



Article Study of Salmonella spp. from Cage Papers Belonging to Pet Birds in an Argentinean Canary Breeder Championship

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Simple Summary: This study was conducted to estimate the rate of *Salmonella* spp. isolation from cage papers, located in the bottom of canaries' cages and those of other exotic pet birds, in the 2015 Argentinean canary breeder championship. Furthermore, we determined the antimicrobial resistance profile of the isolates for antibiotics and commercial disinfectants. One pool of 258 cage paper pools was positive for *Salmonella* spp. (0.4%). Two strains of *Salmonella* serotype Glostrup were isolated, which were only resistant to sulfonamides and erythromycin and sensitive to alkali-based product PL301 AS. Although the rate of *Salmonella* spp. isolation from cage papers in an Argentinean canary breeder championship is low, this is the first study conducted in Argentina on *Salmonella* spp. isolation from these materials of pet birds. However, *Salmonella* ser. Glostrup isolated strains could be a source of human *Salmonella* outbreaks, and they show high resistance to disinfecting products.

Abstract: Birds, including canaries and other birds, have become increasingly popular as pets. Bird fairs, where breeders gather and show their production in a championship setting, present a setting for possible *Salmonella* spp. contamination and transmission. Therefore, this study estimated the rate of *Salmonella* spp. isolation from cage papers, located in the bottom of cages of exotic pet birds, including canaries. Collected *Salmonella* isolates were used to determine the antimicrobial resistance profile to 52 antibiotics and 17 commercial disinfectants, based on pure or a mixture of acids, alcohols, aldehydes, alkalis, halogens, peroxygen, and quaternary ammonium compounds. The samples consisted of 774 cage papers taken in the 2015 Argentinean canary breeder championship, pooling three cage papers into one sterile sampling bag. Only one pool of the cage papers was positive for *Salmonella* spp. (0.4%), which belonged to the sample from three frill canary cages. Two strains of *Salmonella* serotype Glostrup were isolated, which were only resistant to sulfonamides and erythromycin and sensitive to alkali-based product PL301 AS. Although the rate of *Salmonella* spp.



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). isolation from cage papers in an Argentinean canary breeder championship is low, it should not be discounted because *Salmonella* ser. Glostrup can be a source of human *Salmonella* outbreaks and they show high resistance to disinfecting products.

Keywords: canary; Salmonella; bird fairs; Argentina; antibiotic; disinfectant

1. Introduction

Different species of birds have become increasingly popular as pets. Of all pet birds, *Serinus canarius* (canary) is considered a domesticated species [1]. There are three groups of canaries: song, color, and form. Song canaries include Harzer (Germany), Malinois (Belgium), Timbrado (Spain), and American singers (USA). Color canaries are divided into two groups: melanin (black, brown, agate, and isabel birds) and lipochrome (white, red, and yellow). Form canaries are a diverse group, distributed between frill, type, shape, crested (and crest-bred) birds, and feather-pattern birds [2].

Salmonella enterica is a diverse bacterial species, currently divided into six subspecies and more than 2500 serovars [3]. Many serotypes are important pathogens in humans and animals with varying levels of host specificity [4]. Avian species without ceca, like the canary, or with involute ceca appear to be more susceptible to *Salmonella* infections than birds with fully functioning ceca [5]. This bacterium produces one of the most important bacterial diseases in canaries [2].

Whether or not to treat *Salmonella* infections in companion birds is controversial. However, due to public health hazards, antibiotic treatment is usually recommended for clinically affected birds and companion birds that are identified as carriers [5]. A culture and antibiotic sensitivity test result is performed to determine the most effective antibiotic to administer [2,5]. There are few studies of *Salmonella* susceptibility to different antimicrobial agents in canaries [6–9]. Furthermore, chemical disinfectants are often the first line of defense against pathogens, and these antimicrobial products vary in their ability to destroy microorganisms. Reducing the amount of pathogens on surfaces lowers the chance of exposure and illness risks, safeguarding the health of animals and people who interact with them [10]. However, guidelines for disinfectant selection and usage often are defined less strictly. Although different studies are available on the in vitro efficacy testing of commercial disinfectants against *Salmonella* serovars with different contamination methods [11–14], they do not use strains isolated from canaries or their environment.

There are competitions and exhibitions of canariculture at local, regional, national, international, and global scales [15]. In Argentina, the Argentinean Federation of Canary Breeders organizes a championship every year, which is held over a few days, and exotic pet bird breeders present their bird pets, especially different types of canaries. Although the National Poultry Health Plan includes some Salmonella serovars in the control plan for poultry in Argentina, utilizing culture methods in the laboratory procedure [16], canaries and other pet birds are usually not vaccinated or monitored for diseases/microorganisms. These birds can be clinically affected by *Salmonella* serovars or subclinical carriers and can serve as a reservoir for an aviary [5,7]. Consequently, these infected birds can contaminate the environment through cage papers, feces, or feathers. They may also transmit infections to humans and other birds either indirectly or directly from handling [17]. Paper and paper products are materials often used for bedding in bird cages because it is very easy to change, cheaper than other materials, relatively inexpensive, and allows for the visual monitoring of droppings. However, it is a rich environment for bacterial growth because of the contamination of dropped food, spilled water, and bird feces [18]. For that reason, this study was conducted to (1) study the rate of Salmonella spp. isolation from papers, located in the bottom of the cage of canaries and other exotic pet birds during an Argentinean canary breeder championship; and (2) determine the antibiotic and disinfectant resistance profile of the isolated bacteria.

