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Antibiotic resistance assessment in bacteria isolated in migratory Passeriformes transiting through the Metaponto territory (Basilicata, Italy)

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Abstract

Background: Wild birds are considered to be reservoirs of human enteric pathogens and vectors of antimicrobial resistance dissemination in the environment. During their annual migration, they play a potential role in the epidemiology of human associated zoonoses. The aim of this study was to investigate the frequency of isolation and antimicrobial susceptibility profiles of microorganisms found in the cloaca of common European passerines.

Methods: One hundred and twenty-one cloacal swabs were collected during a monitoring program of migratory birds in the Forest Reserve for Protection "Metaponto" (Basilicata, Italy). All samples were cultured using standard bacteriological methods and antibiotic susceptibility testing (agar disk diffusion test) of isolated strains was performed.

Results: The bacteriological analysis produced 122 strains belonging to 18 different species. The most commonly isolated species were *Enterobacter cloacae* and *Providencia rettgeri* (21 strains, 17.2%). Potentially pathogenic species including *Klebsiella pneumoniae*, *Serratia marcescens* and *Pseudomonas* spp. have also been identified. Isolates showed significant frequencies of antimicrobial resistance. The highest frequency of resistance was observed against amoxicillin ($n = 79$, 64.8%); ampicillin ($n = 77$, 63.1%); rifampicin ($n = 75$, 61.5%); amoxicillin–clavulanic acid ($n = 66$, 54.1%). Thirty-one strains (25.4%) showed resistance to imipenem and 8 (6.6%) to meropenem.

Conclusions: Migratory birds play an important role in the ecology, circulation and dissemination of potentially pathogenic antimicrobial resistant organisms. They can therefore be considered sentinel species and environmental health indicators. Our results suggest that the integration of epidemiological surveillance networks during ringing campaigns of wild species can be an effective tool to study this phenomenon.

Keywords: Passeriformes, Cloacal swabs, Bacteriological test, Antimicrobial resistance

Background

Several studies have shown that migratory wild birds play an important role in the ecology, circulation and dissemination of enteric human pathogens such as *Campylobacter*, *Salmonella*, toxin-producing *Escherichia coli* and antimicrobial resistant organisms (Reed et al. 2003; Abulreesh et al. 2007; Foti et al. 2011; Magda et al. 2013).

Although these birds come rarely in contact with antimicrobial agents, they could serve as reservoirs and potential disseminators of resistant bacteria in the environment through fecal depositions (Guenther et al. 2010; Jarhult et al. 2013; Shobrak and Abo-Amer 2015). Resistant bacteria of human and veterinary origin are believed to be transmitted to wild birds through contaminated food or water (Abulreesh et al. 2007; Bonnedahl et al. 2009; Guenther et al. 2010; Radhouani et al. 2012).

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Residues of antibiotics and bacteria carrying antibiotic resistance may be introduced into the environment due to the spread of manure from medicated livestock and urban effluents into agricultural land (Blanco et al. 2009). At rest sites, birds of different species often congregate and the horizontal transmission of pathogens occurs due to interindividual and interspecies contact (Hubàlek 2004), including interaction with sedentary birds.

Furthermore, heavy stress and immunosuppression related to migration could promote the onset of infectious diseases and the spread of infectious agents. Other factors contributing to the prevalence of resistant bacterial strains in wild birds are the environmental contamination, the presence of livestock and human density (Skurnik et al. 2006; Allen et al. 2010). Several studies have shown a wide spread of antibiotic resistant enterobacteria in bird populations sympatric to areas inhabited by people and areas with a high density of livestock (Camarda et al. 2006; Literak et al. 2010; Elmberg et al. 2017).

