Title: Bacteria and Antibiotic Resistance detection in fractures of Wild birds from wildlife rehabilitation centres in Spain

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Abstract

Anatomic adaptations make birds more prone to open fractures with exposed bone parts losing vascularization. As a result of this exposure, fractures are colonized by different microorganisms, including different types of bacteria, both aerobic and anaerobic, causing osteomyelitis in many cases. For this reason, antibiotic treatment is common. However, carrying out antibiotic treatment without carrying out a previous antibiogram may contribute to increased resistance against antibiotics, especially in migratory wild birds. In this paper, bacterial counts regarding fracture type, bacterial identification and antibiotic resistance have been analyzed in wild birds from wildlife rehabilitation centres in Spain. The results obtained showed that open fractures had higher bacterial counts (CFU/mL) than closed ones. Bacteria in family *Enterobacteriaceae*, identified were *Escherichia* spp., *Enterobacter* spp., *Shigella* spp., *Hafnia alvei, Proteus mirabilis*, *Leclercia adecarboxylata* and *Pantoea agglomerans*. Other bacteria present in wild birds' fractures were *Aeromonas* spp., *Enterococcus* spp. *Bacillus wiedmannii* and *Staphylococcus sciuri*. All species found presented resistance to at least one of the antibiotics used. Wild birds can be implicated in the introduction, maintenance and global spreading of antibiotic resistant bacteria and represent an emerging public health concern. Results obtained in this paper support the idea that it is necessary to take this fact into account before antibiotic administration to wild animals, since it could increase the number of bacteria resistant to antibiotics.

Keywords: Antimicrobial Resistance, Bacterial Counts, Bone Fractures, Fracture Contamination, Wild Birds.

Introduction

Adaptations of birds for flying, such as reduction of weigh and bone modifications, predispose these animals to suffer from fractures in case of traumatic injuries, as collisions with electric lines, shots, car crashes, among others [1–5]. The anatomical adaptation makes birds more prone to open fractures with exposed bone parts losing vascularization. In fact, lack of irrigation through the periosteum, medullary, metatarsal and epiphyseal blood vessels, which are responsible for nourishing the bone and the exposure of the fracture to external contaminants, favor the appearance of osteomyelitis and infections of adjacent tissues, as well as necrosis [6,7]. Osteomyelitis is the infection of bone by pathogens such as fungi or bacteria, both aerobic and anaerobic, as a result of trauma or previous infection [6,8].

Osteomyelitis in birds does not affect systemically, unlike what occurs in mammals. However, if osteomyelitis is found in pneumatic bones, such as the humerus or femur, the infection is in direct contact with the air sacs inside the medullary canal and, therefore, with the whole respiratory system [9]. As osteomyelitis could have multiple possible etiologies, treatment with antimicrobial drugs is diverse. Different authors recommend treatments based on clindamycin, while others use ceftiofur, cefotaxime or enrofoxacin [6,10–12]. Nonetheless, the massive use of antibiotics is not recommended. According to World Health Organization, “antibiotic resistance is one the biggest threats to global health, food security, and development”. In fact, this International Organization reaffirmed its global action plan on antimicrobial resistance, one of its five strategic objectives being to optimize the use of microbial agents in 2019 [13].

Most studies reported antibiotic-resistant bacteria in many parts of the world, even remote areas [14]. This antibiotic-resistant bacteria have been found in different types of wild animals, both mammals and birds [15–20]. In that sense, some authors indicate that wild birds could be an important reservoir of resistance to antibiotics, particularly wild migratory birds for their ability for long range movements [21–25].

The aim of this work is to compare the bacteriological contamination in open and closed fractures in birds from wildlife rehabilitation centres in Spain. Bacterial species and antimicrobial resistance were also evaluated.

Materials and methods

Sample and data collection

All animals were handled according to the principles of animal care published by Spanish Royal Decree 53/2013 [26]. Sampled collection was approved by the Ethics Committee and Animal Experimentation of UCH-CEU University. Sample collection was carried out in three different wildlife rehabilitation centres in Spain during the period between February and Juny 2019. A total of 27 birds were sampled and 36 fractures of these birds were analysed. Specimens were collected using sterile cotton swab (AMIES sterile transport swabs, Deltalab Barcelona, Spain) by rotating the swab on the bone surface and then transported under refrigeration to the microbiological laboratory for bacterial isolation.