2. Materials and Methods

2.1. Sample Collection

Approximately 13,000 canaries, 73 canary hybrids and 117 other exotic pet birds from 44 associations, that belonged to the Argentinean Federation of Canary Breeders arrived at La Rural property, Buenos Aires City, Argentina, on 30 June 2015 to participate in the 65th Argentine Canary Breeder Championship [19,20]. Upon arrival, the birds received electrolytes in their drinking water to decrease their susceptibility to potential pathogens due to stress during transport. During the entire championship, the birds that presented clinical signs were monitored and medicated if needed.

The cage white paper (one/cage) was placed in the bottom of each cage (30 × 23 × 25 cm) on the morning of 31 June 2015. A total of 774 cage papers, used as bedding, were randomly removed, using disinfected gloves, from the cages of birds on 2 July 2015. This also included the bird feces and the remainder of their food, feathers, and water that was on the paper. The Argentinean Federation of Canary Breeders approved this sampling work. Three cage papers were pooled and put into one sterile sampling bag. Each sterile bag was labeled with cage numbers to document which bird's cage paper was being tested. Two hundred fifty-eight sterile bags, which included cage papers from 744 canaries, 15 canary hybrids, and 15 exotic pet birds (Table 1), were transferred to the INTA Bacteriology Laboratory of Poultry Department (Concepción del Uruguay, Entre Ríos, Argentina) in ice chests for *Salmonella* spp. isolation.

Pet Bird		Name/Type	Number of Cage Papers Sampled
Canaries	Group	Color	607
	-	Form	137
Canary hybrids		Hooded Siskin (<i>Carduelis magellanica</i>) \times canary	7
		European Goldfinch (<i>Carduelis carduelis</i>) × canary	3
		Eurasian Siskin (<i>Carduelis spinus</i>) \times canary	2
		House Finch (<i>Carpodacus mexicanus</i>) \times canary	2
		European Greenfinch (<i>Chloris chloris</i>) \times canary	1
Other pet birds		Zebra finch (<i>Taeniopygia guttata</i>)	6
		Fischer's lovebird (Agapornis fischeri)	3
		Cut-throat finch (Amadina fasciata)	2
		Rosy-faced lovebird (Agapornis roseicollis)	2
		Red-headed finch (Amadina erythrocephala)	1
		Cockatiel (Nymphicus hollandicus)	1

Table 1. Pet birds whose cage papers were sampled in the 65th Argentine canary breeder championship for *Salmonella* spp. isolation.

2.2. Salmonella spp. Isolation and Identification

In the laboratory, 100 mL of buffered peptone water (Acumedia-Neogen, Lansing, MI, USA) was added to each sterile plastic bag. The mixture was incubated at 35 ± 2 °C for 18–24 h. One milliliter of incubated broth was transferred to 10 mL of tetrathionate broth base (Acumedia-Neogen), containing 20 mL/L of iodine potassium iodide solution (6 g of iodine; 5 g of potassium iodide; 20 mL of demineralized water), brilliant green 0.1% (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and 40 mg/mL of novobiocin (Sigma-Aldrich Chemie GmbH), and incubated at 35 ± 2 °C for 18–24 h. After that, samples were streaked on two selective and differential media, xylose lysine desoxicholate agar (XLD; DifcoTM, Sparks, MD, USA), and EF-18 agar (Acumedia-Neogen) and incubated at 35 ± 2 °C for 18–24 h.

Two or more presumed *Salmonella* colonies on each selective–differential agar plate were picked and biochemically confirmed using triple-sugar iron agar (Acumedia-Neogen), lysine iron agar (Acumedia-Neogen), ortho-nitrophenyl-β-galactoside (Laboratorios Britania, Buenos Aires, Argentina), Simmons citrate (Merck, Darmstadt, Germany), sulfide indole motility medium (Merck), Jordan's tartrate agar, phenylalanine agar (Hi-Media, Mumbai, India), and urea agar (Laboratorios Britania). If no bacterial colonies comparable with *Salmonella* spp. in a selective–differential agar plate were present, two atypical *Salmonella* spp. colonies were picked and tested using the same biochemical tests listed above. All *Salmonella* isolations were preserved on nutritive (Merck) slant agar until serotyping, which was carried out according to the White–Kauffmann–Le Minor scheme, with somatic and flagellar antigens [21].

