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Microbiological survey of birds of prey pellets

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ABSTRACT

A microbiological survey of 73 pellets collected from different birds of prey species housed at the Wildlife Rescue and Rehabilitation Center of Napoli (southern Italy) was performed. Pellets were analyzed by culture and biochemical methods as well as by serotyping and polymerase chain reaction. We isolated a wide range of bacteria some of them also pathogens for humans (i.e. *Salmonella enterica* serotype Typhimurium, *Campylobacter coli*, *Escherichia coli* O serogroups). This study highlights the potential role of birds of prey as asymptomatic carriers of pathogenic bacteria which could be disseminated in the environment not only through the birds of prey feces but also through their pellets.

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1. Introduction

Pellets are regurgitated oblong masses of the undigested remains of prey ingested by a bird of prey. Pellets usually consist of fur, bones, claws, and teeth. Pellet formation occurs within the gizzard. Muscular contractions then push the pellet up into the lower esophagus. From there, antiperistaltic waves move the pellet toward the oropharynx where it is expelled. The volume, appearance and timing of pellets varies according to diet fed and, to a lesser extent, the individual bird [1].

Because pellets are characterized by survival of a high proportion of skeletal material they were used to collect information in taphonomic, environmental and biological studies [2–4]. Furthermore, it is possible that viable pathogens such as viruses and bacteria may be present in pellets becoming themselves a risk to human health. In this respect, outbreaks of salmonellosis associated with dissection of owl pellets were reported at two elementary schools by Smith et al. [5] in the USA.

In light of the above, the present study was undertaken with the aim to perform a microbiological survey of birds of prey pellets with specific reference to zoonotic bacteria.

2. Materials and methods

2.1. Sampling

During the period January 2012–January 2014, a total of 73 birds of prey housed at the Wildlife Rescue and Rehabilitation Center (WRRC) of Napoli (southern Italy) was examined. Birds belonged to several avian species. In particular, there were $n = 26$ Common kestrel (*Falco tinnunculus*), $n = 14$ Peregrine falcon (*Falco peregrinus*), $n = 13$ Common buzzard (*Buteo buteo*), $n = 6$ Eurasian sparrowhawk (*Accipiter nisus*), $n = 5$ Barn owl (*Tyto alba*), $n = 4$ Tawny owl (*Strix aluco*), $n = 2$ Eurasian eagle-owl (*Bubo bubo*), $n = 2$ Short-eared owl (*Asio flammeus*), $n = 1$ Short-toed eagle (*Circaetus gallicus*). Each bird of prey was temporarily placed in a cardboard box. For each bird, pellet was collected at the time of regurgitation by using a sterile surgical drape placed on the base of the cardboard box. For the majority of birds, sample collection was on the day of admission (before treatment administration) and before housing in the hospitalization cage or aviary. Each pellet was weighed and five equal size samples were collected from the innermost part by using sterile lancet. Bird-handling procedures were performed according to the Office of Animal Care and Use guidelines.

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Table 1
Bacteria isolated from pellets collected from 73 birds of prey and related results of antimicrobial susceptibility test.

Bacteria	Antimicrobial susceptibility test (%)[a]							
	Number tested	AMC 30 μg[b]	TE 30 μg[b]	CAZ 30 μg[b]	CN 10 μg[b]	ENR 5 μg[b]	SXT 25 μg[b]	CIP 5 μg[b]
Gram-negative bacteria								
Enterobacter amnigenus	20	85	80	85	75	70	80	70
Citrobacter freundii	24	50	75	50	83	83	67	83
Enterobacter cloacae	18	100	83	83	72	72	89	72
Citrobacter youngae	2	0	100	0	100	100	100	100
Citrobacter brakii	2	0	100	50	100	100	100	100
Salmonella Typhimurium	2	50	0	50	0	100	50	100
Klebsiella pneumoniae	12	17	83	42	75	83	67	83
Escherichia coli	48	92	73	92	94	94	63	92
Achromobacter xyloxidans	10	50	50	80	20	30	80	30
Alcaligenes faecalis	2	50	50	50	0	100	50	100
Gram-positive bacteria								
coagulase-negative *Staphylococcus* spp.	38	58	79	53	84	82	76	79
Staphylococcus aureus	26	38	58	42	73	77	65	81

AMC = amoxycillin/clavulanic acid, TE = tetracycline, CAZ = ceftazidime, CN = gentamicin, ENR = enrofloxacin, SXT = sulfamethoxazole/trimethoprim, CIP = ciprofloxacin.
[a] Percentage of susceptibility.
[b] Concentration of the disk used for testing.

