

## Identification and antimicrobial resistance of *Salmonella enterica* isolated from live birds at commercial resellers

### Identificação e resistência a antimicrobianos de *Salmonella enterica* isoladas de aves vivas em revendas comerciais

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#### Abstract

The objective of this study was to investigate the sanitary and management characteristics of live-bird resellers as well as identify and undertake an antigenic characterization of *Salmonella enterica* and its sensitivity to antimicrobials. Structured questionnaires were applied and 627 samples were collected from the cages, consisting 209 samples of excreta, 209 of feed and 209 drinker swabs. These were processed by conventional bacteriology. The obtained isolates were subjected to the susceptibility test and to 12 antimicrobial tests by the disk diffusion method. Of the studied resellers, 91.7% house *Gallus gallus domesticus*, together with other animal species; sell birds with little zoosanitary documentation; have unsatisfactory active surveillance; and use and sell antimicrobials indiscriminately. The presence of *Salmonella enterica* was detected in 1.4% (9/627) of the samples analyzed in the cages, with 1.9% (4/209) found in excreta, 0.95% (2/209) in feed and in 1.4% (3/209) in drinker swabs. These were characterized antigenically as *Salmonella* Heidelberg, Gallinarum, Risen, Ndolo, Saint Paul, Mbandaka and subsp *enterica* O:6,7. When susceptibility to antimicrobials was determined, 44.4% resistance (4/9) was detected for trimethoprim-sulfamethoxazole, 33.3% (3/9) for enrofloxacin, 22.2% (2/9) for ciprofloxacin, ceftiofur and amoxicillin and 11.1% (1/9) for tetracycline and fosfomicin. *Salmonella* Heidelberg, as well as serovars Gallinarum, Risen, Saint Paul and Mbandaka, showed resistance to at least one of the tested antimicrobials. Trimethoprim-sulfamethoxazole and enrofloxacin were the antimicrobials that showed the least efficacy. Serovars such as Heidelberg, Gallinarum and Mbandaka have multiresistance to antimicrobials commonly used in human and veterinary medicine, implying potential risks to One Health.

**Keywords:** Chickens. Excreta. Feed. Salmonellosis. Water.

#### Resumo

Objetivou-se com este trabalho investigar as características sanitárias e de manejo das revendas de aves vivas, bem como a identificação e caracterização antigênica de *Salmonella enterica* e sua sensibilidade a antimicrobianos. Foram aplicados questionários estruturados, além de 627

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amostras coletadas nas gaiolas, sendo 209 de excretas, 209 de ração e 209 suabes de bebedouros. Essas amostras foram processadas por bacteriologia convencional. Os isolados obtidos foram submetidos ao teste de suscetibilidade e a 12 antimicrobianos pelo método de difusão em disco. Verificou-se que 91,7% das revendas estudadas alojam *Gallus gallus domesticus* juntamente a outras espécies animais, comercializam aves com escassa documentação zoonosológica, a vigilância ativa é insatisfatória e utilizam e comercializam antimicrobianos de forma indiscriminada. Detectou-se a presença da *Salmonella enterica* em 1,4% (9/627) das amostras analisadas nas gaiolas, sendo 1,9% (4/209) em excretas, 0,95% (2/209) em ração e em 1,4% (3/209) em suabes de bebedouros, as quais foram caracterizadas, antigenicamente, como *Salmonella* Heidelberg, Gallinarum, Risen, Ndolo, Saint Paul, Mbandaka e subesp. enterica O:6,7. Na determinação da suscetibilidade aos antimicrobianos, obteve-se resistência de 44,4% (4/9) para Trimetoprim-sulfametoxazol, 33,3% (3/9) para enrofloxacin, 22,2% (2/9) para ciprofloxacina, ceftiofur, amoxicilina e 11,1% (1/9) para tetraciclina e fosfomicina. *Salmonella* Heidelberg, além dos sorovares Gallinarum, Risen, Saint Paul e Mbandaka, todos mostraram resistência a pelo menos um dos antimicrobianos testados. Trimetoprim-sulfametoxazol e enrofloxacin foram os antimicrobianos que apresentaram menor eficácia. Sorovares como Heidelberg, Gallinarum e Mbandaka apresentam multirresistência aos antimicrobianos de uso comum em medicina humana e veterinária, o que implica em riscos potenciais para saúde única.

