

Original Research Article

<https://doi.org/10.20546/ijcmas.2022.1106.009>

Investigation of Antimicrobial Resistance of *Salmonella* Isolated from Foods, in Minas Gerais, Brazil, from 2001 to 2021

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ABSTRACT

Keywords

Salmonella,
resistance
antimicrobial,
foods, foodborne
and sanitary
inspection.

Article Info

Received:

06 May 2022

Accepted:

31 May 2022

Available Online:

10 June 2022

Salmonella has been considered the major cause of foodborne diseases in the world. In this study, we evaluated the phenotypic resistance antimicrobial of 133 isolates of *Salmonella*, collected from different types of foods, in Minas Gerais - Brazil, from 2001 to 2021. The antibiogram results showed that 82.7 % of *Salmonella* isolates were resistant to at least one of the 16 antimicrobials tested, with highlight to resistance tonalidixic acid (51.9 %), nitrofurantoin (46.6 %), streptomycin(13.5 %), ampicillin (13.5 %) and tetracycline (12.8%). There were differences in antimicrobial resistance among *Salmonella* serotypes. *S. Typhimurium* showed resistance to a greater number of therapeutic agents (15 of 16 antimicrobial tested) and *S. Enteritidis* a high rate of resistance to nitrofurantoin (89.5 %), nalidixic acid (77.0 %) and ciprofloxacin (54.1 %). Thirty isolates of *Salmonella* (22.5%) were classified as multidrug resistant due the resistance to at least one antimicrobial from three different classes. *Salmonella* isolated from animal products showed significant resistance ampicillin, chloramphenicol, gentamicin, streptomycin, sulfamethoxazole-trimethoprim and tetracycline, when compared to *Salmonella* collected from other foods. The results this study reinforced the need to implement an integrated surveillance system to monitor the antimicrobial resistance in *Salmonella* in the country.

Introduction

Salmonella has been considered the major cause of foodborne diseases and serious public health problem in the world due the spread of antimicrobial resistant strains (Ejo *et al.*, 2016). The development of resistance antimicrobial is a natural phenomenon and can occur in the bacterial evolutionary process. However, the overuse and the misuse of

antimicrobial drugs in humans and in animals have increased resistance antimicrobial selective pressure (Rodrigues *et al.*, 2020). The abusive use of antimicrobials as a prophylactic agent and improver of zoo technical indexes in herds has been widely implicated in the selection of bacterial resistance (Quesada *et al.*, 2016; Rau *et al.*, 2021). It is believed that by 2030, the global consumption of antimicrobials in livestock is expected to increase by

67%, and in Brazil, Russia, India, China and South Africa, this increase will be 99%, almost seven times more than human population growth (Boeckel *et al.*, 2015).

The increasing antimicrobial resistance in *Salmonella* isolated from food can limit therapeutic possibilities, increase morbidity rates and worsen hospitalizations (Li *et al.*, 2019; Mukherjee *et al.*, 2019). According to Fardsanei *et al.*, (2016), although most infections by *Salmonella* are characterized by a mild to moderate and self-limiting gastrointestinal disease, in risk group patients, the infection can be severe and the microorganism can spread from the intestinal site to other tissues, causing bacteremia, septicemia, endocarditis or meningitis. In this situation, the use of effective antimicrobials is essential for treatment, mainly against resistant pathogens. In this scenario, the slow progress in the development of new antimicrobials is worrying, since the 1960s, only four new classes of antimicrobials have been introduced, and none of them have had a major impact yet (Fischbach and Walsh, 2009).

According to O'Neill (2016), approximately 700 thousand people die annually worldwide from infections associated with antimicrobial resistant microorganisms and, by 2050, it is estimated that about 10 million deaths a year will occur as a result of this resistance. Given this reality, epidemiological and clinical large-scale systematic studies can help to monitor the resistance of the main human pathogens and to determine which therapeutic classes should be used in antimicrobial therapy for the treatment of salmonellosis and others diseases (Abatcha *et al.*, 2014). In this sense, *in vitro* antimicrobial susceptibility testing is an important tool to monitor resistance trends in microorganisms and to guide the most appropriate therapy (Wang *et al.*, 2019).