Most of the information regarding bacterial enteropathogens in wild birds stems from the application of traditional microbiological techniques adapted to the study of those species that are most likely to affect human health. In a previous research carried out by Giacopello et al. (2016) the most frequently diffused resistances among Enterobacteriaceae isolated from passerines in a wildlife rescue centre in Sicily were found to be trimethoprim/sulfamethoxazole (100%), streptomycin (56.2%), amoxicillin/clavulanic acid (62.5%), ampicillin (50%) and tetracycline (31.2%) (Giacopello et al. 2016). Strains of *Escherichia* spp. isolated from migratory wild birds from different areas of Saudi Arabia displayed resistance to chloramphenicol (100%), oxytetracycline (100%), ciprofloxacin (87.5%), ampicillin (75%), cefaclor (62.5%), cephalexin (62.5%) and amoxicillin (50%) (Shobrak and Abo-Amer 2015). Guenther et al. (2010) evaluated the susceptibility of 187 *Escherichia coli* isolates from 226 European wild birds (117 of which belonging to the order Passeriformes) to different antimicrobials and found resistance to ampicillin, cephalotin, tetracycline and neomycin in 60, 46.6, 46.6 and 33.3% of the isolates, respectively (Guenther et al. 2010).

In 2010, the Territorial Office for Biodiversity of the Italian Forestry Corps had set up a monitoring program in the Forest Reserve for Protection "Metaponto", located in the Matera province (Italy), with the aim of studying the migratory avifauna along the Ionian Basilicata Coast. The research activities are becoming especially intense during autumn, when several species of migratory passerines (especially intrapaleartic) stop to rest in extended formations of Mediterranean maquis and in the remaining retrodunal wetlands.

During the tracking season a population of migratory birds has been subjected to health evaluation through various laboratory tests. The study aimed to acquire new data about the bacterial flora of migratory populations passing through Italy by focusing on the isolation of Enterobacteriaceae and by recording the eventual presence of pathogens in all captured specimens. Furthermore, the antimicrobial susceptibility of the isolated strains was tested in order to highlight the possible spread of the antimicrobial resistance in animals that, surely, have never received therapeutic protocols and can therefore be considered environmental sentinels.

Methods

Sampling

The catches were made near the mouth of the river Bradano using 276 m of mist-net 12 × 2, kept open from dawn to dusk and monitored every hour.

In 121 subjects belonging to the Order Passeriformes (Table 1) cloacal swabs were obtained by inserting a sterile culture swab impregnated with buffered peptone

Table 1 Classification of sampled avian species

Family	Common name	Scientific name	Number of samples
Emberizidae	Reed Bunting	*Emberiza schoeniclus* (Linnaeus, 1758)	1
Fringillidae	Chaffinch	*Fringilla coelebs* (Linnaeus, 1758)	2
Sylviidae	Chiffchaff	*Phylloscopus collybita* (Vieillot, 1817)	6
	Firecrest	*Regulus ignicapilla* (Temminck, 1820)	1
	Eurasian Blackcap	*Sylvia atricapilla* (Linnaeus, 1758)	39
Turdidae	European Robin	*Erithacus rubecula* (Linnaeus, 1758)	68
	Blackbird	*Turdus merula* (Linnaeus, 1758)	2
	Song Thrush	*Turdus philomelos* (Brehm, 1831)	2
Total			121

water (Oxoid, Basingstoke, UK) into the cloaca and gently rotating the tip against the mucosa.