**Palavras-chave:** Água. Excretas. Galinhas. Ração. Salmonelose.

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## Introduction

The resale of live birds, a common practice throughout Brazil, takes place in commercial establishments where there is accommodation and movement of birds of different species, origins and destinations. In this environment, birds of the species *Gallus gallus domesticus* coexist with other animals such as parrots, anseriforms, reptiles, passerines and mammals, which are sold for small-scale production. These resellers are inspected on several parameters according to the legislation; however, they have shown to be insufficient in overcoming health issues effectively. This is especially true regarding the control of pathogens in resale houses, alternative farming systems and free-range systems <sup>(1)</sup>.

Even the National Poultry Health Program (*Programa Nacional de Sanidade Avícola*, PNSA), instituted in Brazil in 1994, which was structured upon legal and sanitary aspects of risk mitigation and traffic control in poultry establishments<sup>(1)</sup>, did not address actions concerning the resale of live poultry, leaving the states with this attribution. The PNSA instituted the monitoring of *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella pullorum* and *Salmonella gallinarum* in *Gallus gallus domesticus* for breeding and commercial broiler and laying establishments. However, the program did not include the control of these agents at resellers; in alternative farming systems; and/or in free-

range systems, shifting the responsibility to the states.

The protection of the poultry industry is important not only in terms of productivity, but also for health protection, since the industry in the state of Goiás is represented by eight hatcheries, 63 parent-stock breeders, seven grower-poultry breeders, two great-grandparent-stock breeders, one grandparent-stock breeder and 975 commercial broiler and laying-hen establishments<sup>(1)</sup>.

Birds, as well as different animal species, can harbor pathogenic bacteria, such as *Salmonella*, in the intestinal tract, constituting potential reservoirs of zoonotic diseases. In addition, evidence indicates that these birds can disseminate antimicrobial drug resistance genes<sup>(2)</sup>. Chemotherapeutics are relevant to poultry production, where they are used with therapeutic and prophylactic functions and as growth modulators<sup>(3)</sup>, besides possibly promoting animal welfare. However, they may have impacts on health, and their abusive use, according to the World Health Organization, can boost antimicrobial resistance, which reinforces the need to adopt measures to limit the spread of resistance<sup>(4)</sup>.

It is important to note that facilitated use of antimicrobials can imply potential risks for the selection of resistant bacteria, which may interfere with the control of important diseases that are common among birds and man<sup>(2)</sup>. Therefore, the present study was developed to investigate the sanitary and management characteristics of live-bird resellers in the metropolitan region of Goiânia, GO, Brazil, as well as identify the presence of *Salmonella enterica* by undertaking an antigenic and sensitivity characterization of the isolates to antimicrobial agents.

## Material and Methods

The project was approved by the Ethics Committee on Animal Use (Comissão de Ética no Uso de Animais, CEUA)/Federal University of Goiás (Universidade Federal de Goiás, UFG) (approval no. 107/17) and authorized by the owners of the resale establishments, who signed the Consent Term (Termo de Consentimento Livre e Esclarecido, TCLE). Analyses were performed at the Bacteriology Laboratory of the Department of Veterinary Medicine of the School of Veterinary and Animal Science (Escola de Veterinária e Zootecnia, EVZ) at UFG. The antigenic characterization of the isolates was carried out at the Oswaldo Cruz Laboratory (Laboratório Oswaldo Cruz, FIOCRUZ), in Rio de Janeiro.

For the collection of samples, 60 establishments were selected by convenience, which were distributed across 12 municipalities in the metropolitan region of Goiânia and which housed *Gallus gallus domesticus*. The number of studied resellers varied based on the number existing in each municipality. Resellers were visited and structured questionnaires made up of closed questions were answered by the heads of establishments to obtain information on the origin of birds, source and destination of the water used, destination of dead birds and waste, feed supplied, marketing and antimicrobial use.