On the other hand, data from each animal were collected by a questionnaire to determine the possible risk factors for fracture infection. Data included was taxonomic order, nocturnal or diurnal life, causes of bone fractures, fractured bone and fracture type (open or closed). All questionnaires were completed and submitted together with the samples to the Laboratory of "*Group Microbiological Agents Associated with Animal Reproduction (PROVAGINBIO)*", UCH-CEU University.

Bacterial isolation

Swabs were introduced in 10 ml sterile tubes containing 5 ml of phosphate buffered saline (PBS) and vortexed during 1 minute. Serial dilutions to 10^{-5}were performed using PBS. Solutions were then plated in two different solid mediums simultaneously, Blood Agar (BD Columbia Agar with 5% Sheep Blood, BD, Madrid, Spain) as a general bacterial growth medium and MacConkey agar medium (BD MacConkey II Agar, BD, Madrid, Spain) for selective growth and enumeration of *Enterobacteriaceae*. All plates were incubated under aerobic conditions for 24-48h at 37ºC. After the incubation, bacterial count was assessed by quantifying the number of colony forming units per milliliter (CFU/ml). Moreover, morphologically different colonies obtained were freeze at -80ºC and stored in BHI broth medium with 50% glycerol until their used.

Determination of antibiotic susceptibility

Antibiotic susceptibility profile of bacterial isolates was conducted using the agar disk diffusion method according the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [27]. Antibiotics employed in our study were selected

according the common antibiotics used to treat birds' fractures in the wildlife recovery centers where the animals came from. The source for zone diameters used for interpretation of the test was http://www.eucast.org/clinical_breakpoints/. Zone diameters were interpreted and categorized as susceptible, intermediate or resistant according to the EUCAST clinical breakpoint tables and manufacturer's standards for each antimicrobial agent. Antimicrobial agents used and their concentrations were cefazoline (30 mcg), cefotaxime (30 mcg) and clindamycin (2 mcg) (BD BBL Sensi-Disc antimicrobial susceptibility test discs, BD, CA; USA), ceftiofur (30 µg), and enrofloxacin (5 µg) (Antimicrobial Susceptibility Disks, TermoFisher, Oxoid, Valencia, Spain). The measured diameters by the disk diffusion method were interpreted for correlation with the MIC values by agar dilution and compared in database from tables M100S from CLSI.

Identification of bacterial isolates

Genomic DNA extraction of bacterial isolates were isolated using an DNeasy UltraClean Microbial Kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions. Identification of bacterial isolated were performed by PCR amplification and sequencing of 16S rRNA gene using bacterial universal primers (27F 5′-AGAGTTTGATCCTGGCTCAG and 1492R 5′-GGTT ACCTTGTTACGACTT) (Kumar et al., 2017). The PCR was performed in 25 µl reaction volumes containing 2X Taq Master Mix, 0.25 mM forward primer, 0.25 mM reverse primer and 0.4 ng of genomic DNA and nuclease-free water to make volume 25 µl. Temperature cycling conditions for PCR were as follows: an initial heating of 95ºC for 3 min, followed by 40 cycles of denaturation at 95ºC for 30 sec, annealing at 55ºC for 30 sec and extension at 72ºC for 90 sec, and termination step was realized with a 5 min of 72ºC. The PCR

products were examined with electrophoresis on a 1.5% w/v agarose gel, stained by Safe Lab nucleic acid stain. The PCR products were purified using QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions.

Purified PCR products were sequenced using the ABI 3730 XL Analyzer, with BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA). Sequences of approximately 1000 bases were obtained. The bacterial identification was obtained by comparison with 16S rRNA gene sequences of GenBank database from National Center of Biotechnology Information (www.ncbi.nlmnih.gov) through the basic local alignment search tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), using database 16S ribosomal RNA sequences (Bacteria and Archae), and using Megablast (optimize for highly similar sequence) with general parameters. Sequence with more than 4 ambiguous characters or shorter that 500 bases were discarded, and it was considered at least 94% similarity.

