

Antimicrobial resistance patterns of Enterobacteriaceae in European wild bird species admitted in a wildlife rescue centre

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Keywords

Antimicrobial resistance,
Enterobacteriaceae,
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Wild birds.

Summary

Wild birds have been considered to be reservoirs of enteric human pathogens and vectors of resistance dissemination to the environment. During annual migration, they potentially play a 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 Enterobacteriaceae members isolated from cloacal swabs of common European wild birds. Fifty-five cloacal swabs were taken during birds' entrance evaluation in a rescue centre for injured wild birds in the Province of Messina (Sicily, Italy). All samples were examined for the presence of members of the family Enterobacteriaceae using standard methods and on the isolated strains antibiotic susceptibility testing was performed. Eighty three Enterobacteriaceae strains were isolated from raptors, waterbirds and passerines. The bacterial species isolated were: *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Salmonella* Typhimurium, *Escherichia vulneris*, *Enterobacter amnigenus* biogroup 2, *Salmonella* Duesseldorf and *Hafnia alvei*. The isolates showed significant frequencies of antibiotic resistance. Multiresistance to three or more groups of antibiotics also occurred. None of them have shown a phenotypic Extended Spectrum Beta Lactamase (ESBL) profile.

Antibiotico resistenza in ceppi di Enterobacteriaceae isolati da avifauna europea ricoverata presso un centro di recupero per la fauna selvatica

Parole chiave

Antibiotico resistenza,
Centro di recupero,
Enterobacteriaceae,
Uccelli selvatici.

Riassunto

Gli uccelli selvatici possono veicolare enterobatteri umani patogeni e rappresentare un mezzo di diffusione di ceppi antibiotico resistenti nell'ambiente. Durante la migrazione annuale possono giocare un ruolo importante nella diffusione di zoonosi. Lo scopo della nostra ricerca è stato quello di valutare la frequenza d'isolamento e la suscettibilità agli antimicrobici di ceppi appartenenti alle Enterobacteriaceae isolati da tamponi cloacali effettuati in uccelli selvatici appartenenti a specie comunemente diffuse in Europa. A tal fine, presso un centro di recupero per l'avifauna selvatica della provincia di Messina (Sicilia, Italia), sono stati eseguiti tamponi cloacali al momento della visita d'ingresso di 55 esemplari rappresentati da diverse specie di rapaci, uccelli acquatici e passeriformi. Tutti i campioni sono stati sottoposti a metodiche standard per la ricerca di Enterobacteriaceae ed è stata valutata la suscettibilità agli antibiotici dei ceppi isolati. Sono stati isolati 83 ceppi appartenenti alla famiglia delle Enterobacteriaceae. Le specie isolate sono state: *Escherichia coli*, *Escherichia vulneris*, *Proteus mirabilis*, *Proteus vulgaris*, *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Salmonella* Typhimurium, *Enterobacter amnigenus* biogruppo 2, *Salmonella* Duesseldorf e *Hafnia alvei*. Gli isolati hanno mostrato significative frequenze di resistenza agli antibiotici testati, diversi ceppi hanno presentato multiresistenza a 3 o più gruppi di antibiotici. Nessun ceppo ha presentato un profilo fenotipico caratteristico di ceppi produttori di Beta Lattamasi a Spettro Esteso (ESBL).

Introduction

The emergence of multiresistant bacteria in natural environments represents a potential hazard to both human and animal health (Allen et al. 2010). It is recognised that wild birds are reservoirs of enteric human pathogens such as *Campylobacter*, *Salmonella*, and toxin-producing *Escherichia coli* (Abulreesh et al. 2007, Magda et al. 2013). Moreover, because of their ability to cover long distances during annual migration, they could potentially play an important role in the epidemiology of human zoonoses (Abulreesh et al. 2007, Foti et al. 2009, 2011). Although these birds are rarely in contact with antimicrobial agents, they could serve as reservoirs and potential spreaders of resistant bacteria in the environment via their faecal deposits (Guenther et al. 2010, Jarhult et al. 2013). Water contact and acquisition via food are the supposed ways of transmission of resistant bacteria of human and veterinary origin to wild birds, such as passerines, waterbirds, and raptors (Abulreesh et al. 2007, Bonnedahl et al. 2009, Guenther et al. 2010, Radhouani et al. 2012). The aim of this study was to assess the frequency of isolation of members of the family *Enterobacteriaceae* in the gut flora of common European wild birds and to determine the antimicrobial susceptibility of the isolates.