2.3. Antibiotic Susceptibility Test

The antibiotic susceptibility test was performed by the standard disk diffusion method in Mueller-Hinton agar (Difco, Sparks, MD, USA) and the results were expressed as susceptible, intermediate, or resistant, according to the Clinical and Laboratory Standards Institute [22–24]. The strains were screened for resistance to 52 antibiotics belonging to 14 antibacterial classes (Table 2). Pseudomonas spp. breakpoints were adopted from the Clinical and Laboratory Standards Institute [22] for colistin, as no Enterobacterales criteria for disc diffusion antibiotic resistance assay has been defined either by this Institute or the European Committee on Antimicrobial Susceptibility Testing. The zone diameter breakpoint used for fosfomycin/tylosin was the same as for fosfomycin. Escherichia coli ATCC 25922 was used as control strain. An isolate was classified as multidrug-resistant (MDR) if it resulted as non-sensitive to at least one agent in three or more antibacterial classes [25]. Multiple antibiotic resistance index (MARI) for each resistance pattern was calculated too, by employing the formula proposed by Krumperman [26]: MARI = number of resistance antibiotics/total number of antibiotics tested. Isolates classified as intermediate on the basis of inhibition zone were considered as sensitive for the MARI [27]. Additionally, we evaluated two more methods for testing colistin, a commercial spot agar (ColTest, Laboratorios Britania) that uses a proprietary concentration of colistin (3 μ g/mL), using the same procedure as the one described by Pasteran et al. [28], and a broth disk elution [23].

Antimicrobial Class	Antimicrobial Agent	Concentration (µg/disc)	
	Amoxicillin ^a	10	
Penicillins	Ampicillin ^a	10	
	Piperacillin ^b	100	
	Amoxicillin-clavulanate ^a	20–10	
	Ampicillin–sulbactam ^c	10–10	
B-lactams combination agents	Ceftazidime-avibactam ^a	10–4	
	Ceftolozane-tazobactam ^b	30–10	
	Piperacillin–tazobactam ^c	100-10	
	Cefazolin ^b	30	
	Cephalothin ^c	30	
	Cefaclor ^b	30	
	Cefoxitin ^a	30	
	Cefotaxime ^a	30	
Cephems	Ceftazidime ^a	30	
Cepitents	Cefpodoxime ^a	10	
	Cefixime ^a	5	
	Ceftibuten ^b	30	
	Ceftiofur ^a	30	
	Cefepime ^c	30	
	Ceftaroline ^b	30	
Monobactams	Aztreonam ^c	30	

Table 2. Antimicrobial agents and disc concentrations used for antibiotic susceptibility test over

 Salmonella spp. strains.

Antimicrobial Class	Antimicrobial Agent	Concentration (µg/disc)	
	Doripenem ^b	10	
D	Ertapenem ^b	10	
Penems	Imipenem ^a	10	
	Meropenem ^c	10	
	Streptomycin ^b	10	
	Kanamycin ^a	30	
	Amikacin ^a	30	
Aminoglycosides	Tobramycin ^b	10	
	Gentamicin ^a	10	
	Netilmicin ^b	30	
	Neomycin ^a	30	
Ealata antheorem anto ann.' '	Sulfonamides ^a	300	
Folate pathway antagonists	Trimethoprim-Sulfamethoxazole ^a	23.75-1.25	
Phosphonic acid derivatives and	Fosfomycin ^c	200	
combinations	Fosfomycin–Tylosin ^c	160-40	
Lipopeptides	Colistin ^a	10	
M 1:	Azithromycin ^a	15	
Macrolides	Erythromycin ^a	15	
Nitro-heterocyclics	Nitrofurantoin ^c	300	
- I	Chloramphenicol ^a	30	
Phenicols	Florfenicol ^a	30	
	Nalidixic acid ^a	30	
	Ciprofloxacin ^a	5	
Quinolones and Fluoroquinolones	Norfloxacin ^a	10	
Quinoiones and Photoquinoiones	Ofloxacin ^b	5	
	Levofloxacin ^c	5	
	Enrofloxacin ^a	5	
	Tetracycline ^a	30	
Tetracyclines	Doxycycline ^a	30	
Tetracyclines	Minocycline ^c	30	
	Tigecycline ^a	15	

Table 2. Cont.

^a Oxoid, Basingstoke, UK. ^b Liofilchem, Via Scozia, Italy. ^c Britania, Buenos Aires, Argentina.

