of analyzed broiler carcasses, as shown in Table 1.

In fresh carcass samples, *Salmonella panama* (14.3%) was isolated. Refrigerated carcasses showed a high rate of contamination with *Salmonella enteritidis* and *Salmonella newport* (16.7%). Frozen carcasses showed the lowest contamination rate, with the isolation of *Salmonella enteritidis* (5.26%). *Salmonella* contamination among carcass samples varied; however, these differences were not statistically significant ($p<0.05\%$).



Graph 1 - Percentage of *Salmonella* strains isolated in broiler carcasses.

Among all the isolated strains, *Salmonella enteritidis* presented the highest rate of isolation (50%), followed by *Salmonella panama* (33.3%) and *Salmonella newport* (16.6%).

The results of antimicrobial sensibility tests are presented in Table 2. The behavior of the isolated strains from broiler carcasses relative to the action of antibiotics showed that all the isolated strains were resistant to Ampicillin and Tetracyclin. Four isolates (*Salmonella panama* -1 and *Salmonella enteritidis* -3) were resistant to Amoxycillin. Three isolates (*Salmonella panama* -1 and *Salmonella enteritidis* -2)

Table 1 – Isolation and characterization of *Salmonella* in broiler carcasses.

Carcasses	Isolation (%)	Samples (n[1])	Isolated strains (n[2])
Fresh	2 (14.3)	14	*S.* panama (2)
Refrigerated	3 (16.7)	18	*S.* newport (1); *S.* enteritidis (2)
Frozen	1 (5.26)	19	*S.* enteritidis (1)
Total	6 (11.8)	51	*S.* enteritidis (3); *S.* panama (2); *S.* newport (1);

n - number of samples. Not statistically different ($p<0.05\%$).

Table 2 - Antimicrobial sensitivity test of Salmonella strains isolated from broiler carcasses.

Salmonella	N. of samples	Resistance to antibiotics (µg)									
		AMP(10)	TET(30)	AMO(10)	AMI(30)	GEN(10)	NET(30)	SUF(300)	NOR(10)	CLO(30)	CAR(100)
Panama	2	2	2	1	-	-	-	1	1	-	-
Newport	1	1	1	-	-	-	-	-	-	-	-
Enteritidis	3	3	3	3	1	-	-	2	-	-	-
Total (%)	100,0	100,0	100,0	66,7	16,7	-	-	50,0	16,7	-	-

Legend: AMP – Ampicillin, TET – Tetracycline, AMO – Amoxycillin, AMI – Amikacin, GEN – Gentamycin, NET – Netilmycin, SUF – Sulfonamide, NOR – Norfloxacin, CLO – Chloramphenicol, CAR – Carbenicillin.

were resistant to Sulfonamide, while only one isolate of *Salmonella enteritidis* presented resistance to Amikacin, and one isolate of *Salmonella panama* presented resistance to Norfloxacin.

DISCUSSION

Salmonella is one of the most important sources of food-borne illness in humans, mainly due to the complexity of Salmonellosis epidemiology (Zancan *et al*. 2000). Broiler meat is a high risk product, according to Berchieri (2000), and production systems and processing facilitate the presence of *Salmonella* in the final product.

There are several methods to verify the contamination of *Salmonella* in samples (Read *et al*. 1994). The bacterial analysis of excreta is a sensitive test (Aho, 1992), and present better results as compared to the methods using cloacal swabs and antibody research in avian serum. Other advantages are that it does not cause stress, and is more representative of bird contamination (Giessen *et al*. 1991). The absence of *Salmonella* in the 63 excreta samples from broiler flocks produced in the state of Ceará implies that management and hygiene are good in these farms. These results are in agreement with Moreira (2002), who did not find *Salmonella* in day old broiler chicks reared by poultry companies in the same region. However, Gama (2001) found *Salmonella enteritidis* contamination in commercial layer chicks, and Zancan *et al.* (2000) verified 44.45% of *Salmonella* contamination in the transport boxes of commercial layer chicks.

On the other hand, all carcass categories (fresh, refrigerated, and frozen) presented some kind of contamination, as shown in Table 1.

Salmonella panama was isolated in 14.3% of the fresh carcasses. In refrigerated carcasses, there was a high level of contamination (16.7%) with *Salmonella enteritidis* and *Salmonella newport*. The lower rate of contamination occurred in frozen carcasses (5.26%) with the isolation of *Salmonella enteritidis*. The total rate of *Salmonella* contamination of broiler carcasses was 11.8%, which is considered high, The *Codex Alimentarius* established 0% contamination in 25 g of analyzed food, including poultry meat and eggs (Santos *et al*., 2000). This percentage is lower than that found by Santos *et al*. (2000), with 32% of contamination; by Machado & Bernardo (1990), with 57% of contamination; by Plummer *et al*. (1995), with 23%; and Arvanitidou *et al*. (1998), with 69%. Our results are consistent with the percentage verified by Sharma (1992), with 9.21% of contamination of broiler carcasses. However, our findings are higher than those reported by Verde *et al*. (2003), who found 3.6% of *Salmonella* contamination in broiler carcasses, and of the USDA, which reported that the national pathogen-reduction program reduced the incidence of *Salmonella* contamination in broiler carcasses to less than 10% in 2000 (Food, 2000).