Materials and methods

Sample collection

From March to June 2013, 55 cloacal swabs were collected from common European wild bird species during their entrance examination in a rescue centre for injured wild birds of the Province of Messina (Sicily, South Italy). Birds' admission to the centre was due to several causes: trauma of unknown origin, gunshot, predator attack, wing injuries, inability to fly, young orphaned, hook or fishing line injuries. Birds were identified by species and age category (juvenile or adult). Individuals belonging to the following orders were sampled: Falconiformes (*Falco tinnunculus*, *Buteo buteo*, *Circus macrourus*, *Pernis apivorus*, *Falco peregrinus*, *Falco eleonora*), Accipitriformes (*Hieraaetus pennatus*), Strigiformes (*Strix aluco*, *Athene noctua*), Charadriiformes (*Larus michahellis*, *Chroicocephalus ridibundus*), Ciconiiformes (*Ciconia ciconia*, *Ardea cinerea*, *Nycticorax nycticorax*, *Egretta garzetta*), and Passeriformes (*Sturnus vulgaris*, *Corvus monedula*, *Corvus corone cornix*, *Garrulus glandarius*, *Turdus merula*, *Passer domesticus*, *Carduelis carduelis*). For the purpose of this study, birds were classified into 3 main groups, represented by raptors (belonging to the orders Falconiformes, Strigiformes, Accipitriformes),

waterbirds (belonging to the orders Charadriiformes and Ciconiiformes) and passerines (of the order Passeriformes). Due to differences in the frequency of entrance in the wildlife rescue centre, several bird species were represented by larger numbers of sampled animals, while only 1 sample was collected from the species *Pernis apivorus*, *Hieraaetus pennatus*, *Falco peregrinus*, *Falco eleonora*, *Athene noctua*, *Ciconia ciconia*, *Ardea cinerea*, *Nycticorax nycticorax*, *Egretta garzetta*, *Corvus corone cornix*, *Garrulus glandarius*, *Turdus merula*, and *Carduelis carduelis*. Orders, species and number of individuals sampled for each species are reported in Table I. The sampling

Table I. Order, species, and number of birds sampled for the isolation of *Enterobacteriaceae* in a rescue centre in the province of Messina (Italy).

Order	Species	Number
Falconiformes	Common kestrel, <i>Falco tinnunculus</i> , Linnaeus 1758	12
	Common buzzard, <i>Buteo buteo</i> , Linnaeus 1758	8
	Pallid harrier, <i>Circus macrourus</i> , Gmelin 1770	4
	European honey-buzzard, <i>Pernis apivorus</i> , Linnaeus 1758	1
	Peregrine falcon, <i>Falco peregrinus</i> , Tunstall 1771	1
Accipitriformes	Eleonora's falcon, <i>Falco eleonora</i> , Gené 1839	1
	Booted eagle, <i>Hieraaetus pennatus</i> , Gmelin 1788	1
Strigiformes	Tawny owl, <i>Strix aluco</i> , Linnaeus 1758	3
	Little owl, <i>Athene noctua</i> , Scopoli 1769	1
Charadriiformes	Yellow-legged gull, <i>Larus michahellis</i> , Naumann 1840	6
	Black-headed gull, <i>Chroicocephalus ridibundus</i> , Linnaeus 1766	2
Ciconiiformes	White stork, <i>Ciconia ciconia</i> , Linnaeus 1758	1
	Grey heron, <i>Ardea cinerea</i> , Linnaeus 1758	1
	Black-crowned night heron, <i>Nycticorax nycticorax</i> , Linnaeus 1758	1
	Little egret, <i>Egretta garzetta</i> , Linnaeus, 1766	1
Passeriformes	Common starling, <i>Sturnus vulgaris</i> , Linnaeus 1758	3
	Eurasian jackdaw, <i>Corvus monedula</i> , Linnaeus 1758	2
	Carrion crow, <i>Corvus corone cornix</i> , Linnaeus 1758	1
	Eurasian jay, <i>Garrulus glandarius</i> , Linnaeus 1758	1
	Common blackbird, <i>Turdus merula</i> , Linnaeus 1758	1
	House sparrow, <i>Passer domesticus</i> , Linnaeus 1758	2
	European goldfinch, <i>Carduelis carduelis</i> , Linnaeus 1758	1
Total		55

was performed within 1 hour from birds' admission. Cloacal swabs were obtained by inserting a sterile culture swab impregnated with buffered peptone water (Oxoid, Basingstoke, UK) into the cloaca and gently rotating the tip against the mucosa. The swabs were immediately transported under refrigeration to the microbiology laboratory for bacteriological investigations of the Department of Veterinary Sciences (University of Messina, Messina, Italy). All samples were examined using standard methods for the presence of members of the family *Enterobacteriaceae* and antibiotic susceptibility test of the isolates was also performed.