Laboratory procedures

Bacterial isolation and identification

Samples were transported in refrigeration conditions to the laboratory of Microbiology of the Department of Veterinary Sciences, University of Messina (Italy) and then submitted to standard bacteriological examination for detection of Enterobacteriaceae. After an enrichment in buffered peptone water, the samples were streaked into MacConkey Agar plates (Oxoid, Basingstoke, Hampshire, UK) using sterile loops. Isolates were subcultured in Blood Agar plates for identification by mass spectrometry MALDI–TOF (matrix assisted laser desorption/ionisation–time of flight mass spectrometry). The isolated colonies were seeded in a 48-well metal plate with disposable loops, using as a reference strain *Escherichia coli* ATCC 8739. The results were analyzed with the VITEK MS system (bioMérieux SA, Marcy l'Etoile, France), using the software Axima (Shimadzu Kyoto, Japan)-SARAMIS database (Spectral ARchive And Microbial Identification System) (AnagnosTec, Berlin, Germany).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the bacterial isolates was performed by disk diffusion method (Bauer et al. 1966) on Mueller–Hinton agar (Oxoid, Basingstoke, UK) in accordance to international standards (CLSI 2013). Susceptibility to 18 antimicrobial agents belonging to 9 antibiotics classes was evaluated: amikacin (AK, 30 µg), amoxicillin (AML, 30 µg), amoxicillin/clavulanic acid (AUG, 30 µg), ampicillin (AMP, 10 µg), aztreonam (ATM, 30 µg), cefotaxime (CTX, 30 µg), cefotaxime/clavulanic acid (CTL, 40 µg), ceftazidime (CAZ, 30 µg), ceftazidime/clavulanic acid (CAL, 40 µg), ciprofloxacin (CIP, 5 µg), enrofloxacin (ENR, 5 µg), gentamicin (CN, 10 µg), imipenem (IMI, 10 µg), meropenem (MEM, 10 µg), rifampicin (RD, 30 µg), tetracycline (TE, 30 µg), tobramycin (TOB, 10 µg), trimethoprim/sulfamethoxazole (SXT, 50 µg) (Liofilchem, Teramo, IT). Isolates were considered resistant or susceptible according to the manufacturer's instructions based on CLSI guidelines (Liofilchem® 2016). Isolates showing intermediate susceptibility were considered as resistant. Strains were considered multidrug resistant (MDR) when showing resistance to three or more antimicrobial classes (Schwarz et al. 2010).

Statistical analysis

The statistical analysis of the results was made using the *z*-test. Differences were considered significant at values of $p < 0.05$.

Results

Bacterial isolation and identification

One hundred and twenty-two strains were isolated. Of these 114 belonged to 10 different genera and 8 were unidentified (Table 2).

The most commonly isolated species were *Providencia rettgeri* (23 strains, 18.9%), *Enterobacter cloacae* (21 strains, 17.2%) and *Leclercia adecarboxylata* (16 strains, 14.7%). Potentially pathogenic species including *Klebsiella pneumoniae*, *Serratia marcescens* and *Pseudomonas* spp. have also been identified.

There were no significant differences in the frequencies of microorganism among the most common bird species (Table 3).

Antimicrobial susceptibility testing

Isolates displayed significant frequencies of antibiotic resistance (Table 4).

Twenty-four strains showed resistance to more than 50% of the tested molecules. The resistance patterns varied from one to sixteen of the antibiotics tested. The resistance to amoxicillin ($n = 79$, 64.8%) was the most frequent, followed by ampicillin ($n = 77$, 63.1%), rifampicin ($n = 75$, 61.5%) and amoxicillin–clavulanic acid ($n = 66$, 54.1%).

Table 2 Identification of bacterial species

Isolated species	Number of strains
Aeromonas spp.	1
Aeromonas punctata	3
Citrobacter spp.	6
Citrobacter braaki	1
Citrobacter farneri	2
Citrobacter freundii	4
Enterobacter spp.	4
Enterobacter asburiae	2
Enterobacter cancerogenus	8
Enterobacter cloacae	21
Enterobacter cowanii	1
Hafnia alvei	4
Klebsiella oxytoca	2
Klebsiella pneumoniae	2
Leclercia spp.	2
Leclercia adecarboxylata	16
Proteus mirabilis	1
Providencia rettgeri	23
Pseudomonas putida	2
Pseudomonas stutzeri	2
Serratia liquefaciens	1
Serratia marcescens	6
Unidentified	8
Total	122

Table 3 Distribution of isolated strains in different species of birds grouped into their genus

Bird species	Number of strains for genus										
	Aeromonas	*Citrobacter*	*Enterobacter*	*Hafnia*	*Klebsiella*	*Leclercia*	*Proteus*	*Providencia*	*Pseudomonas*	*Serratia*	Unidentified
Reed Bunting						1					
Chaffinch		1	1				1				
Chiffchaff	1	1	2					3			
Firecrest											1
Eurasian Blackcap	1	4	8		1	8		10	1	2	4
European Robin	2	7	23	4	3	7		10	3	5	3
Blackbird			2								
Song Thrush						2					