2.4. Testing of Disinfectants

The strains were in vitro screened in nine concentrations of 17 commercial disinfectants used in animal production, based on pure or mixture of acids, alcohols, aldehydes, alkalis, halogens, peroxygen, and quaternary ammonium compounds (Table 3). These concentrations included those recommended by the manufacturers. The dilutions of the products were prepared with sterile deionized water on the day of the test. The Mueller-Hinton agar (Laboratorios Britania) plates were inoculated by dipping a sterile swab into $\sim 1.5 \times 10^8$ CFU/mL (turbidity visually comparable to that of the 0.5 McFarland turbidity standard) Salmonella spp. strain suspension in tryptic soy broth (Neogen, USA) and streaking it across the surface of the agar in three directions. The plates were dried at ambient temperature for 15 min before applying 15 μ L of each disinfectant dilution. Each plate was inoculated with 3 doses of one product and after 30-45 min it was incubated at 37 ± 1 °C for 18–24 h. A control was included in which the disinfectant was substituted by sterile deionized water. The minimum inhibitory dose (MID) was considered as the lowest concentration of a disinfectant necessary to inhibit visible growth (the diameter of the zone showing no obvious, visible growth, >5 mm, measured in two directions by a ruler) of the strain in the plate. The strains were reported as sensitive and intermediate to each disinfectant tested when the MID was less than or equal to the doses recommended by the manufacturer, respectively. The strains were considered resistant when the MID was greater than the maximum dose recommended by the manufacturer. The disinfectant doses recommended for each product by manufacturers are detailed in Table 3. Furthermore, a mixture of 15 µL of a mixture of 1% PL 308 DA (Proar Pilar, Fatima, Argentina) with 1% Profoam (Diversey), concentrations as it is used in the poultry industry, was applied on the plate with the strain as described above.

Table 3. Doses of commercial disinfectants used for antimicrobial susceptibility testing of *Salmonella* spp. strains.

Name of the Disinfectant (Company)	Ingredients	Manufacturer's Dose Recommendation (%)	Tested Doses (%) from Original Product	
PL301 AS (Proar Pilar S.A., Villa Bosch, Argentina)	Caustic soda, alkyl glucoside, polyalkali, sodium gluconate, water	3–5		
PL308 DA (Proar Pilar S.A., Villa Martelli, Argentina)	Alkaline agents, non-ionic surfactants, ethylenediaminetetraacetic acid (EDTA), corrosion inhibitors, water	1		
Ucarsan 420 (Ruminal, Villa Martelli, Argentina)	Glutaraldehyde, detergent	0.5		
Ruminal 50 (Ruminal)	Alkyl dimethyl benzyl ammonium chloride	0.04		
End Bac 2 (Diversey, Villa Bosch, Argentina)	Sodium hypochlorite	0.55–2.5		
GLUTASEPT (Nieser, Argentina S.A., Pilar, Buenos Aires)	Formaldehyde, glutaraldehyde, alkyl dimethyl benzyl ammonium chloride, glyoxal	0.25-0.50		
Cid 20 (CID LINES, Ieper, Belgium)	Alkyl dimethyl benzyl ammonium chloride, glyoxal, glutaraldehyde, isopropanol, formaldehyde	0.25-0.50	0.03; 0.06; 0.12; 0.25; 0.5; 1; 2, 4; 8	
X5 (Laboratorios Bimex S.R.L., Ciudad Autonoma de Buenos Aires, Argentina)	Formaldehyde, dimethyl benzyl alkyl ammonium chloride, glutaraldehyde, glyoxal, isopropanol	0.5–2		
Squad (Nieser Argentina S.A., Pilar, Argentina)	Mixed alkyl dimethyl benzyl ammonium chlorides, formaldehyde, ethyl alcohol	1		
TH4+ [®] (Phibro Animal Health, Grand Bourg, Argentina)	Didecyl dimethyl ammonium, alkyl dimethyl benzyl ammonium chloride, octyl dicyldimethyl ammonium chloride, dioctyl dimethyl ammonium chloride, glutaraldehyde, pine oil, terpinol	0.2–0.5		
Zix Virox [®] (Vetanco S.A., Villa Martelli, Argentina)	Hydrogen peroxide, peracetic acid	0.2–1.5		
El Rey Granel	Alcohol vinegar	pure		
Pramolipa (Pramol Química, Piñeyro, Argentina)	Isopropyl alcohol	70	20; 30; 40; 50; 60; 70; 80; 90; pure	
Germatron (Quimax S.A., Ciudad Autonoma de Buenos Aires, Argentina)	Pine oil, quaternary ammonium	0.82–1	0.05; 0.1; 0.2; 0.4, 0.8; 1.6;	
OmnicideTM (Ensol, Villa Bosch, Argentina)	Glutaraldehyde, dimethyl cocobenzyl ammonium chloride	0.2–0.3	3.2; 6.4, 12.8	
Profoam (Diversey, Villa Bosch, Argentina)	Potassium hydroxide, tripolyphosphate sodium, sodium hypochlorite, adjuvants	3–10	0.16; 0.31; 0.62; 1.25; 2.5; 5; 10; 20, 40	
Aminol 50 (Laboratorios Weende, Villa Maria, Argentina)	N-alkyl dimethyl benzylammonium chloride	0.02-0.04	0.0025; 0.05; 0.1; 0.2; 0.4; 0.8; 1.6; 3.2; 6.4	

3. Results

Out of 258 samples, which included three cage papers each, only one pool was positive for *Salmonella* spp. (0.4%). From 1032 bacterial colonies taken from agar plates, two strains of *Salmonella* ser. Glostrup were isolated from EF-18 agar. These belonged to a pool of cage papers from three frill (Gibber italicus, Frisé Suisse and Fiorino) canary cages, from three different canary breeders.