Statistical methods

Statistical analysis was performed with statistical package R Commander and RcmdrPlugin. The 95% confidence intervals for prevalence estimates were calculated using the Wilson score interval method. Variables were compared with Pearson's Chi-squared test and Fisher exact tests. Colony-forming units per ml (CFU/ml) were analysed with a non-parametric test (Krustal-Wallis test) to test the difference between groups. Shapiro-Wilk test for normality and Levene's test for homoscedasticity were used to detect significant difference among group variances. All results were expressed as mean ± SD and the statistical significance was accepted at p-value <0.01.

Results

Data collected in the questionnaire about taxonomic order showed that a total of 27 birds were analysed and showed in table 1. The causes of 36 fractures found in a total of 27 birds were varied, with the majority being fractures caused by trauma (n=26) and nest fall (n=3). Other causes were falconry (n=2), collision with power line (n=2), shooting (n=1), electrocution (n=1) and crash against fencing (n=1). In most individuals, the fractured bone was the humerus (n=16) and ulna bone (n=11) with or without radius and metacarpus bones. Other fractures were metacarpus (n=5) and tarsus (n=4). Finally, open fractures were much higher than closed ones (24 *versus* 12).

As it is shown in table 1, when these variables were correlated with bacterial counts (CFU/mL) in Blood Agar and MacConkey agar, only type of fracture influences the bacterial growth in both culture media and CFU/mL, being higher in open fractures than in closed ones ($p<0.01$) (Table 2). Bacterial species detected in fractures are shown in table 3..

Regarding antimicrobial resistance, 85.42% of the isolates presented resistance to clindamycin, whereas 47.92% were resistant to cefazoline. Low levels of resistance were observed towards ceftiofur (only 24.49%) and cefotaxime (20.83%). Table 4 shows the bacteria found, as well as the species of wild birds where they have been isolated and the antibiotic resistance they presented.

Discussion

Results obtained in our study showed that most of the fractures observed in birds from the wildlife rehabilitation centres in Spain were caused by trauma and most of them were open fractures. This is in accordance with another retrospective study made in Spain, were they also found that the main causes of morbidity in wild raptor populations admitted at a wildlife rehabilitation centre were trauma [28]. Bacterial presence and bacterial counts (CFU/mL) were significantly higher in open fractures compared to closed ones. Vergneau-Crosset et al. (2020) [29] showed a worse prognosis in open fractures of wild birds, probably due to a higher incidence of bacterial infections.

It is known, that wild birds have been considered to be reservoirs of resistant pathogens and they can disseminate zoonotic resistant bacteria to the environment during migration [16]. In our work, we identified different bacteria from birds' fractures and we studied antimicrobial susceptibility of all isolates to the antibiotics usually used in the wildlife recovery centres.

Bacterial identification revealed the presence of species in family *Enterobacteriaceae*, as *Escherichia* spp., *Enterobacter* spp., *Shigella* spp., *Hafnia alvei, Proteus mirabilis, Leclercia adecarboxylata* and *Pantoea agglomerans*. Some of the bacteria in family *Enterboacteriaceae* found in our study has been previously identified in European wild bird species admitted in wildlife rescue centres [16].

E. fergusonii plays an important role in human and animal infections [30]. This bacterium causes different pathologies in animals, such as fibrino-necrotic typhlitis in ostriches, or gastrointestinal problems in goat and horses [31–33]. The presence of *E. fergusonii* in

wild birds has been previously reported [34]. In our results, two strains of *E. fergusonii* presented resistance to cefazoline whereas all strains presented resistance to clindamycin. It is known that this bacterium possesses an extended spectrum of resistance to antibiotics [35,36]. Specifically, antimicrobial resistance of *E. fergusonii* isolated from broiler chickens has also been reported [37,38]. Specifically, beta-lactamase gene that confer resistance to ampicillin and cephalosporins has been found in plasmids of *E. fergusonii* isolated in farm animal [39]. Regarding the other *Escherichia* specie found in our study, *E. marmotae*, it has been previously reported as a potential invader pathogen in wild animals as rodents [40]. In our work, this bacterium was resistant to clindamycin.