In this study, we evaluated the antimicrobial susceptibility profile of 133 isolates of *Salmonella*, collected from different types of foods by sanitary inspection and investigation of outbreaks of human

salmonellosis services, in Minas Gerais - Brazil, from 2001 to 2021, against 16 antimicrobials belonging to eight therapeutic classes.

Materials and Methods

Bacterial isolates

In the current study, 133 isolates of *Salmonella* (24 different serotypes) were analyzed. They were collected from different types of foods by sanitary inspection and investigation of outbreaks of human salmonellosis services, in Minas Gerais - Brazil, from 2001 to 2021. The bacteria belong to the microbial culture collection at the Central Laboratory of Public Health of Minas Gerais and were stored at -80 °C. Before antimicrobial susceptibility analyzes, the isolates were inoculated in brain heart infusion broth (Kasvi) and incubated in aerobiosis at 37°C for 18-24 hours.

After this period, each isolate was spread in xylose lysine deoxycholate agar (Oxoid) and hektoen enteric agar (Difco) and again incubated in aerobic conditions at 37°C for 18-24 hours. Then the bacterial were submitted to re-identification using the VITEK[®] II compact system (Biomérieux) and Matrix-Assisted Laser Desorption Ionization-time of flight *mass spectrometry* - MALDI-TOF MS (Bruker Datltonics) systems. The serotyping of isolates of *Salmonella* was conducted by the National Reference Laboratory for Enterobacteria, Foundation Oswaldo Cruz, Rio de Janeiro - Brazil.

Antimicrobial susceptibility

Antimicrobial susceptibility testing was carried out using the Kirby Bauer disk diffusion techniques, as described in Bauer *et al.*, (1966). Five colonies of each *Salmonella* isolated, previously cultivated on PCA agar (Difco) were inoculated in 3mL of 0.9% sodium chloride solution to obtain a concentration corresponding to 0.5 on the McFarland nephelometric scale. After this step, a sterile swab was immersed in the solution and its volume was dispensed over the entire muller hinton agar (Difco).

The antimicrobial disks were applied at a minimum distance of 24 mm from each other, and the plates were incubated at 37°C in aerobic conditions for 18-24 hours. The growth inhibition halos were measured with the aid of a calibrated caliper and the results classified as resistant, partially sensitive (intermediate) and sensitive, according to criteria described in the Clinical and Laboratory Standards Institute M100-ED 30:2020 (CLSI, 2020). All bacterial classified as intermediate resistant were considered non-susceptible (Borges *et al.*, 2019).

Those that presented resistance to three or more classes of antimicrobials were regarded as multidrug resistant (Borges *et al.*, 2019). The multiple antimicrobials resistance (MAR) index was calculated as previously described (Krumperman, 1983) using the following formula: a/b , where a represents the number of antimicrobials to which a particular isolate was resistant and b the total number of antibiotics tested. MAR values above 0.2 characterize multidrug resistant. *Escherichia coli* (ATCC 25922) and two repetitions of the tests was used for internal quality control of the experiment.

The antimicrobials (Oxoid) were included: nalidixic acid (NAL, 30 µg), ampicillin (AMP, 10 µg), azithromycin (AZI, 15 µg), aztreonam (ATM, 30 µg), ceftazidime (CAZ, 30 µg), chloramphenicol (CLO, 30 µg), ciprofloxacin (CIP, 30 µg), streptomycin (EST, 5 µg), fosfomicin (FOS, 200 µg), gentamicin (GEN, 10 µg), imipenem (IPM, 10 µg), nitrofurantoin (NIT, 300 µg), sulfamethoxazole-trimethoprim (SUT, 1.25 µg / 23.75 µg) and tetracycline (TET, 30 µg).

Statistical analysis

Fisher's tests were used to analyze the susceptibility of the isolates of *Salmonella* to the different antimicrobials according to its source of isolation (sanitary inspection or foodborne diseases) and type of food (products of animal origin or other foods). The software GraphPad Prism was used to Fisher's exact test.