Table 4 Number of resistant strains for single molecules and single bacterial species

Class	Antibiotics	Bacterial species[a]	Total number of resistant strains
Aminoglycosides	Amikacin	*Citrobacter farneri* (1) *Citrobacter freundii* (3) *Enterobacter asburiae* (1) *Enterobacter cancerogenus* (2) *Enterobacter cloacae* (6) *Hafnia alvei* (1) *Klebsiella pneumoniae* (1) *Leclercia adecarboxylata* (2) *Providencia rettgeri* (6) *Pseudomonas putida* (1) *Serratia marcescens* (1)	25 (20.5%)
	Gentamicin	*Citrobacter freundii* (3) *Citrobacter* spp. (2) *Enterobacter asburiae* (1) *Enterobacter cancerogenus* (2) *Enterobacter cloacae* (6) *Klebsiella oxytoca* (1) *Klebsiella pneumoniae* (1) *Leclercia adecarboxylata* (2) *Providencia rettgeri* (1) *Pseudomonas putida* (1)	20 (16.4%)
	Tobramicina	*Citrobacter freundii* (2) *Citrobacter* spp. (1) *Enterobacter cancerogenus* (3) *Enterobacter cloacae* (7) *Klebsiella oxytoca* (1) *Klebsiella pnemoniae* (1) *Leclercia adecarboxilata* (3) *Providencia rettgeri* (3) *Serratia marcescens* (1)	22 (18%)
Cephalosporins	Cefotaxime	*Citrobacter freundii* (1) *Enterobacter asburiae* (1) *Enterobacter cancerogenus* (3) *Enterobacter cloacae* (6) *Klebsiella pneumoniae* (1) *Providencia rettgeri* (3) *Pseudomonas putida* (1)	16 (13.1%)
	Cefotaxime–clavulanic acid	*Citrobacter freundii* (2) *Enterobacter asburiae* (1) *Enterobacter cancerogenus* (2) *Enterobacter cloacae* (6) *Klebsiella pneumoniae* (1) *Providencia rettgeri* (3) *Pseudomonas putida* (1)	16 (13.1%)
	Ceftazidime	*Citrobacter farneri* (1) *Citrobacter freundii* (2) *Citrobacter* spp. (1) *Enterobacter asburiae* (1) *Enterobacter cancerogenus* (3) *Enterobacter cloacae* (8) *Klebsiella pneumoniae* (1) *Leclercia adecarboxylata* (2) *Providencia rettgeri* (4) *Pseudomonas putida* (1)	24 (19.7%)
	Ceftazidime–clavulanic acid	*Citrobacter farneri* (1) *Citrobacter freundii* (2) *Citrobacter* spp. (1) *Enterobacter asburiae* (1) *Enterobacter cancerogenus* (3) *Enterobacter cloacae* (8) *Klebsiella pneumoniae* (1) *Providencia rettgeri* (7) *Pseudomonas putida* (1)	25 (20.5%)