Sample collections were performed according to the number of batteries in each

establishment. These were divided into five strata with collection of 100%, 80%, 60%, 40% and 20% of the batteries with *Gallus gallus domesticus*, in a total of 209 sampled batteries. Thirty grams of excreta from the trays, 30 g of feed from the feeders and three swabs from the drinkers constituted a sample, in a total of 627 samples.

After collection, the samples were homogenized, identified and transported in cooler boxes containing reusable ice to the laboratory for processing. The samples were then processed according to Brasil<sup>(5,6)</sup>, with modifications: 25 g of excreta and feed were weighed; then, all samples, including drinker swabs, received 225 mL of 1% peptone water and were homogenized and finally incubated at 37 °C for 24 h.

Next, the samples were homogenized and 1 mL of the solution was removed and transferred to 9 mL of selenite cystine broth and 0.1 mL to 10 mL of Rappaport Vassiliadis broth, which were incubated at 37 °C for 18 to 24 h. At the end of this period, aliquots were plated on Hektoen agar, XLT4 agar and brilliant green agar and incubated at 37 °C for 18 to 24 h.

Three and five colony-forming units (CFU) of each medium, per plate, with morphological characteristics of *Salmonella*, were grown on triple sugar iron (TSI) agar and incubated at 37 °C for 18 to 24 h. After this period, the tubes containing TSI suggestive of growth of *Salmonella* were subjected to the urease, indole and H<sub>2</sub>S production, methyl red, motility, lysine decarboxylase, malonate utilization and Simmons' citrate tests. When biochemical reactions compatible with *Salmonella* occurred, the samples were subjected to serological testing with polyvalent anti-O *Salmonella* serum and the samples with positive results were sent to the Osvaldo Cruz Institute (FIOCRUZ-RJ) on nutrient agar for typifying the isolated serovar.

The antimicrobial susceptibility profile was determined by the disc diffusion method, according to the Clinical and Laboratory Standards Institute - CLSI(7). The tested antibiotics were amoxicillin (10 mcg), ciprofloxacin (5 mcg), doxycycline (30 mcg), enrofloxacin (5 mcg), fosfomicin (200 mcg), florfenicol (30 mcg), gentamicin (10 mcg), neomycin (30 mcg), ceftiofur (30 mcg), tetracycline (30 mcg), trimethoprim/sulfamethoxazole (1.25/23.75 mcg) and chloramphenicol (30 mg). *Salmonella enterica* ATCC 14028 was used as the reference strain.

## Results and Discussion

The answers obtained in the structured questionnaire on the sanitary and management characteristics of live-bird resellers are described in Table 1. According to the results, the resellers sold several animal species, with 8.3% (5/60) of them housing only the species *Gallus gallus domesticus* and 91.7% (55/60) housing chickens and other species of birds and mammals. The existence of different species of animals at these resellers is worrying because of the possibility of them harboring pathogenic bacteria in the intestinal tract such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, among others, constituting potential reservoirs of zoonotic diseases of importance in human and veterinary medicine<sup>(2,8)</sup>.

**Table 1.** Sanitary and management characteristics of live-bird resellers investigated in the metropolitan region of Goiânia in the years 2017 to 2018.

<b>Sanitary and management aspects</b>	<b>Number of resellers</b>
<b><i>Number of resellers housing Gallus gallus domesticus</i></b>	
Without other species	05 (8.3%)
With parrots, quails, turkeys, mallards and mammals	55 (91.7%)
<b><i>Use of Animal Movement Permit (Guia de Trânsito Animal, GTA)</i></b>	
Yes	19 (31.7%)
No	41 (68.3%)
<b><i>Origin of water used at resellers</i></b>	
Sanitation company	52 (86.6%)
Artesian well	08 (13.4%)
<b><i>Destination of water used at resellers</i></b>	
Sewers	41 (68.3%)
Septic tank	19 (31.7%)
<b><i>Destination of dead birds</i></b>	
Landfill only	47 (78.3%)
Landfill and burial	13 (21.7%)
<b><i>Diet</i></b>	
Formulated feed only	56 (93.3%)
Feed and fresh vegetables	04 (6.7%)
<b><i>Use of antimicrobials</i></b>	
Yes	48 (80.0%)
No	12 (20.0%)

Source: developed by the authors (2020).