Laboratory procedures

Cloacal swabs collected into buffered peptone water were incubated at 37°C for 24 hours. A loopful of each culture was streaked onto MacConkey agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 18-24 hours. One to 3 morphologically different colonies growing on each agar plate were included in further processing. The isolates were identified on the basis of their colony morphology, Gram's staining technique, and by commercial biochemical identification methods (API strips, bioMerieux). *Salmonella* spp. isolates were serotyped by slide agglutination with commercial antisera (Diagnostics Pasteur, Staten Serum Institut, Copenhagen, Denmark).

Antimicrobial susceptibility testing of the bacterial isolates was performed by disk diffusion method on dry Mueller-Hinton agar (Oxoid, Basingstoke, UK), evaluating the activity of 16 antimicrobial agents (Oxoid, Basingstoke, UK): ampicillin (10 µg), amoxicillin/clavulanic acid (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), gentamicin (10 µg), amikacin (30 µg), streptomycin (10 µg), tobramycin (10 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (50 µg), imipenem (10 µg), and meropenem (10 µg).

For each isolate, the zone of inhibition around each disk was measured, after incubation at 37°C for 24 hours. The isolates were classified as susceptible, intermediate or resistant, in accordance with breakpoints proposed by the Clinical and Laboratory Standards Institute (CLSI 2010¹).

Isolates showing resistance to cefotaxime, ceftazidime or ceftriaxone were also screened for Extended Spectrum Beta Lactamase (ESBL) production by Phenotypic Confirmatory Test (PCT) using ceftazidime

and cefotaxime disks either alone or in combination with clavulanic acid (ceftazidime/clavulanic acid - 30 µg, cefotaxime/clavulanic acid - 30 µg) (Clinical and Laboratory Standards Institute 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

All the sampled birds carried members of the family *Enterobacteriaceae*. Eighty-three bacterial isolates, representing 7 genera, were cultured from 55 cloacal swabs. In 24 of 55 samples, coexistence of 2 up to 3 species was detected. Isolated bacterial species were: 30 *Escherichia coli* (36.1% of isolates; 54.5% of individuals), 15 *Proteus mirabilis* (18.1% of isolates; 27.3% of individuals), 9 *Proteus vulgaris* (10.8% of isolates; 16.4% of individuals), 9 *Cytrobacter freundii* (10.8% of isolates; 16.4% of individuals), 6 *Enterobacter cloacae* (7.2% of isolates; 10.9% of individuals), 6 *Klebsiella oxytoca* (7.2% of isolates; 10.9% of individuals), 2 *Salmonella* Typhimurium (2.4% of isolates; 3.6% of individuals), 2 *Escherichia vulneris* (2.4% of isolates; 3.6% of individuals), 2 *Enterobacter amnigenus* biogroup 2 (2.4% of isolates; 3.6% of individuals), 1 *Salmonella* Duesseldorf (1.2% of isolates; 1.8% of individuals), and 1 *Hafnia alvei* (1.2% of isolates; 1.8% of individuals). The prevalence of isolated bacterial species varied among host types (Table II).

The isolates displayed significant frequencies of antibiotic resistance. Antibiotic resistance rates showed little variation among host types (Table III).

Table II. Prevalence of *Enterobacteriaceae* among raptors ($n = 32$), waterbirds ($n = 12$) and passerines ($n = 11$) sampled in a rescue centre in the province of Messina (Italy).

