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Original article

Antimicrobial susceptibility of *Salmonella* spp. strains isolated from free-living birds

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Abstract

Salmonella is one of the most common causes of food poisoning in the European Union and the United States of America. Free-living birds are known as a reservoir for the different serovars of *Salmonella*, including *S*. Typhimurium, *S*. Enteritidis, *S*. Infantis, *S*. Newport and *S*. Hadar, which may play an important role in the epidemiology of salmonellosis in farm animals, particularly poultry. Also, the antibiotic resistance of *Salmonella* spp. is a growing, public health emergency.

In the present study, the authors examined 36 *Salmonella* spp. strains, which belonged to 3 subspecies; *enterica, salamae* and *houtenae*. All of them were obtained from 13 species of free-living birds in Poland. The antimicrobial susceptibility of these *Salmonella* strains was determined, using commercial Sensititre™ Salmonella, MIC plates, for fourteen antimicrobials, from nine antimicrobial groups: sulfonamides, aminoglycosides, fluorochinolones, cephalosporines, beta-lactams, tetracyclines, phenicols, polymyxins and trimethoprim. The prevalence of selected genes which determine antimicrobial resistance; i.e. *aadB, aacC, blaTEM, blaPSE-1, blaOXA, tetA, tetB, tetC, tetG, cat1, cat2, cat3* and *floR* was also tested. Among all of the examined strains, no resistance was detected in relation to gentamicin, cefotaxime and ceftazidime, while most strains (94.5%) were resistant to sulfamethoxazol. Among the 36 examined bacteria isolates, twenty were resistant to more than one antimicrobial agent. The antimicrobial resistant gene, *floR* was most frequently detected among all examined strains (50%).

Key words: Salmonella spp., antimicrobial susceptibility, resistance, wild birds

Introduction

Salmonellosis is one of the major human foodborne diseases, with a high incidence of infection causing gastroenteritis (EFSA Report 2015). According to recent reports, published by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), the two most commonly reported *Salmonella* serovars, in confirmed human cases from the European Union (EU) in the last few years, were *Salmonella* (*S.*) *enterica* subsp. *enterica* ser. Enteritidis and ser. Typhimurium (EFSA Report 2015). Poultry meat (especially chicken) and eggs have consistently been among the most frequently implicated sources of human *Salmonella* outbreaks (Gast 2008). Free-living

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birds are also recognized as a reservoir for *Salmonella* and they play an important role in the transmission of pathogens (Daoust and Prescot 2007). Some wild bird species, including garden birds and pigeons, seem to be more sensitive to infections or present a host adaptation to some *Salmonella s*erovars, especially to *S.* Typhimurium (Tizard 2004, Daoust and Prescot 2007, Hughes et al. 2008, Lawson et al. 2011). The majority of salmonellosis cases in wild birds worldwide, are caused by this serovar (Tizard 2004). Potentially, it is being transmitted from wild birds to people and animals (Daoust and Prescot 2007). The antibiotic resistance of bacteria, including *Salmonella* spp., is a growing public health emergency. The problem concerns bacteria carrying different resistance mechanisms, which can be transferred to humans and animals, and can therefore result in a carrier state (WHO 2014). It has been reported that the number of animals affected by *Salmonella* spp. or other bacteria has shown marked antibiotic resistance (Cole et al. 2005, Dobbin et al. 2005). Some reports showed higher resistance of various *Salmonella* serovars isolated from birds than from other animals (Molina-Lopez et al. 2015). The presence of dangerous, multi-drug resistance (MDR) has also been highly detected among *Salmonella* strains obtained from birds. The frequent detection of *Salmonella* and other bacterial strains, resistant to commonly used antimicrobials, within the livestock but also within environment and wildlife, is alarming (Camarda et al. 2006, Heuer and Smalla 2007). One of the most important determinants of bacterial contagion is the presence of antibiotic resistance-associated genes. The occurrence of *Salmonella* strains being increasingly resistant to multiple, antimicrobial agents is associated with the acquisition of multiple resistance genes. Among the many genes known to be associated with the resistance of *Salmonella* strains to antimicrobials, which are commonly used in human and veterinary medicine, the most likely to occur are: *aadB* and *aacC* (the determination of resistance with aminoglycosides), *blaTEM*, *blaPSE1*, *blaOXA* (β-lactams), *floR, cat1, cat2, cat3* (phenicols) *tetA-tetE, tetG* and *tetL* (tetracyclines, where the most popular are *tetA, tetB and tetC*) as well as mutations in *gyrA*, resulting in Ser→83→Tyr substitution, or Asp→87→Asn substitution, which determine resistance to fluorochinolones (Carlson et al. 1999, Gallardo et al. 1999, Reche et al. 2002, Randall et al. 2004, Fonseca et al. 2006).