According to the questionnaire, only 31.7% (19/60) of the resellers had documentation in accordance with the legislation<sup>(9)</sup>, whereas 68.3% (41/60) did not possess zoosanitary documents, revealing a lack of control of entry and exit of birds. The data included in the Animal Movement Permit (*Guia de Trânsito Animal, GTA*) (e.g. origin, destination, vaccinations and laboratory tests) allow traceability and health control; thus, the absence of these documents leads to deficiency of information to the official protection service about the housed birds.

Regarding the origin of the water used for the animals to drink and for sanitation, 86.6% (52/60) of the resellers were serviced by public sanitation companies and 13.4% (8/60) used water from artesian wells. Most of the water used, 68.3% (41/60), had an ecologically correct destination; however, 31.7% (19/60) of the resellers used a septic tank, which allows the introduction of toxic substances and pathogenic microorganisms that can easily reach the aquifer by percolation through the vadose zone and be extracted through the wells<sup>(10)</sup>.

As for the destination of waste and dead birds, 78.3% (47/60) were directed to landfills and 21.7% (13/60) were deposited in landfills and buried. Landfills, the places where solid waste is deposited directly on the soil, increase the risk of contamination of the air, the surface and underground water, in addition to favoring the maintenance and proliferation of disease vectors and synanthropic animals and attracting people and animals in the condition of social vulnerability<sup>(11)</sup>. Thus, the deposit of carcasses in landfills poses a risk of infection for individuals who live close by and, mainly, for free-living animals. Nevertheless, the percentage extracted from the information given in the questionnaire can be considered satisfactory, considering that the Health Defense Service of the state of Goiás receives complaints about poultry establishments that deposit waste on public roads.

Most resellers (48/60) used antimicrobials in the housed birds, mainly in chicks immediately after reception. The most used antimicrobials included oxytetracycline (42/60), trimethoprim-sulfamethoxazole (38/60) and enrofloxacin (14/60). This indiscriminate use of antimicrobials in housed animals, which are exposed and sold to small farms, implies potential risks for the selection of resistant bacteria and for the control of important diseases common to birds and humans. Because the molecules of these drugs are not fully metabolized in the animal organism, residues are the release into the environment<sup>(12)</sup> and genes are transferred horizontally to other microorganisms. As a consequence, microorganisms have their resistance to antimicrobial agents increased and toxicological problems are caused to living organisms<sup>(3)</sup>.

Table 2 shows the results of the *Salmonella* investigation by sample category, reseller municipality, hatchery and state of origin of the birds.

**Table 2.** *Salmonella* serovars identified in samples obtained from resellers, from different origins, located in three municipalities of Goiás

*Municipality of resale	**Hatchery/State	Serovar identified
<b>Excreta</b>		
1	C/SP	<i>Salmonella enterica</i> subsp <i>enterica</i> O:6,7
2	A/GO	<i>Salmonella enterica</i> subsp <i>enterica</i> O:6,7
2	Not identified/GO	<i>Salmonella enterica</i> serovar Mbandaka
3	B/DF	<i>Salmonella enterica</i> serovar Gallinarum
<b>Drinker swabs</b>		
1	C/SP	<i>Salmonella enterica</i> subsp <i>enterica</i> O:6,7
2	A/GO	<i>Salmonella enterica</i> serovar Heidelberg
1	C/SP	<i>Salmonella enterica</i> serovar Risen
<b>Feed</b>		
1	C/SP	<i>Salmonella enterica</i> serovar Saint Paul
2	A/GO	<i>Salmonella enterica</i> serovar Ndolo

Legend: \*Municipalities: 1, 2, 3. \*\*A/GO, B/DF and C/SP: different resellers from hatcheries in the states of Goiás, Distrito Federal and São Paulo, respectively.

Source: developed by the authors (2020).