Results and Discussion

The antibiogram results showed that 82.7% (110/133) of *Salmonella* isolates were resistant to at least one of the 16 antimicrobials tested. In this regard, *Salmonella* showed resistance mainly tonalidixic acid (51.9%), nitrofurantoin (46.6%), streptomycin (13.5%), ampicillin (13.5%) and tetracycline (12.8%) (Figure 1). Intermediate resistance was identified among ciprofloxacin (36.0%), nitrofurantoin (13.3%) and streptomycin (11.3%). Fosfomicin was the most efficient inhibitor of bacterial growth of *Salmonella*, with only 0.8% of the isolates resistant to this drug. No antimicrobial agent was efficient in inhibiting the growth of 100% of *Salmonella* tested.

There were important differences in antimicrobial resistance among *Salmonella* serotypes, as described in table 1. *S. Typhimurium* showed resistance to a greater number of therapeutic agents (15 of 16 antimicrobials tested) (Table 1). The serotype *S. Enteritidis* showed a high rate of resistance to ciprofloxacin (54.1%), nalidixic acid (77.0%) and nitrofurantoin (89.5%) and *S. Typhimurium* isolates to nalidixic acid (53.6%), nitrofurantoin (64.3%) and streptomycin (67.9%). Susceptibility against all antimicrobials tested was observed only among serotypes *S. abaeetuba*, *S. mbandaka*, *S. morehead*, *S. newport* and *S. ohio*. However, this result can be influenced by the reduced number of isolates among the serotypes (only one per serotype). Multiple antimicrobial resistance (MAR \geq 0.2), according index Krumperman, was observed among isolates of *S. typhimurium*, *S. anatum*, *S. schwarzengrund*, *S. corvallis*, *S. gaminara* and *S. Panama*.

Forty-one resistance profiles were observed in the 133 isolates of *Salmonella*, as showing in table 2. The CIP-NAL-NIT resistance profile was the most prevalent (21.9%) among *Salmonella* isolates, followed by the NAL-NIT (9.77%) and NIT (9.33%) profiles. A total of 80 (60.1 %) *Salmonella* isolates showed resistance profiles of one to three antimicrobials. Seventeen (12.7%) *Salmonella*, resistance profiles of four to six antimicrobials and

twelve (9.0%) isolates, resistance profiles ranging from six to nine antimicrobials. Thirty *Salmonella* isolates (22.5%) presented a phenotype of resistance to at least one drug from three different therapeutic classes, a circumstance that classifies these isolates as multidrug resistant.

A total of 68.3% (48/70) and 84.3% (59/70) *Salmonella*, originating from foods involved in food poisoning outbreaks, were more strongly associated ($p < 0.05$) with resistance to nalidixic acid and nitrofurantoin antimicrobials, respectively, when compared with bacteria originating from food from sanitary inspection (table 3). Regarding the food source of the bacteria, *Salmonella* isolated from animal products showed significant resistance to streptomycin (49.0%), ampicillin (32.7%), tetracycline (26.0%), chloramphenicol (20.4%), gentamicin (16.3%) and sulfamethoxazole-trimethoprim (10.2%) when compared to *Salmonella* collected from other foods. A combined analysis of these two variables (origin and food) showed that *Salmonella* coming from food collected by the sanitary inspection service and from products of animal origin are, notably, more susceptible to resistance to the antimicrobials ampicillin, chloramphenicol, streptomycin, gentamicin and tetracycline. For the other antimicrobials, there was no significant difference in the percentage of resistant *Salmonella*, regardless of the origin or type of food in which they were isolated.

In this study, we evaluated the antimicrobial susceptibility profile of 133 isolates of *Salmonella*, collected from different types of foods by sanitary inspection and investigation of outbreaks of human salmonellosis services, in Minas Gerais – Brazil, from 2001 to 2021. Unfortunately, the Brazilian health authorities do not have a continuous integrated system to monitor the antimicrobial resistance of the main pathogens of human and animal origin (Borges *et al.*, 2019). In addition, there is an accuracy lack resistance antimicrobial frequency in *Salmonella*, because many isolates are under-investigated for genetic and phenotypic resistance antimicrobial, situation makes difficult

the comprehension of the real level of *Salmonella* resistance antimicrobial in the country (Rodrigues *et al.*, 2020). Faced with this scenario, studies like this try to make up for the absence of official sources of information. According to World Health Organization, the surveillance data on the use of antimicrobials and resistance antimicrobial are valuable information to guide policies on their use from the local to the global level (WHO, 2012).