Table 4 continued

Class	Antibiotics	Bacterial species[a]	Total number of resistant strains
Carbapenems	Imipenem	*Citrobacter farneri* (1) *Citrobacter freundii* (2) *Citrobacter* spp. (1) *Enterobacter asburiae* (2) *Enterobacter cancerogenus* (4) *Enterobacter cloacae* (9) *Klebsiella pneumoniae* (1) *Leclercia adecarboxylata* (2) *Providencia rettgeri* (7) *Pseudomonas putida* (1) *Serratia marcescens* (1)	31 (25.4%)
	Meropenem	*Enterobacter cancerogenus* (2) *Enterobacter cloacae* (1) *Leclercia adecarboxylata* (2) *Providencia rettgeri* (2) *Pseudomonas putida* (1)	8 (6.6%)
Fluoroquinolones	Ciprofloxacin	*Citrobacter freundii* (2) *Citrobacter* spp. (2) *Enterobacter cancerogenus* (1) *Enterobacter cloacae* (6) *Klebsiella pneumoniae* (1)	12 (9.8%)
	Enrofloxacin	*Citrobacter freundii* (2) *Citrobacter* spp. (2) *Enterobacter cancerogenus* (1) *Enterobacter cloacae* (1) *Klebsiella oxytoca* (1) *Klebsiella pneumoniae* (1) *Leclercia adecarboxylata* (1) *Providencia rettgeri* (2) *Pseudomonas putida* (1) *Serratia marcescens* (2)	14 (11.5%)
Monobactams	Aztreonam	*Citrobacter farneri* (1) *Citrobacter freundii* (1) *Citrobacter* spp. (1) *Enterobacter asburiae* (1) *Enterobacter cancerogenus* (4) *Enterobacter cloacae* (7) *Klebsiella oxytoca* (1) *Providencia rettgeri* (5) *Pseudomonas putida* (1) *Pseudomonas stutzeri* (1) *Serratia marcescens* (1) Non-identified (1)	25 (20.5%)
Penicillins	Amoxicillin	*Aeromonas punctata* (1) *Aeromonas* spp. (1) *Citrobacter farneri* (2) *Citrobacter freundii* (4) *Citrobacter* spp. (3) *Enterobacter asburiae* (2) *Enterobacter cancerogenus* (8) *Enterobacter cloacae* (18) *Enterobacter cowanii* (1) *Enterobacter* spp. (4) *Hafnia alvei* (1) *Klebsiella oxytoca* (2) *Klebsiella pneumoniae* (2) *Leclercia adecarboxylata* (6) *Proteus mirabilis* (1) *Providencia rettgeri* (12) *Pseudomonas putida* (1) *Serratia marcescens* (5) Non-identified (5)	79 (64.8%)

Table 4 continued

Class	Antibiotics	Bacterial species[a]	Total number of resistant strains
	Amoxicillin–clavulanic acid	*Aeromonas punctata* (1) *Aeromonas* spp. (1) *Citrobacter farneri* (2) *Citrobacter freundii* (4) *Citrobacter* spp. (3) *Enterobacter asburiae* (2) *Enterobacter cancerogenus* (8) *Enterobacter cloacae* (17) *Enterobacter* spp. (4) *Hafnia alvei* (1) *Klebsiella pneumoniae* (1) *Leclercia adecarboxylata* (3) *Providencia rettgeri* (12) *Pseudomonas putida* (1) *Serratia marcescens* (3) Non-identified (3)	66 (54.1%)
	Ampicillin	*Aeromonas punctata* (1) *Aeromonas* spp. (1) *Citrobacter farneri* (1) *Citrobacter freundii* (4) *Citrobacter* spp. (2) *Enterobacter asburiae* (1) *Enterobacter cancerogenus* (8) *Enterobacter cloacae* (18) *Enterobacter cowanii* (1) *Enterobacter* spp. (4) *Hafnia alvei* (1) *Klebsiella oxytoca* (2) *Klebsiella pneumoniae* (2) *Leclercia adecarboxylata* (5) *Proteus mirabilis* (1) *Providencia rettgeri* (15) *Pseudomonas putida* (1) *Pseudomonas stutzeri* (1) *Serratia marcescens* (4) Non-identified (4)	77 (63.1%)
Rifamycins	Rifampicin	*Aeromonas punctata* (1) *Citrobacter farneri* (2) *Citrobacter freundii* (4) *Citrobacter* spp. (5) *Enterobacter asburiae* (2) *Enterobacter cancerogenus* (7) *Enterobacter cloacae* (16) *Enterobacter cowanii* (1) *Enterobacter* spp. (4) *Hafnia alvei* (3) *Klebsiella oxytoca* (1) *Klebsiella pneumoniae* (2) *Leclercia adecarboxylata* (10) *Leclercia* spp. (1) *Providencia rettgeri* (9) *Pseudomonas putida* (1) *Pseudomonas stutzeri* (1) *Serratia marcescens* (4) Non-identified (2)	76 (61.5%)
Sulfonamides	Trimethoprim/sulfamethoxazole	*Citrobacter freundii* (1) *Enterobacter cloacae* (2) *Leclercia adecarboxylata* (1) *Providencia rettgeri* (7) *Pseudomonas stutzeri* (1) Non-identified (3)	15 (12.3%)