The presence of *Salmonella enterica* was detected in 1.4% (9/627) of the 627 analyzed samples, which came from four resellers located in three different municipalities, originating from hatcheries in the states of Goiás, São Paulo and the Federal District. These data are worrying, considering that the bacterium has a wide variety of hosts and affects most animal species, including mammals, birds, cold-blooded animals and man, which, in the epidemiological chain, can become asymptomatic or sick and disseminators of the bacterium.

Despite the potential of resellers in maintaining and spreading the bacteria, it should be noted that studies related to the frequency of *Salmonella enterica* in birds housed in these places are scarce, in the consulted literature. In the state of Santa Catarina, day-old chicks from farming houses sold for non-industrial farming were necropsied and *Salmonella typhimurium* was isolated from a pool of three livers (2.32%; 3/129). The authors revealed a risk of transmission to humans as well as to other production systems<sup>(13)</sup>. On the other hand, studies carried out on broiler chickens in the state of Paraná pointed to a greater circulation of the bacteria, with 11.4% positive drag-swab samples reported<sup>(14)</sup>. Although obtained under different conditions, these results, among others, demonstrate the importance of the pathogen in *Gallus gallus domesticus* and its potential risk of transmission and spread to other species, as pointed out in this study.

When the sample categories were analyzed, it was found that 1.9% (4/209) of excreta, 1.4% (3/209) of drinker swabs and 0.95% (2/209) of feed samples were positive for the pathogen, which were characterized antigenically as serovars Heidelberg, Gallinarum, Risen, Ndolo, Saint Paul and Mbandaka subsp. *enterica* O:6,7. It is noteworthy that the resellers with positive samples evaluated in this study received birds from hatcheries certified by MAPA. In this respect, it is important to stress that the protection service of the state of Goiás received, in the period from 2018 to June 2019, the notification of 416 outbreaks of *Salmonella*, 396 of which were of atypical salmonellae, seven of *Salmonella Pullorum* and two of *Salmonella gallinarum*, totaling 27,111,306 birds within the foci<sup>(1)</sup>.

The presence of several serovars in the processed samples may have originated from the received birds or even by contamination of the water in the drinkers, and the feed. The availability of feed at resellers can attract flies, which can propagate the bacterium in the environment. This hypothesis was supported by Ugboogu et al.<sup>(15)</sup>, who identified 61.7% (21/34) of flies contaminated with *Salmonella* and thus considered to be the main mechanical vectors of salmonellosis in poultry farms<sup>(16)</sup>.

According to the World Organization for Animal Health (OIE), in the last ten years, the Brazilian poultry sector faced sporadic outbreaks caused by the Gallinarum and Pullorum biovars, mainly in the states located in the south and center-west regions. Fowl typhoid is a serious disease of systemic nature that infects only avian species—chickens, mainly. It is commonly described in adult birds, with vertical and horizontal transmission and low fecal excretion. However, in this study, the bacterium was identified in excreta. Although the fecal excretion of this pathogen is low, this finding suggests the presence of carrier birds housed at the time of collection or the survival of the bacteria in the excreta trays by biofilm production. Biofilms can be considered a survival strategy for

microorganisms in unfavorable environments. Additionally, they can be chronic sources of contamination and increase resistance to environmental factors<sup>(17,18)</sup>.

Another piece of information that reinforces the presence of biofilms was that this reseller had previously reported mortality of backyard chicks. However, at the time of collection, the establishment housed 182 chicks, which were apparently healthy and from a certified hatchery. It is considered that unsatisfactory hygiene, without removal of organic matter adhered to the trays, did not eliminate the bacteria and allowed the formation of biofilms. Gama et al.<sup>(19)</sup> stated that organic matter present in these environments protects and provides nutrients for bacterial multiplication and allows the persistence of *Salmonella* in environmental sources such as fluff, feed and water.