The analysis of the patterns of antimicrobial resistance showed 82.7% (110/133) of the isolates of *Salmonella* resistant to one or more antimicrobials. Significant prevalence of antimicrobial resistance in *Salmonella* isolated from foods also has been reported in other studies (Rowlands *et al.*, 2014; Almeida *et al.*, 2015) and in some international reports (CDC, 2019; EFSA 2021a; EFSA 2021b). The detection of high rates *Salmonella* resistant is worrying, because although most infections human by this pathogen are considered self-limiting, there are reports of prescriptions for antimicrobials many of patients affected by this disease (Souza *et al.*, 2010; Mukeherjee *et al.*, 2019). In addition to public health implications, the detection of *Salmonella* resistant to antimicrobials in food requires the attention, because these bacteria can disseminate, through the food chain, their pattern of resistance to other bacteria in several countries and worsen the international food trade (Borges *et al.*, 2019). In 2014, for example, the Antimicrobial Resistance Monitoring System (NARMS) identified an unusual multidrug resistant (MDR) strain of *S. infantis* in the USA, which spread rapidly through the food chain, representing 25% of human infections by *S. infantis* four years later (CDC, 2019). The significant prevalence of resistance to nitrofurantoin (57.9%) and nalidixic acid (54.2%) identified in the isolates of *Salmonella* was also observed in other reports. In the European continent, 48.8% of the isolates of *Salmonella* from chicken meat are resistant to nalidixic acid (EFSA 2021b). In Brazil, resistance rates to nitrofurantoin (59.2%) and nalidixic acid (34.2%) were also significant in isolates of *Salmonella* identified from human salmonellosis cases (Reis *et al.*, 2018).

Table.1 Frequency of antimicrobial resistance and multiple antimicrobials resistance (MAR) index in different serotypes *Salmonella* collected from different types of foods by sanitary inspection and investigation of outbreaks of human salmonellosis services, in Minas Gerais - Brazil, from 2001 to 2021.

Serotypes	Resistance (%)																	Average MAR
	TOTAL	AMP	ATM	AZI	CAZ	CFO	CLO	CIP	CPM	EST	FOS	GEN	IMP	NAL	NIT	SUT	TET	
<i>S. Enteritidis</i>	48	0	4,2	0	0	0	0	54.1	0	4.2	2.1	0	0	77	89.5	2.1	0	0.14
<i>S. Typhimurium</i>	28	25	3.6	3.6	14.3	14.3	14.3	32.1	3.6	67.9	0	21.4	7.1	53.6	64.3	10.7	21.4	0.22
<i>S. enterica *</i>	9	11.1	0	0	0	0	0	33.3	0	11.1	0	0	0	11.1	11.1	0	0	0.04
<i>S. Anatum</i>	5	100	0	0	0	0	60	20	0	40	0	40	0	40	20	0	60	0.24
<i>S. Heidelberg</i>	5	20	0	0	0	0	20	20	0	20	0	0	0	20	40	20	20	0.11
<i>S. Schwarzengrund</i>	5	40	20	20	0	0	40	60	20	40	0	0	0	80	20	0	60	0.31
<i>S. Corvallis</i>	3	0	33.3	33.3	0	0	0	100	33.3	0	0	0	0	100	66.6	0	0	0.23
<i>S. Infantis</i>	3	0	0	0	0	0	0	0	0	33.3	0	0	0	0	0	0	0	0.02
<i>S. Senftenberg</i>	3	33.3	0	0	0	0	0	33.3	0	100	0	0	33.3	33.3	33.3	33.3	33.3	0.21
<i>S. Abony</i>	2	0	0	0	0	0	0	50	0	50	0	50	0	100	50	0	50	0.08
<i>S. Agona</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	50	50	0	0	0.06
<i>S. Derby</i>	2	0	0	0	0	0	0	50	0	0	0	0	0	50	50	0	0	0.09
<i>S. Gaminara</i>	2	0	50	50	50	50	50	50	50	0	0	0	0	100	0	0	50	0.31
<i>S. Ndolo</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0.03
<i>S. Panama</i>	2	50	0	0	0	50	50	0	0	50	0	0	0	50	50	50	50	0.25
<i>S. Rissen</i>	2	0	50	0	0	0	0	0	50	0	0	0	0	0	50	0	0	0.09
<i>S. Santipaul</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0.03
<i>S. Abaetetuba</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
<i>S. Braenderup</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0.06
<i>S. Cerro</i>	1	0	0	0	0	0	0	100	0	100	0	0	0	0	0	0	0	0.13
<i>S. Hadar</i>	1	0	0	0	0	0	0	0	0	50	0	0	0	0	50	0	0	0.13
<i>S. Mbandaka</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
<i>S. Morehead</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
<i>S. Newport</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
<i>S. Ohio</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00