Leclercia adecarboxylata, previously recognized as *Escherichia adecarboxylata*, causes infections in immunocompromised individuals. It has been related with the production of post-operative orthopedic infection in humans and it is susceptible to most of the common antibiotics [41,42]. In animals, clinical importance is uncertain, although respiratory distress in cows and isolates of *L. adecarboxylata* in manatees with clinical signs as abscesses, debilitation and anorexia has been reported [43,44]. It has been considered as emerging pathogen [45–47]. To our knowledge, our data have shown for the first time the resistance of this pathogen to clindamycin, suggesting that more studies should be carried out.

Regarding *Shigella* spp., they have been previously reported in reptiles, aquatic animals and birds [48,49] and resistance to antibiotics has been also described [50,51]. Mechanism of antimicrobial resistance in *Shigella* spp. has been studied and has been related to plasmid-mediated quinolone and azithromycin resistance genes [52]. In our study, *Shigella* spp. were resistant only to clindamycin. Infectious disease caused by

pathogenic *Shigella* species, includes the ones isolated in our study, *Shigella flexneri*, *Shigella sonnei*, and *Shigella boydii* which can be related with Shigellosis [53]. Although *these bacteria* seem not produce clinical signs in animals, they can be reservoirs of antimicrobial resistant *Shigella* spp. [54].

We have found *E. cloacae* cluster, formed by *E. cloacae*, *E. kobei*, *E. ludwigii* and *E. faecalis* in birds' fractures. Species of the *E. cloacae* complex are widely distributed and they can act as pathogens in most mammals, such as dogs, cat or humans, producing infections in the urinary and respiratory tracts, skin, ear or soft tissues [55,56]. Moreover, Abou-Zahr et al. (2018) [57] found that the most commonly cultured bacteria from superficial chronic ulcerative dermatitis in Psittacine birds were *E. cloacae* . Our results showed *E. cloacae* cluster presented different antimicrobial resistance patterns. A review from 2019 showed these bacteria has been isolated in different animals species, including humans, and were resistant to several antibiotics as cefoxitin, ampicillin, amoxicillin-clavulanic acid and cephalothin [58]. Moreover, previous studies have demonstrated different features of *E. cloacae* conferring antibiotic resistance, as beta-lactamases production by repression of a chromosomal gene or by the acquisition of a transferable ampC gene by mobile elements, acetyltransferase capacity or efflux-pump [55].

Infection by *H. alvei* is associated with the poultry industry and produce anorexia, depression, ruffled feathers and diarrhoea [59]. Moreover, *H. alvei* has been previously detected in European wild bird species in a wildlife rescue center [16]. Its resistance to antimicrobials such as penicillin, oxacillin, amoxicillin plus clavulanic acid and ceftazidime is well known and it has been relate to multidrug resistance clusters genes, multidrug resistance efflux pumps, lysozyme inhibitors and beta-lactam resistance

AmpC-type gene [60,61]. In our study, *H. alvei* presented resistance to clindamycin and cefazoline.

P. mirabilis is the most common pathogen of *Proteus* spp. and its related with urinary infections, mainly in companion animals and humans [62,63]. In agreement with our results, previous studies have isolated *Proteus mirabilis* in wild birds and demonstrated their resistance to different antibiotics [16,64]. Multidrug resistance of *P. mirabilis* could be explained by the ability of this bacterium to form biofilms [65].

Five samples of this study were colonized by *Pantoea agglomerans* a bacterium isolated in poultry farms [66] and related to infections in animals and humans, which causes endophthalmitis, periostitis, endocarditis and osteomyelitis [67]. Resistance to carbapenems, ciprofloxacin, piperacillin and clavulanic acid have been demonstrated [68,69]. Antimicrobial resistance to *P. agglomerans* seems to be encoded by multiple genes [70]. Our work showed resistance to clindamycin.