The presence of *Salmonella panama* (14.3%) in fresh broiler carcasses and the high rate of *Salmonella enteritidis* and *Salmonella newport* in refrigerated broiler carcasses indicates that processing must be better controlled. Navarro (1995) asserts that the number of food-borne disease increased considerably in Central and South America, mainly due to *Salmonella enteritidis* (Santos *et al*., 2000). The importance of the workers in the processing line was studied by several authors, as the lack of hygiene favors the dissemination of contamination of broiler surfaces. Pether & Gilbert (1971) analyzed the level of dissemination of *Salmonella anatum* by the extremity of fingers from previously infected human volunteers. They verified that the isolation of *Salmonella* from the finger extremity of these people was directly proportional to the inoculated bacterial population; the results were 100% positive for 10^6 inoculated bacteria, and 30% for the interval of 10^3 to 10^4 inoculated bacteria.

The isolated strains in refrigerated carcasses were*Salmonella newport* in one case, and *Salmonella enteritidis* in the two other cases. This represents a significant contamination risk, as many authors report that *Salmonella enteritidis*, among the genus

Salmonella, is the main source of food-borne diseases (Banatvala *et al*., 1999; Ferris *et al*., 1999; Plummer *et al*., 1995; Rodrigues *et al*., 1990). According to Costa *et al*. (1996), refrigerated carcasses present high levels of contamination, whereas there are few reports of *Salmonella* in frozen carcasses. Santos *et al*. (2003) verified that, out of 272 isolates of *Salmonella*, 111 were found in frozen carcasses, 126 in food and biological human material involved with cases of food-borne diseases, and 35 in different poultry materials, with the phage type 4 as the most prevalent bacterium. Roberts (1982) observed 80% contamination in frozen carcasses. This result and our experiment indicate the possibility that broiler carcasses, including frozen carcasses, to disseminate *Salmonella* to humans; however, Foster & Mead (1976) assert that the carcass freezing decreases or impairs the survival of the *Enterobacteriaceae* family.

Among isolated and characterized strains (Graph 1), *Salmonella newport* presented the lowest isolation rate in broiler carcasses. According to Uyttendaele *et al*. (1998), *Salmonella newport* was the most prevalent *Salmonella* isolated in turkeys, and this serotype is not commonly isolated from chickens (Bokanyi *et al*., 1990; Ferris *et al*., 1999; Poppe *et al*., 1998; Read *et al*., 1994). *Salmonella enteritidis* was the most prevalent isolate in the present experiment (50%). This result is consistent with the findings of Santos *et al*. (2003), and Rodrigues *et al*. (1990). Kinde *et al*. (1997) found seven isolates of *Salmonella enteritidis* out of 683 isolated strains in waste water from processing plants located in different districts of California. Other contamination sources were described by other authors, such Cortinez *et al*. (1995), who isolated *Salmonella newport* and *Salmonella panama* from river water samples in Argentina, and Hofer *et al*. (2000), who isolated these serotypes in horse meat in the Northeast Region of Brazil.

Salmonella strains resistant to antibiotics are commonly found, and this may be due to the comprehensive use of antibiotics included in feeds as growth promoters. The results of the antimicrobial sensitivity tests carried out in the present experiment are alarming, as all isolates showed 100% resistance to Ampicillin and Tetracycline. These results agree with the findings of Cortinez *et al*. (1995), who found strains sensitive to Gentamicin and Chloramphenicol, and resistant to Tetracycline. Berchieri *et al*. (1983) verified 77% resistance to Tetracycline of Salmonella in poultry feed, and Antunes *et al*. (2003) found 36% of *Salmonella* strains resistant to Tetracycline in broiler carcasses. Bokanyi *et al*. (1990), Lee *et al*. (1993), and Nascimento *et al*. (1997) showed similar results, with 100% of sensitivity to Chloramphenicol in broiler carcasses. Lee *et al*. (1993) and Santos *et al*. (2000) found 100% of resistance to Ampicillin of Salmonella in broiler carcasses. Our results showed that 66.7% of isolates were resistant to Amoxycillin, while Antunes *et al*. (2003) found 19%, 19%, and 3% of resistance to Amoxycillin, Carbenicillin and Chloramphenicol, respectively. The low isolation rates of *Salmonella* strains resistant to sulfonamide is consistent with Arvanitidou *et al*. (1998), who found 19.35% of resistance to Ampicillin, and few strains were resistant to sulfafurazole in chicken carcasses.

According to our results, broilers evaluated during live production in farms located in the state of Ceará presented good microbiological quality in terms of *Salmonella* infection; however, there was a marked decrease of the microbiological quality of poultry products analyzed in this experiment. As to antimicrobial sensitivity tests, the results were alarming, because 100% of the isolates were resistant to Ampicillin and Tetracycline.

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