Previous investigations on antimicrobial susceptibility concerned mostly two groups of free-living birds: raptors and gulls (Ramos et al. 2010, Molina-Lopez et al. 2011, Molina-Lopez et al. 2015). Due to the alarming nature of the prevalence of multi-drug resistant bacteria indicated in these two groups of wild birds there is a need for further research on antimicrobial susceptibility among *Salmonella* isolates obtained from other free-living birds, including migratory waterfowl and garden birds.

The aim of this study was to investigate the role of wild birds as a potential reservoir of antimicrobial resistant bacteria. The prevalence of antibiotic susceptibility and the antimicrobial resistance genes of *Salmonella* strains, isolated from free-living birds focused mostly on epidemiologically meaningful serovars; i.e. *S.* Typhimurium, *S.* Enteritidis, *S.* Virchow, *S.* Infantis and *S.* Hadar (monitored in the EU, in poultry flocks) as well as serovars more meaningful for birds and other animals: *S.* Newport and *S.* Heidelberg, were evaluated. Strains of *Salmonella enterica* subsp. *salamae* and one strain of the subspecies *houtenae*, were also examined, for the first time.

Materials and Methods

Isolates of 36 *Salmonella* spp., obtained from 13 free-living bird species, in Poland, were used in the present research. Bacterial strains obtained from wild birds were collected from 2011 (November) until 2014 (August). Each *Salmonella* was identified using the International Organization for Standarization procedure PN-EN ISO 6579: 2003/A1: 2007. *Salmonella* isolates were serotyped, using a single factor antisera (Sifin, Berlin, Germany) according to the White-Kauffman-Le Minor scheme (Guibourdenche et al. 2010). Identification of *Salmonella* at the genus and subspecies levels was performed in accordance with the method of Lee et al. (2009). All *Salmonella* strains in the present research were isolated from material collected using a general sampling scheme, under the larger project no. NR 12 0126 10, as detailed by Krawiec et al. (2015).

Antimicrobial susceptibility

The antimicrobial susceptibility of each *Salmonella* strain was determined, using commercial Sensititre„ Salmonella, MIC plates (Thermo Fischer Scientific, Tewksbury, MA, USA) with the International Sensititre *Salmonella* Plate Format, in accordance with Treck Diagnostic Systems, for fourteen antimicrobials, from nine antimicrobial groups according to the manufacturer's protocols. Bacterial isolates were categorized as susceptible, intermediate or resistant to antimicrobials, using interpretive criteria published by the Clinical and Laboratory Standards Institute and by the National Antimicrobial Resistance Monitoring System of the USA (streptomycin)

Table 1. Antimicrobial dilution range for 36 *Salmonella* isolates (according to Minimal Inhibitory Concentration values).

µg/ml	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Smx	x	X	x	X	X	X	x	X	X	x	0	0	1	1	0	0	0	34!
Gen	x	X	x	X	x	0	30	4	1	1	0	0	0	x	x	x	X	x
Cip	1	0	26	3	1	2	0	2	0	1	0	x	X	x	x	x	X	x
Amp	0	0	0	0	0	0	0	7	23	3	1	0	0	2	0	0	0	0
Fot	x	X	x	8	19	7	1	1	0	0	X	x	x	x	x	x	X	x
Taz	x	X	x	X	x	23	10	2	0	0	1	0	x	x	x	x	X	x
Tet	x	X	x	X	x	X	x	3	26	2	2	0	0	3!	x	x	X	x
Str*	x	X	x	X	x	X	x	X	1	1	9	12	9	3	1	x	X	x
Tmp	x	X	x	X	x	x	29	2	1	0	1	0	3	x	x	x	X	x
Chl	x	X	x	X	x	x	x	X	3	10	16	5	2	0	x	x	x	x
Col	x	X	x	X	x	x	x	X	28	8	x	x	x	x	x	x	x	x
Ffn**	x	X	x	X	x	x	x	X	6	16	10	2	1	1	x	x	x	x
Kan	x	X	x	X	x	x	x	X	X	31	1	1	1	2	0	x	x	x
Nal	x	X	x	X	x	x	x	X	X	29	4	0	0	3	x	x	x	x