In addition to *Salmonella gallinarum*, a serovar adapted to the host, other non-adapted serovars were identified (Table 2) in the analyzed samples—*Salmonella* Heidelberg, mainly, in the drinker swabs. According to Burt et al.<sup>(20)</sup>, this bacterium stands out as one of the most pathogenic serovars for man, causing serious complications such as septicemia, myocarditis and death. Its identification in water samples can be explained by the presence of nutrients; by contamination of the drinkers by rodents or mechanical vectors, such as flies; or by the formation of biofilms, as already mentioned. The hypothesis that the contamination originates from the water must be taken into account, because although the water offered to the animals originates from public supply, the cleaning and disinfection of both the cages and the resale environments has likely not been performed satisfactorily. In addition, personal protective equipment (PPE) was not used by the employees and, in many resellers, there was an accumulation of organic matter in cages, feeders and drinkers. The handling of birds by persons without PPE can also be considered a potential risk of *Salmonella* persistence, as reported in the study of Namata et al.<sup>(21)</sup>.

Serovars Saint Paul and Ndolo (Table 2) were isolated from feed samples and can be a source of infection for animals and man<sup>(22)</sup>. The availability of feed in a resale environment potentially allows the presence of reservoirs of this bacterium, which play an important role in its transmission and maintenance<sup>(23)</sup>. Contaminated feeds, especially those containing animal ingredients, are identified as a source of infection for poultry farms<sup>(24)</sup>. Additionally, rats, for instance, as common commensals in urban areas, are attracted to places with easy access to food such as feeders or feed storage places. Moreover, they can contaminate the feed supplied to animals through their feces<sup>(25,26)</sup>.

Regarding the susceptibility to antimicrobials in the samples of *Salmonella enterica* isolates, different levels of resistance to the tested active ingredients were observed, as shown below in Table 3. In decreasing order, 44.4% (4/9) were detected for trimethoprim sulfamethoxazole, 33.3% (3/9) for enrofloxacin, 22.2% (2/9) for ciprofloxacin, ceftiofur and amoxicillin an 11.1% (1/9) for tetracycline, fosfomicin, doxycycline, gentamicin, neomycin, chloramphenicol and florfenicol. Heidelberg, Gallinarum and Mbandaka were the only serovars to show resistance to a greater number of the tested antimicrobials.

**Table 3.** Susceptibility profile of *Salmonella* serovars isolated from environmental samples from live-bird resellers in the metropolitan region of Goiânia in the years 2017 and 2018.

<i>Salmonella enterica</i> serovar	Antimicrobial												
	Amo	Cip	Enr	Neo	Gen	Fos	Sut	Dox	Tet	Clo	Flor	Cep	RP*
Heidelberg	-	R	R	-	-	-	-	-	R	-	-	R	4
Gallinarum	-	R	R	-	-	R	R	-	-	-	-	-	4
Risen	R	-	-	-	-	-	R	-	I	-	-	-	2
Serovar 0:6,7	-	-	-	-	-	-	R	-	-	-	-	-	1
Serovar 0:6,7	-	-	-	-	-	-	-	-	-	-	-	-	-
Ndolo	-	-	-	-	-	-	-	-	-	-	-	-	-
Saint Paul	-	-	-	-	-	-	-	-	-	-	-	R	1
Mbandaka	R	-	R	-	-	-	R	-	R	-	-	-	4
Serovar 0:6,7	-	-	-	-	-	-	-	-	-	-	-	-	-
ATCC 4028	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Total</b>	<b>2</b>	<b>2</b>	<b>3</b>	<b>-</b>	<b>-</b>	<b>1</b>	<b>4</b>	<b>-</b>	<b>2</b>	<b>-</b>	<b>-</b>	<b>2</b>	<b>-</b>

Legend: R (resistant); I (intermediate); - (sensitive).

RP\*(Resistance Pattern): 1= single; 2= double; 4= multiple.

Antibiotics: Amo (amoxicillin), Cip (ciprofloxacin), Enr (enrofloxacin), Neo (neomycin), Gen (gentamicin), Fos (fosfomicin), Sut (trimethoprim-sulfamethoxazole), Dox (doxycycline), Tet (tetracycline), Clo (chloramphenicol), Flor (florfenicol), Cef (ceftiofur).

Source: developed by the authors (2020).