Table 4 continued

Class	Antibiotics	Bacterial species[a]	Total number of resistant strains
Tetracyclines	Tetraciclina	*Citrobacter freundii* (2) *Citrobacter* spp. (1) *Enterobacter asburiae* (1) *Enterobacter cancerogenus* (3) *Enterobacter cloacae* (8) *Klebsiella oxytoca* (1) *Leclercia adecarboxylata* (3) *Proteus mirabilis* (1) *Providencia rettgeri* (21) *Pseudomonas putida* (1) *Serratia marcescens* (4) Non-identified (2)	48 (39.3%)

[a] In the brackets are the numbers of resistant strains

Thirty-one strains (25.4%) showed resistance to imipenem and 8 (6.6%) to meropenem. Multidrug resistance occurred in 35/122 strains (28.7%). Among strains resistant to the cephalosporins none showed a phenotypic ESBL profile. Nine strains were susceptible to all tested molecules. Some bacterial species have shown resistance against numerous molecules (Table 5).

Particularly, *Enterobacter cloacae* has shown resistance to 18 molecules (100%); *Enterobacter cancerogenus*, *Citrobacter freundii* and *Providencia rettgeri* to 17 molecules (94.4%); *Pseudomonas putida* to 15 molecules (83.3%); *Klebsiella pneumoniae* to 14 molecules (77.8%); *Citrobacter* spp., *Enterobacter asburiae* and *Leclercia adecarboxylata* to 13 molecules (72.2%). There were no significant differences in the percentage of resistant bacteria among the different bird species (Table 6).

Discussion

Bacteriological analysis led to the isolation of a wide range of bacterial species. Several of the isolated bacteria, such as *K. pneumoniae*, *Enterobacter* spp., *Proteus* spp., *Providencia* spp. and *Citrobacter* spp., are known to cause diseases in avian species, as well as in mammals and humans (Reslinski et al. 2005; Pindi et al. 2013).

Unlike previous studies on wild birds, no strains of *Salmonella* spp. and *Escherichia coli* have been isolated (Hubàlek 2004; Benskin et al. 2009; Guenther et al. 2010; Matias et al. 2016). This result might partially be explained because of diet, as these species were most commonly found in surveys of omnivorous birds as well as carnivorous birds (Bangert et al. 1988), whereas graminivorous birds, such as many passerines, had much lower prevalence (Glunder 1981; Brittingham et al. 1988; Steele et al. 2005). Antimicrobial susceptibility testing revealed a wide spread of strains resistant to some of the molecules tested. The percentage of resistance to penicillins is consistent with the results obtained by other authors (Guenther et al. 2010; Shobrak and Abo-Amer 2015; Giacopello et al. 2016).

The intake of water polluted with faeces or human waste and the acquisition via food seem to be the sources of transmission of resistant bacteria of human and veterinary origin to wild birds (Reed et al. 2003; Pindi et al. 2013; Pinto et al. 2015). Birds not only acquire pathogens from the environment, but also return them via excretion, potentially facilitating the dissemination of pathogenic organisms to both humans and other animals, especially through water (Benskin et al. 2009; Wellington et al. 2013). However, further epidemiological studies are necessary to gain a more detailed understanding of the transmission modality of resistant bacteria to wild birds and their spreading into the environment (Guenther et al. 2010; Radhouani et al. 2012).