Salmonella ser. Golstrup strains were not MDR strains and the MARI for these strains was 0.04 (2/52). They were resistant to sulfonamides and erythromycin and intermediate to cefazolin and ciprofloxacin. One strain was intermediate to ceftaroline and neomycin. The other strain was sensitive to these two antibiotics (Table 4). Both strains were sensitive to the rest of the antibiotics tested and to colistin by the standard disk diffusion method in Mueller–Hinton agar, CBDE (1 μ g/mL), and ColTest.

Antibiotic	Salmonella ser. Golstrup		
	Strain 1	Strain 2	
Cefazolin	Ι	Ι	
Ceftaroline	Ι	S	
Ciprofloxacin	Ι	Ι	
Erythromycin	R	R	
Neomycin	Ι	S	
Sulfonamides	R	R	

Table 4. Antibiotic non-susceptibility profiles of two strains of *Salmonella* ser. Golstrup isolated from a canary pool of cage papers. I = intermediate, R = resistant, S = susceptible.

In reference to disinfectant resistance, both strains were resistant to the mixture of two disinfectants (1% PL 308 DA with 1% Profoam). However, *S*. ser. Golstrup strains isolated showed some differences from the different disinfectants tested (Table 5). Based on the recommended doses for disinfection, both strains were only sensitive to the alkali-based product PL301 AS. One strain was resistant to 13 commercial products and the other showed resistance to eight disinfectants. The last one was sensitive to nine commercial products.

Table 5. The minimum inhibitory dose (MID) of the disinfectants tested against two strains of *Salmonella* ser. Golstrup. Sensitive (S), intermediate (I), and resistant (R) when the MID is less than, equal to, and greater than the maximum dose recommended by the manufacturer, respectively.

Name of the Disinfectant	MID of the Disinfectants against Salmonella ser. Golstru		nonella ser. Golstrup
Name of the Disinfectant	Strain 1		Strain 2
PL301 AS	1 (S)		
PL308 DA		>8 (R)	
Ucarsan 420	8 (R)		1 (R)
Ruminal 50	0.12 (R)		<0.03(S)
End Bac 2	8 (R)		
GLUTASEPT	2 (R)		1 (R)
Cid 20		1 (R)	
X5	2 (I)		0.25 (S)
Squad	1 (I)		0.5 (S)
TH4+®	0.5 (I)		0.25 (S)
Zix Virox [®]	2 (R)		0.06 (S)
El Rey Granel	>pure (R)		60% (S)
Pramolipa	Pure (R)		80 (R)
Germatron	3.2 (R)		
Omnicide TM	0.8 (R)		0.2 (S)
Profoam	40 (R)		5 (S)
Aminol 50		0.08 (R)	

4. Discussion

In this study, the frequency of *Salmonella* spp. isolation serovars from cage papers at a canary breeder championship was 0.4%. There have been several studies on the prevalence of *Salmonella* infection among pet birds kept in cages, which is between 0.6% and 18.1% for canaries [7–9,29]. However, the samples generally included cloacal swabs, freshly dropped feces, and/or carcasses in these studies, but no cage papers like in our study.

Salmonella ser. Glostrup was the only serovar isolated from canary cage papers in the present study. These cage papers belonged to three different canary breeders, and we did not know if this serotype would come from one, two, or three cages. Different studies report that *Salmonella* serovar Typhimurium is the most frequently isolated serotype followed by *Salmonella* ser. Enteritidis in canaries [6–8,29,30]. Our study did not recover any *Salmonella* ser. Typhimurium (Table 4). Furthermore, there is not any information about *S.* ser. Glostrup in canaries. However, this serovar was isolated from poultry

slaughterhouses [31], water from poultry facilities [32], captive reptiles [33], and human fecal samples [34]. Furthermore, it was reported that the family dog could be implicated as source of a human *S*. ser. Glostrup outbreak [35]. Therefore, cage papers from pet animals can represent a potential risk to other birds and humans.

In our study, *Salmonella* strains were sensitive to 46 antibiotics tested and only resistant to sulfonamides and erythromycin (Table 4). There were not any MDR strains, and the MARI was below 0.21, which is considered the lowest value to be considered high-risk [26]. It is known that Gram-negative bacilli, such as *Salmonella* spp., are usually intrinsically resistant to erythromycin, a macrolide antibiotic [36,37]. Rahmani et al. [7] reported that all *Salmonella* isolates from pet birds in Tehran, Iran, were susceptible to danofloxacin, norfloxacin, levofloxacin, amikacin, gentamicin, and tobramycin. Resistance to other antibiacterial agents was variable and ranged from 0 to 57.9%. Although most of the strains were not completely serotyped in this study, six of them belonged to serogroup C like *S*. ser. Glostrup. Furthermore, although erythromycin was not tested in these studies, strains of this serovar, isolated from a duck carcass and human fecal samples, were susceptible to all of the antibiotics tested [31] or only resistant to gentamicin [34], respectively.