Sensitive intermediate Resistance

* In accordance with CLSI norms M100-S24, there are no MIC interpretive standards for streptomycin. This breakpoint was established by NARMS (Zhao et al. 2006);
** norms for florfenicol were taken from CLSI breakpoints and norms M100-S23;
! all of the bacteria grew, even at the highest MIC value of the examined antimicrobial;
No intermediate breakpoint criteria are determined by some antibiotics.
Sulfonamid: smx – sulfamethoxazol; Aminoglycoside: gen – gentamicin, str – streptomycin, kan – kanamycin; fluorochinolones: cip – ciprofloxacin, nal – nalidixic acid; cephalosporines: fot – cefotaxime, taz – ceftazidime; beta – lactams: amp – ampicilin; tetracyclines: tet – tetracycline; phenicols: ffn – florfenicol, chl – chloramphenicol; polymyxins: col – colistin; trimethoprim: tmp – trimethoprim

in accordance with the manufacturer's recommendation (CLSI 2013, CLSI 2014, Zhao et al. 2006).

DNA extraction

Bacteria were incubated overnight, at 37ºC, on a nutrient agar (Merck) followed by genomic DNA extraction, using a Dneasy® Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Bacterial plasmid DNA was isolated, using a GeneJET Plasmid DNA Purification Kit (Thermo Fischer Scientific, USA). DNA concentration was quantified spectrophotometrically (BioPhotometer, Eppendorf, Wesseling-Berzdorf, Germany) and stored at -20ºC.

Detection of antibiotic resistance – associated genes

The prevalence of genes encoding resistance to selected antimicrobials, such as: aminoglicosides, s-lactams, tetracyclines, phenicols and fluoroquinolones, which are widely used in the treatment of people and poultry, was examined in this study. The genes *aadB*, *aacC*, *blaTEM*, *blaPSE-1*, *blaOXA*, *tetA*, *tetB*, *tetC*, *tetG*, *cat1*, *cat2*, *cat3* and *floR*, were examined and detected, using primers and protocols, as reported by Türkyilmaz et al. (2009). PCR reactions were performed separately, on every examined gene adapted to the annealing temperature of the primers. Mutations in the quinolone resistance-determining region (QRDR) of *gyrA* was determined, using the RFLP-PCR method, with protocol primers and a restriction enzyme, as reported by Abdi-Hachesoo et al. (2013) and previously by Ozeki et al. (1997). The *Salmonella* ser. Typhimurium (ATCC # 14028) strain was used as a control.

Results

Among strains used in this research, 24 belonged to the subspecies *enterica*, 11 to subsp. *salamae* and 1 to subsp. *houtenae*. Isolates of *Salmonella enterica* subsp. *enterica* were most often serotyped as ser. Typhimurium (18 strains) and were collected from the following bird species: Eurasian siskin (7), Greenfinch (3), Mallard duck (3), Redpoll (1), Common wood

Table 2. Number of intermediate and resistant strains among *Salmonella enterica* subsp. *enterica* strains, with examined antimicrobials.

Antimicrobials	*Salmonella enterica* subsp. *enterica* serovar:												*Salmonella enterica* subsp.	
	Typhimurium n=18		Newport n=2		Heidelberg n=1		Virchow n=1		Hadar n=1		Infantis n=1		*salamae* and *houtenae* n=12	
	int	res	Int	res	int	res	Int	res	int	res	int	Res	int	res
Smx	2	16	0	2	0	1	0	1	0	1	0	1	0	12
Gen	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cip	2	1	0	0	0	0	0	1	0	0	0	1	1	0
Amp	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Fot	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Taz	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tet	2	0	0	0	0	0	0	1	0	0	0	1	0	1
Str	–	1	–	0	–	0	–	1	–	0	–	0	–	2
Tmp	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Chl	1	1	0	0	0	0	0	1	0	0	1	0	3	0
Col	–	7	–	0	–	0	–	0	–	0	–	0	0	1
Ffn	2	1	0	0	0	0	0	1	0	0	0	1	8	1
Kan	1	1	0	0	0	0	0	1	0	0	0	0	0	0
Nal	–	1	–	0	–	0	–	1	–	0	–	1	–	0