Subtitle: AMP: ampicillin; ATM: aztreonam; AZI: azithromycin; CAZ: ceftazidime; CFO: ceftioxin; CLO: chloramphenicol; CIP: ciprofloxacin; CPM: cefepime; EST: streptomycin; FOS: fosfomicin, GEN: gentamicin; IMP: imipenem, NAL: nalidixic acid; NIT: nitrofurantoin; SUT: sulfamethoxazole-trimethoprim; TET: tetracycline

Table.2 Frequency of the antimicrobial resistance profiles in *Salmonella* collected from different types of foods by sanitary inspection and investigation of outbreaks of human salmonellosis services, in Minas Gerais - Brazil, from 2001 to 2021.

Resistance profiles- Total (%)			
		<i>Multidrug resistant</i>	
Pansensíveis	23 (18.0)	CAZ-ATM-FOS	1 (0.75)
AMP	3 (2.25)	CAZ-NAL-NIT	1 (0.75)
CIP	2 (1.50)	EST-CIP-NIT	1 (0.75)
EST	5 (3.75)	EST-IPM-NIT	1 (0.75)
NAL	4 (3.00)	NAL-TET-NIT	1 (0.75)
NIT	11 (9.27)	NIT-ATM-CPM	1 (0.75)
CIP-NAL	3 (2.25)	CIP-NAL-NIT-ATM	1 (0.75)
CLO-AMP	1 (0.75)	CLO-EST-AMP-NIT	1 (0.75)
EST-NIT	4 (3.00)	EST-CIP-NAL-NIT	1 (0.72)
EST-CIP	2 (1.50)	EST-GEN-AMP-TET	1 (0.75)
EST-TET	1 (0.75)	CIP-NAL-ATM-CPM-AZI	1 (0.75)
IPM-NIT	1 (0.75)	EST-CFO-CAZ-NAL-NIT	2 (1.50)
NAL-NIT	13 (9.77)	EST-AMP-NAL-SUT-TET	1 (0.75)
NAL-TET	1 (0.75)	EST-GEN-CIP-NAL-NIT	1 (0.75)
CIP-NAL-NIT	29 (21.8)	SUT-NIT-CLO-EST-AMP	1 (0.75)
		CLO-AMP-CIP-NAL-TET-NIT	1 (0.75)
		CLO-EST-AMP-CFO-SUT-TET	1 (0.75)
		CLO-EST-AMP-CIP-NAL-TET	2 (1.50)
		CLO-EST-GEN-AMP-NAL-TET	2 (1.50)
		NAL-TET-NIT-ATM-CPM-AZI	1 (0.75)
		CLO-EST-AMP-CIP-NAL-TET-NIT	1 (0.75)
		EST-GEN-CFO-CAZ-IPM-NAL-NIT	1 (0.75)
		EST-GEN-AMP-CIP-NAL-SUT-TET-NIT	1 (0.75)
		CLO-CFO-CAZ-CIP-NAL-TET-ATM-CPM-AZI	1 (0.75)
		CLO-EST-GEN-AMP-CIP-NAL-SUT-TET-NIT	1 (0.75)
		EST-GEN-AMP-CIP-NAL-SUT-TET-NIT-AZI	1 (0.75)
		EST-GEN-CFO-CAZ-CIP-NAL-NIT-ATM-CPM	1 (0.75)