Aeromonas spp. are pathogenic bacteria that can cause digestive, respiratory and urogenital tract infections, as well as wound, soft tissue infections and osteomyelitis [60,71]. Within this genus, the species found in our work were *A. salmonicida*, *A. enteropelogenes* and *A. media*. *A.salmoncida* was found previously in wild birds [72] whereas *A. enteropelogens* has been found in wild animals of aquatic environments. Additionally, to our knowledge this is the first time that the presence of *A. media* in non-marine animals, as molluscs, has been observed [73,74]. Dias et al. (2018) [72] emphasized the resistance of *Aeromonas* spp. to multiple drugs for their ability to form

biofilms. In this work, *Aeromonas* spp. presented resistance to clindamycin and cefazoline.

Enterococci have emerged as opportunistic pathogen in the intestinal microbiota of many humans and birds and they can cause fatal infections of the urinary tract and endocarditis in humans and it has also been related with postoperative trauma surgical infections in humans and poultry symptoms are joint disease, sepsis, and falls in the first week of life [75,76]. Regarding species found in this genus we identified *E. faecalis, E. faecium* and *E. mundtil*, which have already been described in wild birds [77]. These bacteria have been related with a great capacity to acquire antibiotic resistance genes through plasmids or transposons [76,78,79]. Our results showed their resistance to clindamycin, cefazoline, ceftiofur and cefotaxime.

Bacillus wiedmannii is a novel haemolytic specie of *Bacillus cereus* group, isolated in *Alvinocaris longirostris* (shrimp) and from a silo raw milk sample collected from a dairy powder processing plant in the north-eastern USA [80,81]. This haemolytic capacity found in the prior studies and the resistance found in our study to cefazolin, ceftiofur and cefotaxime could be indicators of danger for this recently discovered species. Genes encoding resistance to tetracycline, streptomycin and beta-lactam antibiotics have been found recently in strains of *B. wiedmannii* isolates [82].

Finally, we have demonstrated the presence of *Staphylococcus sciuri* in wild birds and their resistance to clindamycin. *S. sciuri* is a bacterial pathogen associated with infections in animals and humans [83]. Different authors have detected *S. sciuri* in wild birds [84] and it has also been isolated from a skin wound infection of a patient with infective

endocarditis [85]. Furthermore, a strain methicillin-resistant was recently isolated in fecal samples of wild birds, specifically in passerine birds and rooks from urban areas, indicating the presence of these bacteria in the environmental food sources and the spread of these resistant strains [86]. It has been demonstrated that Gene mecA is present in methicillin-resistant strains of *S. aureus* and is a native gene in *S. sciuri* [87].

Antibiotic resistance among wild animals represent an emerging public health concern [84]. Specially, wild birds can be implicated in the introduction, maintenance and global spreading of antibiotic resistant bacteria [88]. Presence and levels of antimicrobial resistant bacteria in wild birds have been related to human and farm activities and waste products [89]. In fact, it has been reported that wild birds could be infected in urban areas and transport theses resistant bacteria thousands of kilometers to other urban or rural areas in other countries, contaminating food, water or animal farms. This strongly reinforce the necessity of global strategies to control anitmicorgial resistance spread in wild animal interface. Our study strongly indicates that bacteria isolated from wild birds' fractures in wildlife rehabilitation centres in Spain could act as a potential source of resistance and further studies are needed to reduce antimicrobial resistance.

Conclusions

Wild birds can be carriers of antibiotic-resistant bacteria and has been suggested as transmitters of microorganisms. Since many of them are migratory birds, this transmission can occur over very long distances. Our work indicates that wild birds present in their fractures a large number of pathogenic and opportunistic pathogenic

bacteria, resistant to different antibiotics, so it is increasingly necessary to carry out studies to reduce this resistance.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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677 **Table 1.** Colonies in Blood agar and MacConkey agar and variables included in the study (*p value < 0.01 was considered significant).