Serovars Heidelberg, Gallinarum and Mbandaka were identified as multidrug-resistant (MDR) phenotypes, which resisted at least four tested antimicrobials. This could reduce the therapeutic options for the treatment of infections in both men and animals and, at the same time, compose intestinal microbiotas.

The presence of these isolates with multidrug resistance at resellers should be analyzed with concern, considering that, in these places, owners, employees and consumers often handle animals and equipment without the necessary protections. This potentially allows the exposure and the spread of bacteria, which can settle in the intestinal microbiota of both man and animals. It is noteworthy that the agent can still survive in different environments and pose a risk of resistance transfer and spread to commensal bacteria. These risks were pointed out by Chantziaras et al.<sup>(27)</sup>, who correlated antimicrobial resistance in food-producing animals, such as birds, with humans; and by Silva et al.<sup>(2)</sup>, who showed evidence that the intestinal microbiota of birds can harbor zoonotic bacteria—pathogenic or not—that can be reservoirs of genes

that confer resistance to antimicrobials, thus playing an important role in the spread of bacterial resistance among different animal species.

As for *Salmonella* Heidelberg, resistance to third-generation quinolones (enrofloxacin and ciprofloxacin) and cephalosporin (ceftiofur) and tetracyclines was observed. The occurrence of *Salmonella* Heidelberg multiresistant to antimicrobials, present at resellers where people circulate and animals are sold for small farms, increases and represents a danger to public health. This multidrug resistance scenario limits therapeutic treatment, since these are drugs used in human and animal salmonellosis; moreover, *Salmonella* Heidelberg is one of the serovars that can induce systemic complications in people, especially children, the elderly or immunocompromised people<sup>(19,28,2)</sup>. Furthermore, it should be noted that there is a potential risk for other animals, besides chickens, and the environment, since the bacteria can be spread by free-living animals such as pigeons, which are common in these resellers.

This scenario is similar to that demonstrated in a study that investigated the frequency of *Salmonella enterica* in frozen chicken carcasses—in the five geographic regions of Brazil—in which detection rates ranging between 11.9% and 23.5% were reported<sup>(13)</sup>. According to these authors, for *Salmonella* Heidelberg, the frequency was 6.4% (16/250). All *Salmonella* isolates tested were resistant to one or more antibiotics, and 133 (53.2%) were characterized as multidrug-resistant. In addition, *Salmonella* Heidelberg was also resistant to ceftriaxone (75.0%, 188/250) and ceftiofur (43.8%, 110/250)<sup>(13)</sup>.

Serovar Gallinarum showed multiple resistance to ciprofloxacin, enrofloxacin, trimethoprim, sulfamethoxazole and fosfomycin, agreeing with the results described by Penha Filho et al.<sup>(30)</sup>, who evaluated the susceptibility profile of *Salmonella* Gallinarum and *Salmonella* Pullorum, isolated from 1987 to 1991 and from 2006 to 2013 in Brazil. Ciprofloxacin, enrofloxacin, trimethoprim and sulfamethoxazole, antimicrobial drugs identified in this study as ineffective against *Salmonella* Gallinarum, are among those used most empirically and indiscriminately, as shown in the questionnaire. This implies potential risks for birds, suggesting the adoption of sanitary procedures and greater hygienic and sanitary control by resellers.

The possibility and propensity of non-pathogenic and beneficial intestinal bacteria in birds acting as potential reservoirs of genes for antimicrobial resistance translates into risks for animal and human health. Thus, we may state that there are serious consequences for the health of birds and in the control of important diseases common to birds and humans.

## Conclusion

Live-bird resellers have similar sanitary and management characteristics: *Gallus gallus domesticus* housed with other species of birds and animals, sale of birds with little zoosanitary documentation, unsatisfactory active surveillance and indiscriminate use and sale of antimicrobials. *Salmonella* Heidelberg, Gallinarum, Risen, Ndolo, Saint Paul and Mbandaka are identified at resellers, with *Salmonella* Heidelberg, Gallinarum and Mbandaka exhibiting multiple resistance to antimicrobials, which implies potential

risks for animals, man and the environment, that is, for One Health. Therefore, the adoption of sanitary procedures and greater hygienic and sanitary control by resellers is suggested.

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