Subtitle: AMP: ampicillin; ATM: aztreonam; AZI: azithromycin; CAZ: ceftazidime; CFO: cefoxitin; CLO: chloramphenicol; CIP: ciprofloxacin; CPM: cefepime; EST: streptomycin; FOS: fosfomycin. GEN: gentamicin; IMP: imipenem. NAL: nalidixic acid; NIT: nitrofurantoin; SUT: sulfamethoxazole-trimethoprim; TET: tetracycline

Table.3 Frequency of antimicrobial resistance in *Salmonella* collected by sanitary inspection and investigation of outbreaks of human salmonellosis services from foods derivatives of products of animal and other types of foods, in Minas Gerais - Brazil, from 2001 to 2021.

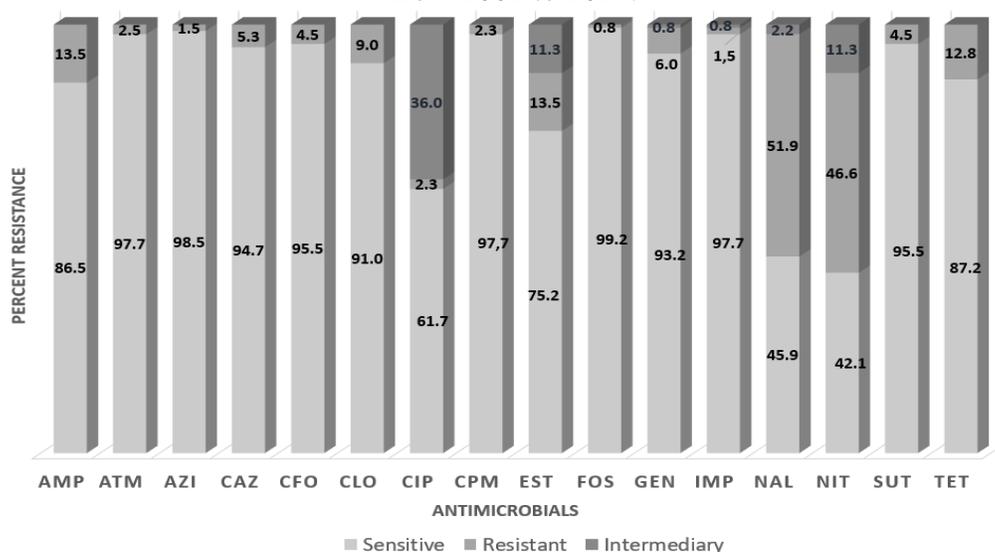
Drugs	Origin			Food		
	Foodborne disease – 70(%)	Sanitary inspection 63 (%)	<i>P</i> (Exato Fischer)	Products of animal origin- 49(%)	Others foods 84(%)	<i>P</i> (Exato Fischer)
APM	1 (1.4) ^a	17 (27.0) ^b	0.00	16 (32.7) ^a	2 (2.4) ^b	0.00
ATM	3 (4.3) ^a	4 (6.3) ^a	0.70	2 (4.1) ^a	4 (4.8) ^a	1.0
AZI	2 (2.9) ^a	2 (3.2) ^a	1.0	1 (2.0) ^a	3 (3.6) ^a	1.0
CAZ	4 (5.7) ^a	3 (4.8) ^a	1.0	3 (6.1) ^a	4 (4.8) ^a	0.70
CFO	3 (4.3) ^a	3 (4.8) ^a	1.0	3 (6.1) ^a	3 (3.6) ^a	0.66
CLO	1 (1.4) ^a	11 (17.5) ^b	0.00	10 (20.4) ^a	2 (2.4) ^b	0.00
CIP	28 (40) ^a	23 (36.5) ^a	0.72	21 (42.9) ^a	30 (35.7) ^a	0.46
CPM	3 (4.8) ^a	2 (3.2) ^a	1.00	1 (2.0) ^a	3 (3.6) ^a	1.0
EST	10 (14.3) ^a	23 (36.5) ^b	0.00	24 (49.0) ^a	9 (10.7) ^b	0.00
FOS	0 (0.0) ^a	1 (1.6) ^a	0.47	1 (2.0) ^a	0 (0.0) ^b	0.36
GEN	1 (1.4) ^a	8 (12.7) ^b	0.01	8 (16.3) ^a	2 (2.4) ^b	0.00
IPM	1 (1.4) ^a	2 (3.2) ^a	0.60	2 (4.1) ^a	1 (1.2) ^a	0.55
NAL	48 (68.6) ^a	24 (38.1) ^b	0.00	24 (49.0) ^a	48 (57.1) ^a	0.37
NIT	59 (84.3) ^a	18 (28.6) ^b	0.00	21 (42.9) ^a	41 (48.8) ^a	0.58
SUT	1 (1.4) ^a	5 (7.9) ^a	0.10	5 (10.2) ^a	0 (0.0) ^b	0.00
TET	4 (5.7) ^a	14 (22.2) ^b	0.02	13 (26.5) ^a	4 (4.8) ^b	0.00