It is considered that the disk diffusion test, commonly used in clinical laboratories, is not reliable for measurements of in vitro colistin resistance because large molecular weight antibiotics, such as polymyxins, diffuse slowly into agar, resulting in small differences in the size of inhibition zones between susceptible and non-susceptible isolates [38]. However, *S.* ser. Glostrup strains were sensitive to colistin by three methodologies in our study: the standard disk diffusion method in Mueller–Hinton agar, CBDE, and ColTest. Recently, Pasteran et al. [28] reported that ColTest showed 97.9% categorical agreement for Enterobacterales with standard broth microdilution.

The selection of an appropriate disinfectant for an animal facility is a complex, multifactorial decision requiring consideration of spectrum of activity, human safety, environmental safety, and behavioral effects on animals [39]. Different reports showed that the ability of disinfectants to eliminate *Salmonella* spp. is influenced by the type of disinfectant chosen and its concentration [11,12]. In this way, we found some differences in disinfectant resistance profiles in the two *S*. ser Golstrup tested. Based on the maximum dose recommended by the manufacturer, both strains were only sensitive to product PL301 AS. Most of the commercial disinfectants that we tested were composed of a mix of substances. One strain was resistant to 13 commercial products and the other showed resistance to eight disinfectants. Therefore, in many cases, the recommended concentration of the products will not be efficient in eliminating these strains. Furthermore, although bleach (sodium hypochlorite) is the standard recommendation made by most Environmental Health and Safety departments [40,41], in our study (Table 5), no *Salmonella* strains were growth-inhibited when the recommended concentration of the commercial product End Bac 2, which contains sodium hypochlorite as the main component, was used.

5. Conclusions

This is the first study conducted in Argentina on *Salmonella* spp. isolation from the cage papers of pet birds. Although the rate of *Salmonella* isolation spp. from cage papers, located in the bottom of the cages of canaries and other exotic pet birds situated in an Argentinean canary breeder championship, is low, it should not be discounted since *S.* ser. Glostrup can be a source of human *Salmonella* outbreak and it shows high resistance to disinfect products (PL308 DA, Ucarsan 420, End Bac 2, GLUTASEPT, Cid 20, Pramolipa, Germatron, Aminol 50). On the other hand, multi-resistance is not a problem in the isolated strains, because they were only resistant to erythromycin and sulfonamides, and multi-resistance has not been a problem in these strains until now. Additional research to generate a larger number of isolates is necessary to adequately evaluate the prevalence of antimicrobial resistance among *Salmonella* isolated from cage papers. Finally, cage paper should be considered as a sample to add in the study related to diseases in pet birds, because these materials also can

contain the feces of birds, feathers, food, and water, which can be a source of *Salmonella* infecting birds and humans.

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Institutional Review Board Statement: Not applicable because this study involved cage papers and not humans or animals.

Informed Consent Statement: The Argentinean Federation of Canary Breeders agreed to study the rate of *Salmonella* spp. isolation from papers, located in the bottom of the cages of canaries and other exotic pet birds, and determine the antibiotic and disinfectant resistance profile of these bacteria, isolated in the 65th Argentine Canary Breeder Championship, which took place between June and July 2015.

Data Availability Statement: The data used in the present work are presented in the tables.

