

## **SALMONELLA SPP. IN RAW BROILER PARTS: OCCURRENCE, ANTIMICROBIAL RESISTANCE PROFILE AND PHAGE TYPING OF THE *SALMONELLA* ENTERITIDIS ISOLATES**

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### **ABSTRACT**

The present study was carried out to evaluate the occurrence of *Salmonellae* in raw broiler parts and to determine the antimicrobial resistance profile of the isolated strains. Twenty-four (39.3%) broiler parts samples were positive for *Salmonella* and twenty-five *Salmonella* strains were isolated, since two different serovars were detected in one single positive sample. *Salmonella* Enteritidis was the most prevalent serovar. Among *Salmonella* Enteritidis isolates, 95.2% belonged to Phage Type 4 (PT4) (20/21) and 4.8% to PT7 (1/21). Twenty-two (88%) strains of *Salmonella* were resistant to at least one antimicrobial agent, generating eight different resistance patterns. The *S. Typhimurium* (n: 1) and *S. Hadar* (n: 3) isolates presented multiple resistance. Three *S. Enteritidis* isolates were susceptible to all antimicrobials tested, two were resistant only to tetracycline. The high prevalence of *Salmonella* in the broiler parts strengthens the importance of the use of good manufacturing practices (GMP), and HACCP. The results also emphasize the need for the responsible use of antimicrobials in animal production.

**Key words:** *Salmonella*, broiler parts, antimicrobial resistance, phage typing

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### **INTRODUCTION**

Food from animal origin are important elements within the human food supply chain, but sometimes they can be a source of food-borne pathogens, such as *Salmonella*, especially in the case of poultry products, which are recognized as frequent vehicles of transmission of that microorganism (29).

*Salmonella*, once introduced by live chickens or other means into the processing plant, will progress along the processing line (19), jeopardizing the product's final microbiological quality for human consumption. The occurrence of *Salmonella* in broiler chicken cuts, in studies undertaken in different countries, can vary for instance from 1.5% in Northern Ireland (26) to 51.1% in Belgium (31).

Antimicrobials have been used in poultry as growth promoters, and for prophylactic or therapeutic purposes.

However, their indiscriminate use is causing increasing resistance amongst *Salmonella* strains and other bacteria (4), which may be present in foods, and thus transmitted to humans through the food chain (1).

The present study was carried out to evaluate the occurrence of *Salmonella* in broiler chicken parts, to estimate the resistance profiles of the isolates and to determine the phage type the *Salmonella* Enteritidis isolates.

### **MATERIALS AND METHODS**

#### **Collection of Samples**

The study was carried out using 61 broiler chicken parts (wings, whole legs, boneless breasts and backs) collected in the period from September, 30 to December, 20, 1996, in a processing plant located in Southern Brazil.

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### Isolation and Identification Procedure

*Salmonellae* were isolated using the microbiological method recommended by the Brazilian Agriculture Ministry (5). Briefly, 25g of skin and muscle, collected from each broiler chicken part under aseptic conditions, were homogenized with 225mL of 1% Buffered Peptone Water (BPW) (Merck AG, Darmstadt, Germany), and incubated  $36\pm 1.0^{\circ}\text{C}$  for 16-20h (pre-enrichment step). One mL of the pre-enrichment broth was transferred into 9 mL of Tetrathionate broth (Merck), and 0.1 mL into 9.9 mL of Rappaport-Vassiliadis broth (Merck), and incubated at  $36\pm 1.0^{\circ}\text{C}$ , and  $41\pm 0.5^{\circ}\text{C}$  respectively (selective enrichment step). After 24h, the selective enrichment cultures were streaked onto XLT4 (Difco, Detroit, MI, USA) and Rambach® (Merck) agar plates and incubated for 18-24h at  $36\pm 1.0^{\circ}\text{C}$ . Typical colonies were identified by biochemical and serological tests.

Complete antigenic characterization and serovar identification was performed by the Enteric Pathogens Laboratory from the Oswaldo Cruz Institute Foundation, Rio de Janeiro (FIOcruz-RJ).

### Antimicrobial Resistance Test

The antimicrobial resistance test was performed using the disk diffusion method on Mueller-Hinton Agar, according to the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) (18). The antimicrobials were ampicillin 10 µg, ciprofloxacin 5 µg, chloramphenicol 30 µg, enrofloxacin 5 µg, gentamycin 10 µg, kanamycin 30 µg, nalidixic acid 30 µg, nitrofurantoin 300 µg, norfloxacin 10 µg, polymixin B 300 I.U., streptomycin 10 µg, and tetracycline 30 µg. *Escherichia coli* ATCC 25922 was used as a reference strain. An isolate was classified as multiple resistant when demonstrated resistance to two or more agents (10).