Subtitle: AMP: ampicillin; ATM: aztreonam; AZI: azithromycin; CAZ: ceftazidime; CFO: cefoxitin; CLO: chloramphenicol; CIP: ciprofloxacin; CPM: cefepime; EST: streptomycin; FOS: fosfomicin, GEN: gentamicin; IMP: imipenem, NAL: nalidixic acid; NIT: nitrofurantoin; SUT: sulfamethoxazole-trimethoprim; TET: tetracycline

Products of animal origin: meats and meat products, cooked or fresh and different types of cheeses. Others food: meals, vegetables, spices, desserts, cakes, snacks, rice, beans, sauces and mayonnaise cream.

^{a,b} Values followed by different letters are distinct by the Fisher test ($P \leq 0.05$)

Fig.1 Frequency of antimicrobial susceptibility in *Salmonella* collected from different types of foods by sanitary inspection and investigation of outbreaks of human salmonellosis services, in Minas Gerais - Brazil, from 2001 to 2021.



Subtitle: AMP: ampicillin; ATM: aztreonam; AZI: azithromycin; CAZ: ceftazidime; CFO: cefoxitin; CLO: chloramphenicol; CIP: ciprofloxacin; CPM: cefepime; EST: streptomycin; FOS: fosfomicin; GEN: gentamicin; IMP: imipenem, NAL: nalidixic acid; NIT: nitrofurantoin; SUT: sulfamethoxazole-trimethoprim; TET: tetracycline

The resistance to these two antimicrobials has been reported as the main pattern in *S. enteritidis* isolated from human and food sources (Fardansey *et al.*, 2016). For some researchers, this high level of resistance to nalidixic acid represents a potential risk to public health, given its positive correlation with reduced susceptibility to ciprofloxacin, a drug of first choice in the treatment of human salmonellosis (Souza *et al.*, 2010; Kagambéga *et al.*, 2018). In this context, our results should be interpreted as a warning sign for health services in Brazil, as 36% of our *Salmonella* isolates showed intermediate resistance to ciprofloxacin. To mitigate the high prevalence of pathogens resistant to nitrofurans and quinolones, Brazilian health authorities have prohibited the use of these therapeutic classes in the animal production chain since 2003 (MAPA 2003; MAPA 2009). It is expected that, in the medium and long term, the ban on the use of nitrofurans in the animal production chain will contribute to a reduction in the selection and circulation of *Salmonella* resistant to these therapeutic classes.