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		Presence Colonies Blood Agar			Presence Colonies MacConkey agar		
Variable categories		No. positive	% positive	X^2 (p-value)	No. positive	% positive	X^2 (p-value)
Order (number of animals)	Accipitriformes (10)	9	36.1	8.84 (0.29)	4	36.1	15.76 (0.02)
	Falconiformes (3)	1	11.1		0	11.1	
	Charadriiformes (1)	0	2.8		0	2.8	
	Strigiformes (5)	4	19.4		2	19.4	
	Ciconiiformes (4)	4	13.9		4	13.9	
	Pelecaniformes (2)	4	11.1		4	11.1	
	Apodiformes (1)	1	2.8		1	2.8	
	Passeriformes (1)	1	0		1	2.8	
Type of life	Diurnal	20	80.6	0.35 (0.55)	14	80.6	0.89 (0.35)
	Nocturnal	4	19.4		2	19.4	
Fracture type	Open	22	68.6	18.9 (< 0.01)*	15	68.6	8.67 (< 0.01)*
	Closed	2	31.4		1	31.4	
Overall		24			16		

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Table 2. Number of colonies (CFU/ml) in Blood agar and MacConkey agar (mean ± SD) and variables included in the study (*p value < 0.01 was considered significant).

Variable categories		CFU/mL Blood Agar (mean ± SD)	Krustal-Wallis chi-squared (p-value)	CFU/mL MacConkey Agar (mean ± SD)	Krustal-Wallis chi-squared (p-value)
Order	Accipitriformes	$3.2*10^6 \pm 5.6*10^6$	10.56 (0.15)	$3.4*10^6 \pm 10.1*10^6$	13.09 (0.07)
	Falconiformes	37.5 ± 75		0	
	Charadriiformes	0		0	
	Strigiformes	$4.4*10^6 \pm 5.3*10^6$		$2.6*10^6 \pm 5.9*10^6$	
	Ciconiiformes	$6.5*10^6 \pm 7.9*10^6$		$5*10^6 \pm 7.4*10^6$	
	Pelecaniformes	$12.3*10^6 \pm 16.3*10^6$		$4.2*10^6 \pm 4.2*10^6$	
	Apodiformes	$4*10^5 \pm$ NA		$4.4*10^6 \pm$ NA	
	Passeriformes	$8.6*10^6 \pm$ NA		$3.6*10^6 \pm$ NA	
Type of life	Diurnal	$4.7*10^6 \pm 8.1*10^6$	0.01 (0.91)	$3.2*10^6 \pm 7.5*10^6$	0.49 (0.48)
	Nocturnal	$4.3*10^6 \pm 5.3*10^6$		$2.6*10^6 \pm 5.9*10^6$	
Fracture type	Open	$6.9*10^6 \pm 8.5*10^6$	16.549 (<0.01)*	$4.6*10^6 \pm 8.3*10^6$	8.45 (<0.01)*
	Closed	120031.8 ± 398050.8		$5.8*10^4 \pm 1.9*10^4$	

687 **Table 3.** Bacterial species identified from the nucleotide sequence of PCR amplified product
688 and number of fractures where these bacterial species are present, accession number of NCBI
689 database and percentage of identification.

Bacterial species (number of fractures)	NCBI Accession number	Perc. Identification
Aeromonas enteropelogenes (1)	NR_116026.1	95.93
Aeromonas media (3)	NR_036911.2	98.16
Aeromonas salmonicida (1)	NR_118945.1	97.19
Aeromonas veronii (1)	NR_112838.1	98.25
Bacillus wiedmannii (2)	NR_152692.1	97.93
Enterobacter cloacae (1)	NR_118568.1	97.13
Enterobacter kobei (1)	NR_028993.1	98.05
Enterobacter ludwigii (1)	NR_042349.1	97.31
Enterobacter faecalis (4)	NR_113901.1	98.16
Enterococcus faecium (1)	NR_113904.1	97.34
Enterococcus mundtil (1)	NR_113906.1	98.17
Escherichia fergusonii (9)	NR_074902.1	98.48
Escherichia marmotae (1)	NR_136472.1	97.68
Hafnia alvei (2)	NR_112985.1	96.86
Leclercia adecarboxylata (1)	NR_114154.1	98.94
Pantoea aggloemerans (5)	NR_041978.1	98.41
Proteus mirabilis (1)	NR_114419.1	96.78
Shigella boydii (1)	NR_104901.1	96.79
Shigella flexneri (5)	NR_026331.1	98.33
Shigella sonnei (1)	NR_104826.1	94.31
Staphylococcus sciuri (2)	NR_025520.1	97.25