Of particular concern is the detection of resistance against two molecules belonging to the family of carbapenems, normally used only in human clinical practice as a last resort for treating infections caused by antimicrobial resistant bacteria. It is unclear how wildlife can acquire such resistance. Different hypotheses have been proposed on the phenomenon's genesis, including the great adaptability of bacteria to a variety of environmental displays, their rapid reproduction, the possibility of genetic material exchange among different species and, especially, an intensive use of antibiotics for the treatment of infections both in human and veterinary medicine. Several studies have shown that soil bacteria can represent an important reservoir of antibiotic resistance determinants, including carbapenemases (Gudeta et al. 2015; Nesme and Simonet 2015).

Our results from Passeriformes with an absence of ESBL-producing bacteria are in agreement with other similar studies carried out in Portugal (Silva et al. 2010) and in Sweden (Jarhult et al. 2013).

Table 5 Number of resistant strains for single bacterial species

Bacterial species	Number of resistant strains																		Total number of molecules
	AK	CN	TOB	CTX	CTL	CAZ	CAL	IMI	MEM	CIP	ENR	AZT	AML	AUG	AMP	RD	SXT	TE	
Aeromonas spp.													1	1	1				3
Aeromonas punctata													1	1	1	1			4
Citrobacter spp.		2	1			1	1	1		2	2	1	3	3	2	5		1	13
Citrobacter farneri	1					1	1	1				1	2	2	1	2			9
Citrobacter freundii	3	3	2	1	2	2	2	2		2	2	1	4	4	4	4	1	2	17
Enterobacter spp.													4	4	4	4			4
Enterobacter asburiae	1	1		1	1	1	1	2				1	2	2	1	2		1	13
Enterobacter cancerogenus	2	2	3	3	2	3	3	4	2	1	1	4	8	8	8	7		3	17
Enterobacter cloacae	6	6	7	6	6	8	8	9	1	6	1	7	18	17	18	16	2	8	18
Enterobacter cowanii													1		1	1			3
Hafnia alvei	1												1	1	1	3			5
Klebsiella oxytoca		1	1								1	1	2		2	1		1	8
Klebsiella pneumoniae	1	1	1	6	1	1	1	1		1	1		2	1	2	2			14
Leclercia spp.																1			1
Leclercia adecarboxylata	2	2	3			2		2	2		1		6	3	5	10	1	3	13
Proteus mirabilis													1		1			1	3
Providencia rettgeri	6	1	3	3	3	4	7	7	2		2	5	12	12	15	9	7	21	17
Pseudomonas putida	1	1		1	1	1	1	1	1		1	1	1	1	1	1		1	15
Pseudomonas stutzeri												1			1	1	1		4
Serratia marcescens	1		1					1			2	1	5	3	4	4		4	10
Unidentified												1	5	3	4	2	3	2	7

Table 6 Number of molecules against which bacterial strains isolated from each single bird species exhibit resistance

Family	Bird species	Number of molecules	
		Range	Average
Emberizidae	Reed Bunting	1	1 (5.6%)
Fringillidae	Chaffinch	4–5	4.5 (25%)
Sylviidae	Chiffchaff	0–15	4 (22.2%)
	Firecrest	4	4 (22.2%)
	Eurasian Blackcap	0–14	4.8 (26.7%)
Turdidae	European Robin	0–16	5.3 (29.4%)
	Blackbird	4–9	6.5 (36.1%)
	Song Thrush	3–5	4 (22.2%)

Conclusions

The results of the present study confirmed that migratory wild birds play an important role in the ecology and circulation of potential zoonotic pathogens. Monitoring antibiotic resistance in wildlife represents a useful method of evaluating the impact of anthropic pressure (Thaller et al. 2010). Furthermore, because migratory birds are recognized as potential reservoirs of pathogenic agents, these birds can be regarded as sentinel species and used as environmental health indicators. All these considerations stimulate discussion about the advantages of an integrated monitoring policy of humans, animals and the environment for the antibiotic resistance control (Köck et al. 2017).

Authors' contributions

All authors made substantial contributions to conception and design, analysis and interpretation of data. In particular: FM, OBM, FV and LPF also contributed to the laboratory analysis and been involved in drafting the manuscript; MA and FE also participated in sampling and data collection operations. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical statement

All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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