In this study, we identified 41 resistance profiles, of which 27 (65.8%) were considered multidrug resistant (MDR). A total of 30 (22.2%) *Salmonella* isolates were characterized as MDR because of the resistance to at least one antimicrobial from three different therapeutic classes. Lower percentages of *Salmonella* MDR (3.4% to 9.7%) were identified in Australia, during the analysis of 54,451 strains, over 35 years (Williamson *et al.*, 2018). In the USA, the MDR of *Salmonella* has remained around 10% in the last 10 years (FDA, 2019; EFSA, 2021; EFSA). A similar percentage our data was observed by the Official European Food Safety Authority when demonstrating that 25.4% of 8,601 isolates of *Salmonella* food and human origin in Europe are MDR, mainly monophasic *S. typhimurium* (73.8%) and *S. typhimurium* (30.9%) serotypes (EFSA 2021a). In the same way, the serotype *S. typhimurium* was also highlighted among the MDR our isolates of *Salmonella*, representing 40% (12/30) of them. This identification of isolates of *Salmonella* MDR has attracted the attention of researchers and governments worldwide, due to the increase in

hospitalizations, deaths and the cost of treatment (Borges *et al.*, 2019). A recent study, for example, reported longer hospital stays, averaging 5.9 days, for patients infected with *Salmonella* resistant against 4.0 days, for patients with isolates pan sensitive (Mukherjee *et al.*, 2019).

The combined analysis of the data (origin and food in which *Salmonella* was isolated) showed that bacteria coming from food collected by the sanitary inspection service and in products of animal origin are, notably, more susceptible to resistance to some antimicrobials. Streptomycin was the antimicrobial with the highest rate (49.0%) of resistance by *Salmonella* isolated of products of *in natura* animal origin (mainly chicken meat).

This result is in line with a high rate of resistance to this antimicrobial (89.3%) in 250 isolates of *Salmonella* originated from chicken carcasses collected by the National Health Surveillance Agency in Brazil (ANVISA, 2012). Some researchers claim that significant levels of resistance to streptomycin in *Salmonella* isolates from food could be associated with its widespread use in animal production, especially poultry and pig farming (Almeida *et al.*, 2018; Reis *et al.*, 2018). Similarly, a high rate of antimicrobial resistance to ampicillin and tetracycline in *Salmonella* isolates from animal products in our study was also observed in extensive meta-analysis research carried out in several Latin American countries (Quesada *et al.*, 2016). This emphasis on antimicrobial resistance of bacteria isolated from animal products is associated with the extensive use of these drugs in livestock for therapeutic purposes and in some illegal situations, as growth promoters at subtherapeutic doses in the animal production (Guardabassi *et al.*, 2008; Korb *et al.*, 2011; Quesada *et al.*, 2016; Raul *et al.*, 2021). Approximately 50% of the antibiotics used in the world are destined for agriculture for the animal treatment, pest extermination and as growth promoters (Arias and Carrilho, 2012). To mitigate this spread of antimicrobial resistance by food chain of products of animal origin, the World Organization for Animal Health, through its 180

member countries, in addition to ratifying its support for the Global Action Plan of the World Health Organization to combat antimicrobial resistance, establishes norms and guidelines for the surveillance and responsible use of antimicrobials in animals destined for food production (OIE, 2016). In this context, it is important to acquire accurate information regarding the level of antimicrobial resistance as well as the data on use of antimicrobials in animal production centers, employing harmonized strategies for sampling, management of data and metadata, and analysis (Rodrigues *et al.*, 2020).

Salmonella collected from food in Minas Gerais - Brazil, from 2001 to 2021, were characterized by a high prevalence of antimicrobial resistance, mainly to nalidixic acid and nitrofurantoin. The presence of multidrug resistant *Salmonella* phenotypes should also be highlighted in this study, as well as a greater association of resistant *Salmonella* with foods of animal origin. These results reinforce the need to implement an integrated surveillance system, in accordance with several international guidelines, to monitor the antimicrobial resistance in *Salmonella* in the country.

Acknowledgement

To the Central Public Health Laboratory of Minas Gerais (Foundation Ezequiel Dias) for providing the biological material that is the subject of this study and the laboratory structure for carrying out the biological assays.

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How to cite this article:

Leandro Leão Faúla. 2022. Investigation of Antimicrobial Resistance of *Salmonella* Isolated from Foods, in Minas Gerais/Brazil, from 2001 to 2021. *Int.J.Curr.Microbiol.App.Sci.* 11(06): 74-84.
doi: <https://doi.org/10.20546/ijcmas.2022.1106.009>