### Phage Typing

Twenty one *Salmonella* Enteritidis isolates were phage typed at the FIOcruz-RJ. The phage types were determined according to Ward *et al.* (32). Samples were inoculated in tubes with 1.5 mL of phage broth and then incubated, with agitation at  $37^{\circ}\text{C}$  for an average of two hours. Samples showing turbidity equivalent to the 0.5 level of the MacFarland scale were checked in a photocolormeter. The selected phage broths were poured on phage agar plates, homogenized, and the excess of fluid was removed with a sterile pipette. The plates were left drying up for 20 minutes. Finally, 10mL from each one of the 10 selected phage solutions were poured onto plates previously divided in ten quadrants, and incubated at  $37^{\circ}\text{C}$  for 24 hours.

## RESULTS AND DISCUSSION

Twenty four out of the 61 raw broiler parts samples (39.3%) were positive for *Salmonella*, which is in line with other papers on the incidence of *Salmonella* in frozen and refrigerated poultry

cuts that reported 41.8% (30) and 51.1% (31) in Belgium, 42.4% in the United States (7), but differ from the 1.5% found in Northern Ireland (26). Surveys performed in Brazil, using broiler chicken cuts, indicated the presence of *Salmonella* in 22.8% (11) and 35% of the samples (12) in São Paulo and 14.2% in Rio de Janeiro (23).

Factors such as the samples origin, the year in which the studies were undertaken, the number of samples and their condition (frozen or refrigerated), the sampling procedure, the flock level of contamination, the quality of sanitation applied in the processing plants, possible cross contamination between the products, and the testing methodology (6,31). must be taken into consideration when comparing the results obtained in the present study.

*Salmonella* Enteritidis was the most frequent serovar, representing 21 (84%) of the 25 *Salmonellae* identified from the 24 positive samples. The other isolates were three *Salmonella* Hadar (12%) and one *Salmonella* Typhimurim (4%). In one positive sample, two serovars were detected, *S. Enteritidis* and *S. Hadar*.

The number of *S. Enteritidis* isolated in this work confirms the existing preoccupation regarding this serovar being frequently involved in food poisoning outbreaks in humans. This was true in Italy, where the evidence of *S. Enteritidis* infection in humans increased from 2.4% to 57.1% between 1982 and 1992, and from 0.5% to 22.8% in foodstuffs (14). Similarly, 150 outbreaks of food poisoning caused by *Salmonella* Enteritidis were recorded in Argentina between 1986 and 1993 (8).

In Brazil, *S. Enteritidis* has been the most prevalent serovar detected in human infections (27), in chicken carcasses (24), in foodstuffs, in the environment, water, sewage, chiller water, animal feed, animal viscera and faeces (28) and also in poultry flocks (15).

The phage typing of *S. Enteritidis* isolates showed that 95.2% (20/21) belonged to phage type 4 (PT4) and 4.8% (1/21) to PT7. Phage type 4 has been shown to be the most common phage type in several countries.

Santos *et al.* (25) showed that 93.3% of *S. Enteritidis* isolated from broiler carcasses in the State of Rio Grande do Sul, Brazil, belonged to the PT4. In Spain, Domínguez *et al.* (13) found PT4 isolates in 58.8% of retail, chicken meat samples analysed, while Liebana *et al.* (16) showed that PT4 was the most common phage type in English poultry farms.

Regarding the antimicrobial resistance, the present results (Table 1) indicated that 88% of *Salmonella* isolates (22/25) were resistant to one or more antimicrobial agent, presenting eight different patterns of resistance (Table 2). Multiple resistance was not observed in five of the 21 *S. Enteritidis* isolates, while 3 were susceptible to all antimicrobials tested and 2 were resistant only to tetracycline. Multiple resistance was also found in the *S. Typhimurium*, and in all three *S. Hadar* isolates.