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Table 4. Species of wild birds analysed, bacteria isolated to bone fracture and resistance to antibiotic found (CL: clindamycin, CZ: cefazoline, CF: ceftiofur, EN: enrofoxacin, CX: cefotaxime).

Order	Wild bird species	Bacterial species isolated (n=number of strains for specie)	Antibiotic resistance		
			Resistant	Intermediate	Susceptible
Accippitriformes	*Accipiter gentilis*	*Enterococcus faecalis*	CL, CZ, CF, CX		EN
	Circus aeroginosus	*Bacillus wiedmannii* (2)	CZ, CF, CX	CL (1 strain)	EN
		Pantoea agglomerans (1)		CL	CZ, EN, CF, CX
		Staphylococcus sciuri (1)		CL	CZ, EN, CF, CX
	Gyps fulvus	*Proteus mirabilis* (1)	CL	CZ	EN, CF, CX
		Shigella flexneri (1)	CL		CZ, EN, CF, CX
		Staphylococcus sciuri (1)	CL	CX	CZ, EN, CF
	Milvus migrans	*Pantoea agglomerans* (1)	CL		CZ, EN, CF, CX
		Shigella boydii (1)	CL		CZ, EN, CF, CX
		Leclercia adecarboxylata (1)	CL		CZ, EN, CF, CX
Ciconiiformes	*Ciconia Ciconia*	*Aeromonas media* (1)	CL, CZ		EN, CF, CX
		Enterobacter kobei (1)	CL		CZ, EN, CF, CX

		Enterococcus faecalis (1)	CL, CZ, CF, CX	EN	
		Enterococcus mundtil (1)	CL, CZ, CF, CX		EN
		Escherichia fergusonii (5)	CL, CZ (1 strain)	CX (1 strain)	EN, CF
		Escherichia marmotae (1)	CL		CZ, EN, CF, CX
		Pantoea agglomerans (1)	CL		CZ, EN, CF, CX
		Shigella flexneri (1)	CL		CZ, EN, CF, CX
Falconiformes	*Falco tinnunculus*	*Hafnia alvei* (1)	CL, CZ		EN, CF, CX
Pelecaniformes	*Bulbucus ibis*	*Aeromonas media* (2)	CL, CZ		EN, CF, CX
		Enterobacter cloacae (1)	CL, CZ		EN, CF, CX
		Enterobacter ludwigii (1)	CL, CZ		EN, CF, CX
		Escherichia fergusonii (2)	CL, CZ	CZ (1 strain)	EN, CF, CX
		Pantoea agglomerans (1)	CL		CZ, EN, CF, CX
	Plegadis falcinellus	*Aeromonas enteropeogenes* (1)	CL, CZ	CF	EN, CX
		Aeromonas salmonicida (1)	CL, CZ		EN, CF, CX
		Aeromonas veronii (1)	CL, CZ		EN, CF, CX
		Shigella flexneri (2)	CL		CZ, EN, CF, CX

Strigiformes	*Bubo bubo*	*Escherichia fergusonii* (2)	CL	CZ	EN, CF, CX
		Enterococcus faecalis (2)	CZ, CF, CX	CL, EN	
		Enterococcus faecium (1)	CL, CZ, CF, CX	EN	
		Escherichia fergusonii (2)	CL,	CZ	EN, CF, CX
		Hafnia alvei (1)	CL, CZ	CX	EN, CF
		Shigella flexneri (1)	CL	CZ	EN, CF, CX
		Shigella sonnei (1)	CL	CZ	EN, CF, CX

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: