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OCCURRENCE OF *SALMONELLA* SPP. IN BROILER CHICKEN CARCASSES AND THEIR SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

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ABSTRACT

The present study was carried out to evaluate the occurrence of *Salmonellae* in broiler chicken carcasses and to determine the antimicrobial resistance profile of the isolated strains. Twenty-five out of the 260 broiler chicken carcasses samples (9.6%) were positive for *Salmonella*. *S.* Enteritidis was the most frequent serovar. Nineteen *Salmonella* isolates were tested for antimicrobial resistance, and the results indicated that 94.7% were resistant to at least one antimicrobial agent. Resistance to streptomycin (73.7%), nitrofurantoin (52.3%), tetracycline (31.6%), and nalidixic acid (21%) were the prevalent amongst *Salmonella* strains tested.

Key words: *Salmonella*, broiler chicken carcasses, antimicrobial resistance.

The consumption of poultry meat has been increasing annually, bringing with it the concern about the cases of foodborne illness events caused by *Salmonella* (30), since poultry have been identified as a significant reservoir of *Salmonella* in the environment (29).

Regarding poultry health, there are three differents salmonellosis syndromes, two of them caused by no motile serovars and host-adapted to poultry, which are Pullorum Disease, caused by *Salmonella* Pullorum, and Fowl Typhoid, caused by *Salmonella* Gallinarum; the other syndrome is Paratyphoid Infections, caused by motile *Salmonella* serovars (29), which are also responsible for outbreaks of foodborne illness in humans.

The presence of *Salmonella* in the intestinal tract, on the skin and among the feathers of chickens, may cause carcasses contamination during slaughtering and processing (16) and is responsible by the introduction of *Salmonella* in the slaughterhouses, where will progress along the processing line, affecting the final microbiological quality and jeopardizing the consumers health(6).

The occurrence of *Salmonella* in chicken carcasses, in studies undertaken in different countries, can vary for instance from 13% in Poland (21) to 66% in Thailand (17). In Brazilian studies, they vary from 5.9% (20) to 42% (14).

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In veterinary medicine, antimicrobial agents are used therapeutically, metaphylatically, prophylactically, and as growth promoters (28). However, their indiscriminate use is causing increasing resistance amongst *Salmonella* strains and other bacteria (7).

The present study was carried out to evaluate the occurrence of *Salmonella* spp. in broiler chicken carcasses and to estimate the resistance profiles of the isolates.

Two hundred and sixty broiler chicken carcasses were purchased in five processing plant located in Northeastern Brazil from April to December 2004. They were collected in sterilized plastic bags and transported, under refrigeration, to the Laboratory of Food Microbiology of the "Laboratório Nacional Agropecuário – Ministério da Agricultura, Pecuária e Abastecimento", Recife, Pernambuco, Brazil.

Salmonella were isolated using the microbiological method recommended by the Brazilian Agriculture Ministry ("Ministério da Agricultura, Pecuária e Abastecimento") (5). Complete antigenic characterization and serovar identification were performed by the Enteric Pathogens Laboratory from Oswaldo Cruz Institute Foundation, Rio de Janeiro (FIOCRUZ-RJ).

Nineteen *Salmonella* strains were tested for antimicrobial resistance by the disk diffusion method on Mueller-Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI, formely NCCLS)(11). The antimicrobials agents were: ampicillin 10 μg, amoxicillin/clavulonic acid, cefotaxime, ciprofloxacin 5 μg, chloranphenicol 30 μg, enrofloxacin 5 μg, gentamicin 10 μg, kanamycin 30 μg, nalidixic acid 30 μg, nitrofurantoin 300 μg, norfloxacin 10 μg, streptomycin 10 μg, polymyxin B 300 I.U., tetracycline 30 μg and trimethoprim/sulfamethoxazole. *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).

Twenty-five out of the 260 broiler chicken carcasses samples (9.6%) were positive for *Salmonellae*. Others researches investigating the occurrence of *Salmonella* in different Brazilian States reported different levels in broiler chicken carcasses, 13% in Santa Catarina (19), 5.9% (20%), 32% (27), and 42% (14) in São Paulo, and 11.8% (23) in Ceará.

Surveys performed in different countries, using broiler chicken carcasses showed 13% in Poland (21), 29.3% in Belgian (31), 29.7% in UK (24), 35.8% in Spain (12), 62.5% in Senegal (3) and 66% in Thailand (17).

Twenty strains of *Salmonella* isolated in this study were identified at serovar level, from where it was identified 11 different *Salmonella* serovars. *S.* Enteritidis was the most frequent serovar (25%). The results obtained agree with the fact that *S.* Enteritidis was the predominant serovar associated with outbreaks in humans, as showed by the WHO *Salmonella* surveillance date for 1990-1995 (15), and with the fact that *S.* Enteritidis is the predominant in poultry carcasses and their products as showed in Belgium (31), in the United Kingdom (24), Spain (12) and Portugal (1).

In Brazil, *S.* Enteritidis has been the most prevalent serovar detected in human infections (13), in broiler chicken carcasses (27), in broiler chicken parts (26), and in poultry flocks (18).

The other isolates were *S.* Typhimurium (3 isolates), *S.* Saintpaul (3), *S.* Mbandaka (2), *S.* Corvallis (1), *S.* Heidelberg (1), *S.* Infantis (1), *S.* Munchen (1), *S.* Newport (1), *S.* Panama (1) and *Salmonella. enterica* subsp. *enterica* (O9,12) (1).

Antimicrobial resistance is a widely studied issue in all bacteria genera, mainly amongst zoonotic bacteria, like *Salmonellae* is.

Nineteen *Salmonella* isolates were tested for antimicrobial resistance, and the results (Table 1) indicated that 94.7% (n: 18) were resistant to at least one antimicrobial

agent. This data is higher than the 58.1% observed in Greece (2), 67.3% in the United States (8), 75% in Portugal (1), but lower than the 100% obtained in *Salmonellae* isolated from broiler chicken carcasses by Cardoso *et al.* (9) in the State of Rio Grande do Sul, Brazil.

Resistance to streptomycin (73.7%), were higher than the 39% obtained by Antunes *et al*., (1) in Portugal, however lower than the 100% obtained by Ribeiro *et al*., (25) in S. Hadar isolated from broiler chicken carcasses in in the State of Rio Grande do Sul, Brazil.

The results regarding the resistance to nitrofurantoin (52.6%) and to tetracycline (31.6%) are supported by Oliveira *et al*. (22), who showed that 52.8% of *S.* Enteritidis strains isolated from bird carcasses, food, humans and poultry-related samples were resistant to nitrofurantoin. As well, it is supported by Antunes *et al*., (1), who found 36% resistance to tetracycline in *Salmonella* isolated from parts and broiler chicken carcasses in Portugal.

Resistance to the nalidixic acid (21%), norfloxacin (5.2%), enrofloxacin (5.2%) and ciprofloxacin (5.2%) were showed by some the *Salmonella* strains.

It was also observed resistence to Kanamycin (15.8%), ampicillin (10.5%), amoxicillin/clavulonic acid (10.5%), chloramphenicol (5.2%), trimethoprim/sulfamethoxazole (5.2%). None of *Salmonella* isolated showed resistance to cefotaxime, gentamicin and polimyxin B.

Fourteen different patterns of resistance were observed (table 3), two of them should be a concern because they showed resistance to eight different antimicrobial agents: CIP, ENR, KAN, NAL, NIT, NOR STR, TET, found in *S.* Newport and AMP, AMC, CHL, KAN, NIT, STR,SXT, TET, found in one *S.* Mbandaka.

Multiple resistance was not observed in 7 out of 19 tested strains, and 6 were resistant only to one antimicrobial agent, nalidixic acid (n: 1), nitrofurantoin (n: 2) and streptomycin (n: 3). One isolate identified as *S.* Muenchen, was susceptible to all antimicrobial agents.

Different factors may contribute to the *Salmonella* entry to the processing plants and to reach processed carcasses. Therefore any successful *Salmonella* control plan will need to implement actions to control contamination and dissemination of the bacteria in poultry raising sites (layers, hatcheries and chicken farms) (4), and in the slaughterhouses with a systematic laborers training, good manufacture practices and HACCP. Regarding the resistance to antimicrobial agents, the data obtained emphasize the need of a judiciously use of these agents in animal production, based on susceptibility tests.

Table 1. Antimicrobial resistence in *Salmonella* strain isolated from broiler chicken carcasses

Samples tested	Number of strains tested	Number of the resistance to antimicrobial agent														
		Amp.	C	Kan	Enr	Nor	Tet	Nal	CTX	Nit	Est	Gen	Cip	SXT	Pol B	AmC
S. Enteritidis	5	-	-	1	-	-	1	-	-	5	3	-	-	-	-	-
S. Typhimurium	3	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-
S. Saintpaul	2	-	-	-	-	-	-	1	-	1	1	-	-	-	-	-
S. Mbandaka	2	1	1	1	-	-	1	-	-	-	2	-	-	1	-	1
S. Infantis	1	-	-	-	-	1	1	1	-	1	1	-	-	-	-	-
S. Muenchen	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
S. Newport	1	-	-	1	1	1	-	1	-	-	1	-	1	-	-	-
S. Heidelberg	1	1	-	1	-	-	1	-	-	1	1	-	-	-	-	1
S. Panama	1	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-
S. Corvallis	1	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-
Salmonella enterica subsp. *enterica* (O9,12)	1	-	-	-	-	-	1	-	-	1	1	-	-	-	-	-
Total	19	2	1	4	1	2	6	4	0	10	14	0	1	1	0	2

Table2. Distribution of antimicrobial resistence patterns in *Salmonella* strains

Patterns	*Salmonella*
Susceptible	*1*
Est.	3
Nal	1
Nit	2
Est, Nal	1
Est, Nit	3
Est, Tet	1
Nit, Tet	1
Est, Kan, Nit	1
Est, Nit, Tet	1
Amp, AmC, Est, Kan	1
Est, Nal, Nor, Tet	1
Amp, AmC, C, Est, Kan, Nit, SXT, Tet	1
Cip, Enr, Est, Kan, Nal, Nor, Nit, Tet	1

AMP: ampicillN; AmC: amoxixilna + ácido clavulônico, CHL: chloramphenicol; CIP: ciprofloxacin; ENR: enrofloxacin, STR: streptomycin; KAN: Kanamycin; NAL: nalidixic acid, NIT: nitrofurantoin; NOR: norfloxacin, SXT: sulfametoxazol + trimetoprim, TET: tetracycline

Note: The opinions in this article are those of the authors and do not necessarily reflect views and policies of the organization to which they are affiliated.

RESUMO

Ocorrência de *Salmonella* spP. em carcaças de frango e sua suscetibilidade a agentes antimicrobianos

O presente estudo teve como objetivo verificar a ocorrência de *Salmonellae* em amostras de carcaças de frango e a suscetibilidade dos isolados a agentes antimicrobianos. Das 260 carcaças analisadas, 25 (9,6%) foram positivas para *Salmonella*. *Salmonella* Enteritidis foi o sorovar predominante. Com relação à suscetibilidade a agentes antimicrobianos, 94,7% das cepas de *Salmonella* testadas, apresentaram resistência a um ou mais agentes antimicrobianos. Os perfis de resistência mais comumente observados entre os isolados foram a resistência à estreptomicina (73,7%), nitrofurantoína (52,3%), tetraciclina (31,6%) e ácido nalidíxico (21%).

Palavras-Chave: *Salmonella*, carcaças de frango, resistência antimicrobiana

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