"–" norms for intermediate strain values were not determined
Sulfonamid: smx – sulfamethoxazol; Aminoglycoside: gen – gentamicin, str – streptomycin, kan – kanamycin; fluorochinolones: cip – ciprofloxacin, nal – nalidixic acid; cephalosporines: fot – cefotaxime, taz – ceftazidime; beta – lactams: amp – ampicilin; tetracyclines: tet – tetracycline
phenicols: ffn – florfenicol, chl – chloramphenicol; polymyxins: col – colistin; trimethoprim: tmp – trimethoprim

pigeon (1), Blue tit (1), Great tit (1) and Blackbird (1). Other serovars of *Salmonella enterica* subsp. *enterica*, such as Infantis (1), Hadar (1), Virchow (1), Heidelberg (1), and Newport (2) were isolated from; Eurasian siskin (1), Mallard duck (1), Eurasian siskin (1), Greenfinch (1), Rook (1) and Great tit (1). *Salmonella enterica* subsp. *salamae* were obtained from Eurasian tree sparrow (1), Great cormorant (1), Great tit (8) and Common swift (1), and subsp. *houtenae* from 1 Mallard duck. No *Salmonella* Enteritidis was detected among isolates obtained from wild birds in the present study. The results obtained in this study show the high variability of antimicrobial susceptibility, detected by MIC, among *Salmonella* strains, isolated from free-living birds, in Poland. MIC values for the examined *Salmonella* strains are shown in Table 1. Among all examined strains, resistance to gentamicin, cefotaxime and ceftazidime was not detected, while most strains (94.5%) were resistant to sulfamethoxazole. Among the thirty-six examined *Salmonella* strains collected from free-living birds, twenty were resistant to more than one antimicrobial. The most multi-drug resistant strain was *Salmonella enterica* ser. Virchow collected from young, common starling. This bacteria showed resistance to nine antimicrobials: sulfamethoxazol, ciprofloxacin, ampicilin, tetracycline, streptomycin, chloramphenicol, florfenicol, kanamycin and nalidixic acid. Another strain, resistant to five antimicrobials, was *Salmonella enterica* ser. Infantis. This strain was collected from another young, common starling and showed resistance to sulfametoxazol, ciprofloxacin, tetracycline, florfenicol and nalidixic acid. *Salmonella* strains which were resistant to ciprofloxacin (3 strains) and nalidixic acid (3 strains) as well as most strains resistant to colistin (7 strains) belonged to the subspecies *enterica*. All three strains resistant to trimethoprim, belonged to the subspecies *salamae*. Among three strains resistant to tetracycline, two of them belonged to the subspecies *enterica* and one to *salamae* (Table 2).

The presence of selected genes encoding antimicrobial resistance in *Salmonella* spp. isolates, collected from free-living birds in Poland, is shown in Table 3. The highest prevalence of the antimicrobial resistant gene *floR*, was most frequently detected among all examined strains (50%). This gene was found in 62.5% of *Salmonella enterica* subsp. *enterica* strains and in only 25% of strains belonging to subsp. *sala-*

Table 3. Distribution of antibiotic resistance genes in strains of *Salmonella* spp. isolated from free-living birds.

Salmonella enterica subsp.	Nos. (%) of isolates	Genes encoding resistance to selected antimicrobials													
		AMG*		BL			TET				FFN & CAM				FQS
		aadB	*acC*	*bla TEM*	*bla PSE1*	*bla OXA*	*tetA*	*tetB*	*etC*	*etG*	*loR*	*at1*	*at2*	*at3*	*gyr A Mut*
Enterica	24	6	0	2	2	0	4	1	0	2	15	11	0	6	3
	66.7	25.0	0.0	8.3	8.3	0.0	16.7	4.2	0.0	8.3	62.5	45.8	0.0	25.0	12.5
Salamae and *houtenae*	12	2	0	0	0	1	3	1	0	1	3	2	1	1	0
	33.3	16.7	0.0	0.0	0.0	8.3	25.0	8.3	0.0	8.3	25.0	16.7	8.3	8.3	0.0
Total	36	8	0	2	2	1	7	2	0	3	18	13	1	7	3
	100.0	22.23	0.0	5.55	5.55	2.78	19.45	5.55	0.0	8.33	50.0	36.12	2.78	19.45	8.33