Species	Raptors	Waterbirds	Passerines
<i>Escherichia coli</i>	17 (53.1)	4 (33.3)	9 (81.8)
<i>Escherichia vulneris</i>	0	2 (16.7)	0
<i>Proteus mirabilis</i>	8 (25)	6 (50)	1 (9.1)
<i>Proteus vulgaris</i>	6 (18.7)	1 (8.3)	2 (18.2)
<i>Cytrobacter freundii</i>	6 (18.7)	2 (16.7)	1 (9.1)
<i>Enterobacter cloacae</i>	5 (15.6)	1 (8.3)	0
<i>Enterobacter amnigenus</i> 2	1 (3.1)	1 (8.3)	0
<i>Klebsiella oxytoca</i>	2 (6.2)	1 (8.3)	3 (27.3)
<i>Salmonella</i> Typhimurium	2 (6.2)	0	0
<i>Salmonella</i> Duesseldorf	1 (3.1)	0	0
<i>Hafnia alvei</i>	1 (3.1)	0	0

¹ Clinical Laboratory Standards Institute (CLSI). 2010. Performance Standards for Antimicrobial Susceptibility Testing. Twentieth Informational Supplement. CLSI document M100-S20. CLSI. Wayne, Pennsylvania, USA.

Table III. Susceptibility (number/percent of resistant, intermediate and susceptible) to a panel of antimicrobial drugs of isolates from raptors (R, n = 49), waterbirds (W, n = 18) and passerines (P, n = 16) of a rescue centre in the province of Messina.

Antibiotics	Resistant			Intermediate			Susceptible		
	R	W	P	R	W	P	R	W	P
Ampicillin	24 (49.0)	11 (61.1)	8 (50.0)	13 (26.5)	3 (16.7)	6 (37.5)	12 (24.5)	4 (22.2)	2 (12.5)
Amoxicillin / Clavulanic acid	25 (51.0)	10 (55.5)	10 (62.5)	12 (24.5)	5 (27.8)	4 (25.0)	12 (24.5)	3 (16.7)	2 (12.5)
Cefotaxime	5 (10.2)	4 (22.2)	0	2 (4.1)	3 (16.7)	0	42 (85.7)	11 (61.1)	16 (100)
Ceftazidime	1 (2.0)	2 (11.1)	0	0	2 (11.1)	0	48 (97.9)	14 (77.8)	16 (100)
Ceftriaxone	3 (6.1)	5 (27.8)	0	5 (10.2)	3 (16.7)	4 (25.0)	41 (83.7)	10 (55.5)	12 (75.0)
Nalidixic acid	17 (34.7)	8 (44.4)	2 (12.5)	6 (12.2)	4 (22.2)	1 (6.2)	26 (53.1)	6 (33.3)	13 (81.2)
Ciprofloxacin	3 (6.1)	5 (27.8)	0	8 (16.3)	1 (5.5)	2 (12.5)	38 (77.5)	12 (66.7)	14 (87.5)
Norfloxacin	2 (4.1)	4 (22.2)	0	5 (10.2)	1 (5.5)	0	42 (85.7)	13 (72.2)	16 (100)
Gentamicin	5 (10.2)	5 (27.8)	1 (6.25)	17 (34.7)	4 (22.2)	10 (62.5)	27 (55.1)	9 (50.0)	5 (31.2)
Amikacin	3 (6.1)	2 (11.1)	3 (18.7)	20 (40.8)	9 (50.0)	9 (56.2)	26 (53.1)	7 (38.9)	4 (25.0)
Streptomycin	26 (53.1)	12 (66.7)	9 (56.2)	14 (28.6)	4 (22.2)	4 (25.0)	9 (18.4)	2 (11.1)	3 (18.7)
Tobramycin	7 (14.3)	7 (38.9)	2 (12.5)	8 (16.3)	3 (16.7)	9 (56.2)	34 (69.4)	8 (44.4)	5 (31.2)
Tetracycline	27 (55.1)	8 (44.4)	5 (31.2)	10 (20.4)	8 (44.4)	4 (25.0)	12 (24.5)	2 (11.1)	7 (43.7)
Trimethoprim / Sulfamethoxazole	41 (83.7)	17 (94.4)	16 (100)	0	0	0	9 (10.8)	1 (5.5)	0
Imipenem	9 (18.4)	6 (33.3)	5 (31.2)	2 (4.1)	0	1 (6.2)	38 (77.5)	12 (66.7)	10 (62.5)
Meropenem	0	0	0	0	0	0	83 (100)	18 (100)	16 (100)

Table IV. Comparison of antibiotic resistance rates (number/percent) by disk diffusion method between isolates from raptors (n = 49), waterbirds (n = 18) and passerines (n = 16) of a rescue centre in the province of Messina.