2.2. Isolation procedures

The aliquots of pellets were inoculated in buffered peptone water (BPW), *Campylobacter*-selective enrichment broth (CSEB), cooked meat medium (CMM), modified tryptone soya broth (MTSB), phosphate buffered saline (PBS). Samples inoculated into BPW were incubated at 37 °C for 24 h and then were placed into Rappaport-Vassiliadis broth (RV) as well as plated onto Columbia blood agar base (CBA; Oxoid), *Pseudomonas* cetrimide agar (PCA; Oxoid), MacConkey agar (MCA; Oxoid) and Baird-Parker agar (BPA; Oxoid). Samples inoculated into MTSB were incubated at 37 °C for 24 h and then plated onto sorbitol MacConkey agar (SMCA; Oxoid) supplemented with cefixime-tellurite (Oxoid) and chromogenic *E. coli* O157 Agar (CEOA; Biolife Italiana S.r.l., Milan, Italy). Samples inoculated into CSEB were incubated in microaerobic atmosphere (oxygen level of 8–9% and carbon dioxide level below 8%) provided by CampyGen (Oxoid) at 42 °C for 48 h and then plated onto *Campylobacter* blood-free selective agar (CBFA; Oxoid). Samples inoculated into CMM were incubated in anaerobic atmosphere (oxygen level below 0.5% and carbon dioxide level between 9% and 13%) provided by AnaeroGen (Oxoid) at 37 °C for 24 h and then streaked onto anaerobe basal agar (ABA; Oxoid). Samples inoculated into PBS were incubated at 4 °C for 14 days and then streaked onto *Yersinia* selective agar base (cefsulodin-irgasan-novobiocin, CIN Agar; Oxoid) with incubation at 30 °C for 24–48 h. The CBA, PCA, MCA, SMCA, CEOA and BPA plates were incubated at 37 °C for 24–48 h, whereas the RV broths were incubated at 42 °C for 24–48 h and then plated onto both xylose lysine desoxycholate agar (XLD) and brilliant green agar (BGA), the CBFA plates were incubated microaerobically at 42 °C, whereas ABA plates were anaerobically incubated at 37 °C for 48 h and checked daily for a further week before discarding.

2.3. Identification procedures

All strains isolated were primarily identified, selecting 2–3 colonies from plates, on the basis of their colonial morphology, Gram and acid-fastness characteristics, growth requirements, motility tests, pigment production, tube coagulase test, and standard conventional biochemical and phenotypic tests. The isolates were confirmed by using API 20 E, API 20 NE systems (bioMerieux, Mercy-l'Etoile, France) and RapID ANA II, RapID NF PLUS, RapID STAPH PLUS Identification Systems (Oxoid). *E. coli* isolates were serogrouped with antisera poly- and monospecific (Sifin), whereas *Salmonella* isolates were serotyped according to the Kauffman-White scheme in collaboration with the OIE National Reference Laboratory for *Salmonella* (IZSVe, Legnaro, Italy). *Campylobacter* isolates were identified by PCR as reported by Gargiulo et al. [6].

2.4. Antimicrobial susceptibility testing

All isolates were submitted to antimicrobial susceptibility testing using the disk diffusion method. As there are not yet CLSI interpretative criteria for susceptibility breakpoints (disk diffusion) for *C. coli* and anaerobic bacteria, antimicrobial susceptibility testing was not performed for these microorganisms. The antimicrobials tested were amoxycillin/clavulanic acid (AMC; 30 μg), tetracycline (TE; 30 μg), ceftazidime (CAZ; 30 μg), gentamicin (CN; 10 μg), enrofloxacin (ENR; 5 μg), sulfamethoxazole-trimethoprim (SXT; 23.75/1.25 μg) and ciprofloxacin (CIP; 5 μg). In order to evaluate the presence of methicillin-resistant *Staphylococcus* species, oxacillin (1 μg) and cefoxitin (30 μg) disks were also used for *Staphylococcus* spp. isolates. The inhibition zones were measured and scored as susceptible, intermediate and resistant according to the Clinical and Laboratory Standards Institute (CLSI) documents [7–11]. The breakpoints for *A. xyloxidans* and *A. faecalis* were those reported for *Burkholderia* spp. and *P. aeruginosa* [7–11]. When an antimicrobial molecule for a specific agent was not present in the CLSI documents, a similar antimicrobial molecule of the same class was used. Specifically, amoxycillin/clavulanic acid breakpoints used for *A. xyloxidans* and *A. faecalis* were those reported by CLSI for ticarcillin/clavulanic acid of *P. aeruginosa* [10]. Ceftazidime breakpoints for *S. aureus* and coagulase-negative *Staphylococcus* spp. were those reported by CLSI for ceftaroline [10] and cefoxitin [8] breakpoints, respectively. Finally, enrofloxacin breakpoints for Enterobacteriaceae were those suggested in CLSI standards for chicken and turkeys [8]. In contrast, enrofloxacin breakpoints for the remaining bacteria are not available, thus the corresponding ciprofloxacin breakpoints reported by CLSI [8,10] were used. Breakpoints used for disk diffusion were summarized in Table 2. *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were used as control strains.

3. Results

Pellets of birds of prey tested in the present study contained a wide range of bacteria both Gram-negative and Gram-positive species. Different bacterial species were simultaneously recovered from each pellet. Among Gram-negative isolates, *E. coli* was detected from 48/73 (65.8%; 95% Confidence Interval

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