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References

- 1. Kaminsky, A. Do Canaries Make Good Pets? Canar Make Good Pets All Things Natura. 2024. Available online: https://www.allthingsnature.org/do-canaries-make-good-pets.htm (accessed on 4 April 2024).
- Sandmeier, P.; Coutteel, P. Management of canaries, finches, and mynahs. In *Clinical Avian Medicine*; Harrison, G., Light, T., Eds.; Spix Publications: Palm Beach, FL, USA, 2013; Volume 2, pp. 879–913.
- Issenhuth-Jeanjean, S.; Roggentin, P.; Mikoleit, M.; Guibourdenche, M.; De Pinna, E.; Nair, S.; Fields, P.I.; Weill, F.X. Supplement 2008–2010 (no. 48) to the White–Kauffmann–Le Minor scheme. *Res. Microbiol.* 2024, 165, 526–530.
- Ferrari, R.G.; Rosario, D.K.A.; Cunha-Neto, A.; Mano, S.B.; Figueiredo, E.E.S.; Conte-Junior, C.A. Worldwide Epidemiology of Salmonella Serovars in Animal-Based Foods: A Meta-analysis. *Appl. Environ. Microbiol.* 2019, 85, e00591-19. [CrossRef]
- Gerlach, H. Bacteria. In Avian Medicine, Principles and Application; Ritchie, B.W., Harrison, G.J., Harrison, L.R., Eds.; Wingers Publishing: Lake Worth, FL, USA, 1994; pp. 949–983. ISBN 0-9636996-0-1.
- Madadgar, O.; Salehi, T.Z.; Ghafari, M.M.; Tamai, I.A.; Madani, S.A.; Yahyareyat, R. Study of an unusual paratyphoid epornitic in canaries (*Serinus canaria*). Avian Pathol. 2009, 38, 437–441. [CrossRef]
- 7. Rahmani, M.; Peighambari, S.M.; Yazdani, A.; Hojjati, P. Salmonella infection in birds kept in parks and pet shops in Tehran, Iran. *Iran J. Vet. Med.* **2011**, *5*, 145–148. [CrossRef]
- 8. Giacopello, C.; Foti, M.; Fisichella, V.; Lo Piccolo, F. Antibiotic-resistance patterns of Gram-negative bacterial isolates from breeder canaries (*Serinus canaria* domestica) with clinical disease. *J. Exot. Pet. Med.* **2015**, *24*, 84–91. [CrossRef]
- Beleza, A.J.F.; Maciel, W.C.; Carreira, A.S.; Bezerra, W.G.A.; Carmo, C.C.; Havt, A.; Gaio, F.C.; Teixeira, R.S.C. Detection of Enterobacteriaceae, antimicrobial susceptibility, and virulence genes of *Escherichia coli* in canaries (*Serinus canaria*) in northeastern Brazil. *Pesqui Veterinária Bras.* 2019, 39, 201–208. [CrossRef]
- 10. The Center for Food Security and Public Health. Disinfection 101. Iowa State University, College of Veterinary Medicine. 2023. Available online: https://www.cfsph.iastate.edu/Disinfection/Assets/Disinfection101.pdf (accessed on 3 April 2024).
- Drauch, V.; Ibesich, C.; Vogl, C.; Hess, M.; Hess, C. In-vitro testing of bacteriostatic and bactericidal efficacy of commercial disinfectants against *Salmonella* Infantis reveals substantial differences between products and bacterial strains. *Int. J. Food Microbiol.* 2020, 328, 108660. [CrossRef] [PubMed]

- 12. Gosling, R.J.; Breslin, M.; Fenner, J.; Vaughan, K.; West, E.; Mawhinney, I.; Birch, C.; Davies, R.H. An in-vitro investigation into the efficacy of disinfectants used in the duck industry against *Salmonella*. *Avian Pathol.* **2016**, *45*, 576–581. [CrossRef] [PubMed]
- 13. Jang, Y.; Lee, K.; Yun, S.; Lee, M.; Song, J.; Chang, B.; Choe, N.H. Efficacy evaluation of commercial disinfectants by using *Salmonella enterica* serovar Typhimurium as a test organism. *J. Vet. Sci.* **2017**, *18*, 209. [CrossRef]
- 14. McLaren, I.; Wales, A.; Breslin, M.; Davies, R. Evaluation of commonly-used farm disinfectants in wet and dry models of *Salmonella* farm contamination. *Avian Pathol.* **2011**, *40*, 33–42. [CrossRef]
- 15. Cuevas Martínez, R. Los Canarios Lipocrómicos y Melánicos; Editorial Hispano Europea SA: Barcelona, España, 2010.
- 16. National Agrifood Health and Quality Service. Manual de Procedimientos Operativos. Programa de Vigilancia y Control de la Contaminación por *Salmonella* spp. en Granjas Avícolas Comerciales. 2018. Available online: https://www.argentina.gob.ar/sites/default/files/2._manual_de_procedimientos_operativos_vigilancia_y_control_de_salmonella_spp._en_granjas_avicolas_comerciales_-res._senasa_ndeg_86.2016._version_2018_0.pdf (accessed on 4 April 2024).
- 17. Boseret, G.; Losson, B.; Mainil, J.G.; Thiry, E.; Saegerman, C. Zoonoses in pet birds: Review and perspectives. *Vet. Res.* 2013, 44, 36. [CrossRef] [PubMed]
- 18. Highfill, C. Cage Liners: The Good, the Bad and the Ugly. Winged Wisdom Magazine. 1997. Available online: https://www. birdcageliners.com/services.html (accessed on 4 April 2024).
- 19. Federación Argentina de Canaricultura. Catálogo Campeonato 2015 (Championship Catalog Year 2015). 2015. Available online: http://www.faccanarios.com.ar/ (accessed on 3 April 2020).
- Palermo Tour. Canarios en La Rural: La 65 Edición del Campeonato Nacional de Canaricultura. 2015. Available online: https://palermotour.com.ar/tourdenoticias/canarios-en-la-rural-la-65a-edicion-del-campeonato-nacional-de-canaricultura/ (accessed on 4 April 2024).
- 21. Grimont, P.A.D.; Weill, F.-X. *Antigenic Formulae of the Salmonella Serovars*, 9th ed.; World Health Organization Collaborating Center for Reference and Research on Salmonella, Institute Pasteur: Paris, France, 2007.
- 22. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*, 25th ed.; CLSI Supplement M100. 25th ed.; Clinical and Laboratory Standards Institute: Wayne, PN, USA, 2015.
- Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, 32nd ed.; CLSI Supplement M100. 32nd ed.; Clinical and Laboratory Standards Institute: Wayne, PN, USA, 2022.
- 24. *VET01S-Ed5*; Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Clinical and Laboratory Standards Institute: Wayne, PN, USA, 2022.
- 25. Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Harbarth, S.; Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **2012**, *18*, 268–281. [CrossRef] [PubMed]
- 26. Krumperman, P.H. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl. Environ. Microbiol.* **1983**, *46*, 165–170. [CrossRef]
- Singh, S.; Yadav, A.S.; Singh, S.M.; Bharti, P. Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Res. Int.* 2010, 43, 2017–2030. [CrossRef]
- Pasteran, F.; Danze, D.; Menocal, A.; Cabrera, C.; Castillo, I.; Albornoz, E.; Lucero, C.; Rapoport, M.; Ceriana, P.; Corso, A. Simple phenotypic tests to improve accuracy in screening chromosomal and plasmid-mediated colistin resistance in *Gram-negative bacilli*. *J. Clin. Microbiol.* 2020, 59, e01701-20. [CrossRef] [PubMed]
- 29. Georgiades, G.K.; Iordanidis, P. Prevalence of *Salmonella* infection in pigeons, canaries and psittacines. *J. Hell Vet. Med. Soc.* **2018**, 53, 113. [CrossRef]
- 30. Harrington, R.; Blackburn, B.O.; Cassidy, D.R. Salmonellosis in Canaries. Avian Dis. 1975, 19, 827–829. [CrossRef]
- Kidie, D.H.; Bae, D.H.; Lee, Y.J. Prevalence and antimicrobial resistance of *Salmonella* isolated from poultry slaughterhouses in Korea. *Jpn. J. Vet. Res.* 2013, *61*, 129–136. [PubMed]
- De Sousa, E.D.; Werther, K.; Berchieri Junior, A.; Almeida, A.; Ardisson, F.; Silva, A.C.; Candioto, C.G.; Fernandes, S.A. Experimental infection of one-day-old chicks with *Salmonella* Serotypes Previously isolated from poultry facilities, wild birds, and swine. *Rev. Bras. Ciênc. Avícola* 2013, *15*, 301–305. [CrossRef]
- Zając, M.; Skarżyńska, M.; Lalak, A.; Kwit, R.; Śmiałowska-Węglińska, A.; Pasim, P.; Szulowski, K.; Wasyl, D. Salmonella in Captive Reptiles and Their Environment—Can We Tame the Dragon? *Microorganisms* 2021, 9, 1012. [CrossRef]
- 34. Delicato, E.R.; Mikcha, J.M.G.; Fernandes, S.A.; Pelayo, J.S. Resistance profile to antimicrobials of *Salmonella* spp. isolated from human infections. *Braz. Arch. Biol. Technol.* **2004**, *47*, 193–197. [CrossRef]
- 35. Hoelzer, K.; Moreno Switt, A.; Wiedmann, M. Animal contact as a source of human non-typhoidal salmonellosis. *Vet. Res.* 2011, 42, 34. [CrossRef] [PubMed]
- 36. Chambers, H.F. Protein synthesis inhibitors and miscellaneous antibacterial agents. In *The Pharmacological Basis of Therapeutic*, 11th ed.; Brunton, L.L., Lazo, J.S., Parker, K.L., Eds.; The McGraw-Hill Companies Inc.: New York, NY, USA, 2006; pp. 1173–1202.
- Nakajima, Y. Mechanisms of bacterial resistance to macrolide antibiotics. J. Infect. Chemother. 1999, 5, 61–74. [CrossRef] [PubMed]
 Maalej, S.M.; Meziou, M.R.; Rhimi, F.M.; Hammami, A. Comparison of disc diffusion, Etest and agar dilution for susceptibility
- testing of colistin against Enterobacteriaceae: Comparison of methods for testing colistin. *Lett. Appl. Microbiol.* **2011**, *53*, 546–551. [CrossRef] [PubMed]

- 39. Campagna, M.V.; Faure-Kumar, E.; Treger, J.A.; Cushman, J.D.; Grogan, T.R.; Kasahara, N.; Lawson, G.W. Factors in the selection of surface disinfectants use in a laboratory animal setting. *J. Am. Assoc. Lab. Anim. Sci.* **2016**, *55*, 175–188. [PubMed]
- 40. Rutala, W.A.; Weber, D.J. Uses of inorganic hypochlorite (bleach) in health-care facilities. *Clin. Microbiol. Rev.* **1997**, *10*, 597–610. [CrossRef]
- Rutala, W.A.; Weber, D.J. The Healthcare Infection Control Practices Advisory Committee. Guideline for Disinfection and Sterilization in Healthcare Facilities. 2019. Available online: https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfectionguidelines-H.pdf (accessed on 11 February 2024).

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