Antibiotics	Raptors	Waterbirds	Passerines	R/W	R/P	W/P
Ampicillin	24 (49.0)	11 (61.1)	8 (50.0)	>0.05	>0.05	>0.05
Amoxicillin / Clavulanic acid	25 (51.0)	10 (55.5)	10 (62.5)	>0.05	>0.05	>0.05
Cefotaxime	5 (10.2)	4 (22.2)	0	>0.05	<0.05	<0.01
Ceftazidime	1 (2.0)	2 (11.1)	0	>0.05	>0.05	<0.01
Ceftriaxone	3 (6.1)	5 (27.8)	0	>0.05	>0.05	<0.01
Nalidixic acid	17 (34.7)	8 (44.4)	2 (12.5)	>0.05	<0.05	<0.05
Ciprofloxacin	3 (6.1)	5 (27.8)	0	>0.05	>0.05	<0.01
Norfloxacin	1 (2.0)	4 (22.2)	0	<0.05	>0.05	<0.01
Gentamicin	5 (10.2)	5 (27.8)	1 (6.2)	>0.05	>0.05	<0.05
Amikacin	3 (6.1)	2 (11.1)	3 (18.7)	>0.05	>0.05	>0.05
Streptomycin	26 (53.1)	12 (66.7)	9 (56.2)	>0.05	>0.05	>0.05
Tobramycin	7 (14.3)	7 (38.9)	2 (12.5)	<0.05	>0.05	<0.05
Tetracycline	27 (55.1)	8 (44.4)	5 (31.2)	>0.05	>0.05	>0.05
Trimethoprim / Sulfamethoxazole	41 (83.7)	17 (94.4)	16 (100)	>0.05	<0.01	>0.05
Imipenem	9 (18.4)	6 (33.3)	5 (31.2)	>0.05	>0.05	>0.05
Meropenem	0	0	0	-	-	-

The most frequently displayed resistances were to trimethoprim/sulfamethoxazole. Significant levels of resistance to streptomycin, amoxicillin/clavulanic acid and ampicillin were found. Lower levels of antibiotic resistance to tetracycline were shown. All isolates were susceptible to meropenem. The highest levels of antibiotic susceptibilities were to ceftazidime, norfloxacin, cefotaxime, ciprofloxacin,

ceftriaxone, and imipenem. Multiresistance to 3 or more classes of antibiotics occurred in 34/49 strains (69.4%) from raptors, 15/18 strains (83.3%) from waterbirds, and 10/16 strains (62.5%) from passerines. No resistance was found in 3/49 isolates from raptors, including a *Proteus vulgaris* isolate, a *Klebsiella oxytoca* isolate, and the *Salmonella* Duesseldorf isolate. Waterbirds isolates

Table V. Screening results for Extended Spectrum Beta Lactamase production by susceptibility testing to cefotaxime (CTX), ceftazidime (CAZ) and ceftriaxone (CRO) drugs and Phenotypic Confirmatory Test (PCT).

Avian host species resistant to Cephalosporins	Bacterial species	Resistance profiles	Positive to PCT	
<i>Falco tinniculus</i>	<i>Escherichia coli</i>	CTX, CRO	0	
<i>Buteo buteo</i>	<i>Proteus mirabilis</i>	CTX, CRO	0	
Raptors	<i>Buteo buteo</i>	<i>Escherichia coli</i>	CTX, CRO	0
	<i>Buteo buteo</i>	<i>Escherichia coli</i>	CTX	0
	<i>Buteo buteo</i>	<i>Escherichia coli</i>	CTX, CAZ, CRO	0
Waterbirds	<i>Laurus michahellis</i>	<i>Escherichia coli</i>	CRO	0
	<i>Chroicocephalus ridibundus</i>	<i>Proteus mirabilis</i>	CTX, CAZ, CRO	0
	<i>Chroicocephalus ridibundus</i>	<i>Escherichia coli</i>	CTX, CRO	0
	<i>Ciconia ciconia</i>	<i>Escherichia coli</i>	CTX, CAZ, CRO	0
	<i>Laurus michahellis</i>	<i>Escherichia coli</i>	CTX, CRO	0
Passerines	-	-	-	

were significantly more resistant to cephalosporins, quinolones, gentamicin and tobramycin than passerines isolates, and they were also more resistant to norfloxacin and tobramycin compared to raptor isolates (Table IV). Resistances to different antibiotics (cefotaxime and nalidixic acid) in raptors isolates were higher than passerines, except for trimethoprim/sulfamethoxazole. Ten of the 83 isolates were resistant to at least 1 cephalosporin, but none of them was positive to Confirmatory Phenotypical Test for the production of ESBL (Table V). All the isolates from passerines were susceptible to cephalosporins.