* AMG – aminoglycosides; BL – β-lactams; TET – tetracycline; FFN & CAM – phenicols (florfenicol and chloramphenicol); FQS – fluorochinolones

mae and *houtenae*. The percentages of other detected genes were as follows: *cat1* (36.12%), *aadB* (22.23%), *tetA* and cat3 (19.45% for both). Three of these genes; *cat1, cat3* and *aadB*, were most frequently found in isolates of *Salmonella enterica* subsp. *enterica*. Mutation in gyrA and the presence of the genes *tetG*, *blaTEM*, *blaPSE1*, *tetB*, *blaOXA* and *cat2*, was also detected among examined strains but in a lower amount (from 2.78% to 8.33%). Genes *aacC* and *tetC* were not detected in any of the examined *Salmonella* strains.

Discussion

Available data on the antimicrobial resistance of *Salmonella* spp., isolated from free-living birds, is diverse, within varying reports. Most researchers indicate a relatively low resistance rate, among the *Salmonella* spp., obtained from wild birds, though this rate is variable among serovars of *Salmonella* worldwide (Kobayashi et al. 2007, Molina-Lopez et al. 2011, Matias et al. 2016). Despite the quite low prevalence of *Salmonella* spp. strains in the bird population, the high occurrence of many drug resistant strains (including MDR) was reported in free-living raptors (Molina-Lopez et al. 2011) and gulls (Ramos et al. 2010). The high prevalence of MDR, especially in these groups of birds could be connected to their lifestyle and nutrition. Raptors are also more often the patients of veterinary clinics or wildlife rescue centres than other groups of birds such as songbirds. Subsequently, they might have contact with bacterial MDR strains more frequently than other groups of wild birds. In turn, because of feeding habits related to garbage and sewage, gulls have been largely assumed to increase the risk of microbiological infection. But, results obtained in the present study confirm this possibility, as two of the most resistant *Salmonella* strains, i.e. ser. Virchow and ser. Infantis, were detected in the faeces of two young starlings, which lived in nests in the same drainage ditches in suburbs of Wrocław. These drainage ditches were also full of litter and sewage. Garden birds (especially *Passerines)* were treated as not representing a high, zoonotic risk of salmonellosis infection, because the molecular characterisation of isolates suggests that the *S*. Typhimurium infection, in wild passerines, is maintained within wild bird populations and causative strains may be host-adapted (Hughes et al. 2008). However, the rate of *Salmonella* resistance detected in our study, conducted on strains obtained mostly from garden birds and waterfowl, seems to be higher, compared to previous reports. This might be connected with the season in which the samples were collected and with the condition of birds. Most cases of salmonellosis, in free-living birds, are documented as case reports of outbreaks during the winter months, and early spring. Such a phenomenon is connected with increased foraging at feeders, which are likely sites of public exposure to sick or dead garden birds and their faeces (Lawson et al. 2011, Krawiec et al. 2014). The common prevalence of wild birds in urban areas, especially in parks, around ponds or close to gardens and houses (e.g. bird feeders) as well as the frequent occurrence of small *Passerine* birds or common swifts on farms (e.g. birds nesting in henhouses, barns or stables) create the possibility of cross-infection among birds, people and livestock (Tizard 2004, Daoust and Prescot 2007, Hamer et al. 2011, Lawson et al. 2011, Hernandez et al. 2012, Krawiec et al 2015), including an exchange of resistant strains. In results obtained in the present research, only one strain (2.78%) showed susceptibility to all fourteen examined antimicrobials. This bacteria was obtained from a blackbird and was determined as *Salmonella* enterica subsp. enterica ser. Typhimurium. Similarly to our results, Hudson et al. (2000) also reported the highest resistance to sul-