**Table 1.** Antimicrobial resistance in *Salmonella* strains isolated from raw broiler parts.

Serovars	Number of strains tested	Number of resistant strains (%)											
		AMP	CIP	CHL	ENR	GEN	KAN	NAL	NIT	NOR	PB	STR	TET
<i>S. Enteritidis</i>	21	0	0	0	2(9.5)	0	0	14(66.6)	8(38.1)	0	0	0	17(80.9)
<i>S. Hadar</i>	3	0	0	0	0	0	0	0	0	0	0	3(100)	3(100)
<i>S. Typhimurium</i>	1	0	0	0	0	0	0	1(100)	0	0	0	0	1(100)
Total	25	0	0	0	2(8.0)	0	0	15(60.0)	8(32.0)	0	0	3(12.0)	21(84.0)

AMP: ampicillin; CIP: ciprofloxacin; CHL: chloramphenicol; ENR: enrofloxacin; GEN: gentamicin; KAN: kanamycin; NAL: nalidixic acid; NIT: nitrofurantoin; NOR: norfloxacin; PB: polymyxin B; STR: streptomycin; TET: tetracycline.

**Table 2.** Distribution of antimicrobial resistance patterns in *Salmonella* strains.

Patterns	<i>S. Enteritidis</i>	<i>S. Hadar</i>	<i>S. Typhimurium</i>	Total
Susceptible	3	-	-	3
TET	2	-	-	2
NAL, TET	6	-	1	7
STR, TET	-	3	-	3
NIT, TET	2	-	-	2
NAL, NIT	1	-	-	1
ENR, NAL, TET	2	-	-	2
NAL, NIT, TET	5	-	-	5

AMP: ampicillin; CIP: ciprofloxacin; CHL: chloramphenicol; ENR: enrofloxacin; GEN: gentamicin; KAN: kanamycin; NAL: nalidixic acid; NIT: nitrofurantoin; NOR: norfloxacin; PB: polymyxin B; STR: streptomycin; TET: tetracycline.

Resistance to tetracycline was observed in 84% of the isolates, which is higher than that the 46.6% found in Dakar, Senegal (3), the 36% in Porto, Portugal (2) and also the 6.2% in Brazil (24). This elevated resistance may be explained by the possible diffusion of the *tet(A)* resistance gene, which was observed in Italy by Pezzella *et al.* (20) in an epidemiological study with *Salmonella* strains isolated from animals.

Resistance to nitrofurantoin (32%), and streptomycin (16%) was low when compared to the 95% found by Cardoso *et al.* (9) in *S. Enteritidis*, and the 100% found by Ribeiro *et al.* (22) in *S. Hadar*.

The *Salmonellae* presented resistance to nalidixic acid (60%) and enrofloxacin (8%). Other authors like Molbak *et al.* (17) have also observed an increase in quinolone resistance to *Salmonella*, which is a cause for concern, since this resistance is mediated by chromosomes (21). On the other hand, we have not found resistance to norfloxacin and ciprofloxacin, which is in accordance to Cardoso *et al.* (9).

The high frequency of *Salmonella* (39.3%) in broiler parts detected in the present study can be explained by the more extensive handling of the birds during the processing, fact that reinforces the paramount importance of implementing good manufacturing practices (GMP), and Hazard Analysis and Critical Control Points (HACCP) systems. The levels of antimicrobial resistance presented here highlight the need for responsible use of antimicrobial agents in food animals, and indicates the need for continuous surveillance.

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#### RESUMO

##### ***Salmonella* spp. em cortes de frango: ocorrência, resistência antimicrobiana e fagotipificação dos isolados de *Salmonella* Enteritidis**

Este trabalho foi conduzido para avaliar a ocorrência de *Salmonella* em cortes de frango e para determinar o perfil de resistência antimicrobiana das cepas isoladas. Vinte e quatro (39,3%) cortes de frango foram positivas para *Salmonella*, tendo sido isoladas vinte e cinco cepas de *Salmonella*, uma vez que em uma amostra isolaram-se dois sorovares. *Salmonella* Enteritidis foi o sorovar prevalente. Entre as *Salmonella* Enteritidis isoladas, 95,2% pertencem ao Fagotipo 4 (PT4) (20/21) e 4,8% ao PT7 (1/21). Vinte e duas (88%) cepas de *Salmonella* foram resistentes a pelo menos um agente antimicrobiano e oito diferentes padrões de resistência foram observados. *S. Typhimurium* (n:1) e *S. Hadar* (n: 3), apresentaram múltipla resistência. Três cepas de *S. Enteritidis* foram sensíveis a todos os antimicrobianos e duas resistentes somente a tetraciclina. A

elevada ocorrência de *Salmonella* nos cortes de frango utilizados no presente estudo reforça a importância das normas de boas práticas de fabricação, bem como dos controles de perigos e pontos críticos de controle. No tocante aos níveis de resistência antimicrobianos, os resultados enfatizam a necessidade do uso responsável dos mesmos na produção animal.

**Palavras chaves:** *Salmonella*, partes de frango, resistência antimicrobiana, fagotipagem.

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