Discussion

Wild birds have been widely studied and considered important reservoirs and vectors for resistance dissemination in the environment (Guenther et al. 2010, Simões et al. 2010, Jarhult et al. 2013). In our study *Enterobacteriaceae* multiresistant strains were isolated from raptors, waterbirds, and passerines. As transmission of these strains to waterways and other environmental sources may occur via their faecal deposits, wild birds may therefore constitute a considerable hazard to human and animal health. Workers of the rehabilitation centre come into close contact with these birds, their faeces, other body fluids, and soiled bedding materials, so it is essential to determine the zoonotic bacteria and any antibiotic resistance carried by them. Anyway, the risk can be

effectively reduced with proper hygiene, husbandry, and disinfection (Steele et al. 2005). *Escherichia coli* was the most frequently recovered species. Its carriage was highest in passerines (81.8%, n = 9/11), followed by raptors (53.1%, n = 17/32), and it was moderate in waterbirds (33.3%, n = 4/12). *Escherichia coli* producing ESBL has recently emerged as a worldwide public health problem and several studies have focused on its prevalence in the gut flora of different free-living bird species, especially seagull (Bonnedahl et al. 2009, Guenter et al. 2010, 2012, Anunnatsiri et al. 2012). Among the 10 strains that were resistant to the cephalosporins, we found 8 *E. coli* and 2 *Proteus mirabilis*. None of them has shown a phenotypic ESBL profile. A previous study found absence of ESBL producing *E. coli* in migratory passerine birds (Silva et al. 2010). Potentially, pathogenic species including 2 *Salmonella* Typhimurium (from a pallid harrier and a tawny owl) and 1 *Salmonella* Duesseldorf (from a common buzzard) were identified in raptors. However, in accordance to previous studies, we found low isolation rates of *Salmonella* spp. (5.4%, n = 3/55) among wild birds (Steele et al. 2005, Abulreesh et al. 2007, Magda et al. 2013). Waterbird and passerine samples were negative for *Salmonella* spp. Isolated species known as opportunistic human pathogens included *Proteus*, *Citrobacter*, *Enterobacter*, *Klebsiella*, and *Hafnia* spp. (Rešliński et al. 2005, Pindi et al. 2013). The intake of water polluted with faeces or human waste and acquisition via food seems to be the source of transmission of resistant bacteria of human and veterinary origin to wild birds. However, further epidemiological studies are necessary to gain a more detailed understanding of the transmission mode of resistant bacteria to wild birds and back into the environment (Guenther et al. 2010, 2011, Radhouani et al. 2012). The rise of multidrug resistant pathogenic and commensal bacteria is of global concern, because it can lead to increased human and domestic animal healthcare costs and increased morbidity and mortality (Steele et al. 2005). Despite the limited size of the considered sample in this study, the information regarding the antibiotic resistance of the isolates, particularly to carbapenems, will have relevance to alert rehabilitation workers of the importance of using appropriate sanitation measures when handling wild birds. As shown by a recent work, the role of wild birds as a reservoir for carbapenemase-encoding genes should be taken into account (Fischer et al. 2013).

The current worldwide emergence of *Enterobacteriaceae* resistance to carbapenems constitutes an important threat to public health (Gupta et al. 2011, van Duin et al. 2013). Imipenem and meropenem are carbapenems, antimicrobial agents used to treat a variety of serious infections when a microorganism is resistant to the primary

agent of choice. Resistance to these antimicrobial agents is rare and limits therapeutic options. In our study resistance to imipenem occurred in 9/49 strains (18.4%) from raptors, 6/18 strains from waterbirds (33.3%), and 5/16 (31.2%) strains from passerines. No bacteria were resistant to meropenem.

It is unclear how these birds acquired carbapenems resistance, but there is reason for concern. All these considerations stimulate discussions about the expedience of a tight grip policy on antibiotic release for resistance control.

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