fametoxasole, among every resistant *Salmonella* strain. In another study, resistance to sulfametoxasole was checked as a combination of sulfametoxasole--trimethoprim and, in these cases, the resistance of *Salmonella* isolates was lower, compared to separated sulfametoxasole (Matias et al. 2016). During the same study, more frequent resistance to tetracyclines and ampicillin or streptomycin, was detected (Matias et al. 2016). A similar order of increasing resistance to ampicillin and tetracycline, among *Salmonella* strains, was reported in isolates obtained from people (Gallardo et al. 1999) or production animals, including poultry (Seyfarth et al. 1997, Boqvist et al. 2003). Such a situation may suggest the participation of wild birds, in the transport and spread of antimicrobial resistant *Salmonella*, into the environment, which can have an epidemiological importance for people and meat-chain production. The presence of *Salmonella* resistance, to chloramphenicol and kanamycin or colistin, reported in our study, was also reported by other researchers, in strains collected from wild birds and people (Gallardo et al. 1999, Kobayashi et al. 2007, Molina-Lopez et al. 2011). The detection of strains resistant to colistin might be significant. Colistin is the last effective antimicrobial used in the treatment of human salmonellosis and colibacteriosis in poultry and pigs. Detection of bacteria resistant to colistin in the population of wild birds might indicate the prevalence of similar strains in the environment and contamination between the environment, wild birds and livestock (Anjum et al. 2016). The resistance of *Salmonella* strains to nalidixic acid and ciprofloxacin, as detected in our study, has also been reported in free-living birds in more recent studies (Reche et al. 2002, Kobayashi et al. 2007, Matias et al. 2016).

Salmonella Virchow and *Salmonella* Infantis examined in the present study, were more multiple resistant, compared to other isolated *Salmonella* serovars. These two serovars are monitored in poultry breeder flocks for being particularly dangerous and are commonly recognized as food-borne diseases in people. The two isolates of *S*. Newport obtained in this study only show resistance to sulfametoxasole. Serovar *S.* Newport is one of the most commonly detected strains in poultry, particularly turkey (Papadopoulou et al. 2009) and is an important cause of salmonellosis, with strains being increasingly resistant to multiple antimicrobial agents (Lynne et al. 2008). The presence of the three *Salmonella* serovars mentioned above (*S*. Virchow, *S*. Infantis, *S*. Newport), in non-domestic birds, might indicate, once more, the epidemiological importance of wild birds in the transmission and spread of potentially infectious *Salmonella* strains, to people and other animals. Antimicrobial resistance might also be different, among strains from the same serovar. Our results, for *S.* Typhimurium strains, show one isolate as being sensitive to every tested antimicrobial, while another shows multi-drug resistance. A similar situation was observed by Reche et al. (2002) and Kobayashi et al. (2007). There is a lack of information on the antimicrobial resistance of non *S. enterica* subsp. *enterica* strains, but of importance seems to be the fact that strains of *S. enterica* subsp. *salamae* show a different susceptibility to selected antimicrobials, compared with susbsp. *enterica*.

The presence of a mutation in the *gyrA* subunit has been described, within *Salmonella* and other *Enterobacteriacae* strains, as resistant to nalidixic acid, obtained from people and animals, including wild birds (Griggs et al. 1996, Reche et al. 2002). The results obtained in the present study vary slightly, compared to results obtained by Reche et al. (2002) who reported that 7/45 (15.5%) of examined *Salmonella* strains from wild birds were resistant to nalidixic acid and carried a mutation in the *gyrA* subunit. The percentage of strains resistant to nalidixic acid and carrying the mutation in the *gyrA* subunit, in this study, was 8.33% (3/36). Some strains showed the higher prevalence of examined genes, encoding antimicrobial resistance, compared to the antimicrobial resistance determined by MIC. This result suggests that many resistant genes might be inactive and it is worth focusing more attention on the mechanisms of antimicrobial resistance development in many bacteria isolated from free-living birds.

In conclusion, in relation to antibiotic resistance and the prevalence of associated genes in the examined strains, free-living birds can be a reservoir for antimicrobial resistant *Salmonella* in the environment, and can play a role in the transmission and spread of this bacteria to animals and humans.

Ethics approval and consent to participate

The research was conducted with the consent of the 2nd Local Ethical Committee for Animal Experiments (Wrocław, Poland; no. 41/2011) the consent of the General Directorate of Environmental Protection, Poland (nos. 253/2012 and 259/2013) and with the consent of the Regional Directorate of Environmental Protection, Wrocław, Poland, no. WPN. 6205.67.2012.MK.1. Game birds were hunted and collected by hunters in accordance with local hunting laws. Samples of great cormorants were obtained during the annual population cull in Poland, in accordance with the annual specifications of the Regional

Directorate of Environmental Protection. All other information is included in the Materials and Methods section.

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