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Research Article

Physiological and potentially pathogenic microbial flora in stone curlew (*Burhinus oedicnemus*), southeastern Sicily

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Abstract

European stone curlew (*Burhinus oedicnemus*) is a Palearctic species with high conservation interest. This species nests on the ground, in open canopies with sparse herbaceous vegetation, and is typically found next to areas of intense agro-pastoral activity, where it feeds on invertebrates present in ruminant droppings. This study aimed to investigate the enteric, ocular, and oral bacterial flora of stone curlew and determine the possible occurrence of pathogenic bacteria. Furthermore, the study aimed to determine how epidemiological factors shape the bacterial flora. Fecal samples, cloacal, conjunctival and oral swabs from 61 individuals of *B. oedicnemus* were taken in three different agro-pastoral areas of the southeastern Sicily. The presence of commensal and potentially pathogenic bacteria in the samples was evaluated by standard methods. The bacteriological analysis revealed the presence of 215 Gram – and 92 Gram + strains belonging to 23 different genera (12 families). Potentially pathogenic species including *Salmonella enterica*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Enterococcus* spp. have been identified. To our knowledge, this is the first study to determine the presence of potentially pathogenic bacteria in stone curlew living in a semi-natural habitat. Some of the detected bacterial species are potentially pathogenic not only for wild species but also for domestic animals and humans. Altogether, our results suggest that stone curlew from agro-pastoral areas are being colonized with commensal or potentially pathogenic bacteria from agricultural or human sources; the prevalence of bacteria is probably influenced by environmental and alimentary factors. *B. oedicnemus* can, therefore, be considered a good indicator of environmental contamination by bacteria deriving from human activities, which are potentially threatening stone curlew and other wild birds species.

Keywords: Environmental contamination, Gram – bacteria, Gram + bacteria, wild birds

Introduction

European stone curlew *Burhinus oedicnemus* (Linneaus, 1758) (Aves, Burhinidae) is a Palearctic species classified as 'vulnerable' in Annex 1 of the Council Directive 79/409/EEC on the Conservation of wild birds and in the Italian "Red List" of breeding birds (Peronace *et al.* 2012**)** because of its continuous numerical

decrease due to the alteration and fragmentation of the habitat where it spends its reproductive period. *B. oedicnemus* has been included in several conservation programs (Hume and Kirwan 2013), which provide a systematic monitoring scheme, in place land/water protection, and conservation (BirdLife International 2018). This species mostly inhabits semi-natural and dry agricultural grasslands and steppe on poor soil (Tucker and Heath 1994). The nests consist of shallow depressions on the ground, often surrounded by a ring of stones or shells and plant material. *B. oedicnemus* females usually lay two eggs during Spring (Hume and Kirwan 2013). This bird feeds mainly on invertebrates present in ruminant droppings, mostly close to areas of intense agro-pastoral activity (Spena *et al.* 2011). The marked reduction in population size is linked to profound changes in agricultural management (Gaget *et al.* 2019). Causal factors contributing to the decline of *B. oedicnemus* include removal of hedges and other uncultivated areas, intensive grassland management, increases in the use of agro-chemicals such as pesticides, and use of fertilizers which stimulate grass growth, making the area no longer suitable for ground-nesting (Newton 2004). However, very little data is available on the dispersal and ecology of this species, partly because of its elusive behaviour, shyness and excellent camouflage (Green and Taylor 1995, Gaget *et al.* 2019). In particular, little information is present in the literature concerning its health status and potential role in the transmission of infectious diseases. Moreover, the lack of experimental studies limits our understanding of the bacterial microflora of this species. The pathogenic infectious agents isolated in stone curlew are few and are usually isolated from single sick subjects. With regard to bacterial infections in Burhinidae, only one episode of infection by *Chlamidophyla* spp. (Terskich 1964) and one by *Mycoplasma gypis* and *M. falconis* (Schmidt *et al.* 2009) are reported in the literature. Moreover, studies documenting bacterial flora of wild birds are scarce and generally limited to the detection of specific strains of bacteria that may present a potential health threat to humans or domestic animals (Benskin *et al.* 2009). It is well established that both sedentary and migratory wild birds can significantly contribute to the spread of pathogenic bacteria over large distances, transmitting the infection to individuals belonging to the same species or sympatric species (Hubálek 2004). The acquisition of more detailed knowledge of the microbial flora present in wild species can clarify some epidemiological aspects of bacterial diseases that are still poorly understood. The purpose of this study was to investigate the culturable aerobic enteric, conjunctival and oral bacterial flora of stone curlew to determine the physiological bacterial microbiota and to investigate the occurrence of pathogenic bacteria.

Material and methods

Sampling

From July to August 2018, 227 samples (49 fecal samples (F), 59 cloacal swabs (Cl), 58 conjunctival swabs (C), and 61 oral swabs (O)) were collected from 61 individuals of *B. oedicnemus* (Tables 1 and 2).

The sampling was carried out in three different areas of S-E Sicily (Italy) characterized by an agro-pastoral environment, which constitute a suitable habitat for stone curlew nesting (Mascara and Sarà 2007, Spena *et al.* 2011): the Gela Plain (Caltanissetta) (GP), the Magnisi Peninsula (Siracusa) (MP) and the farmlands around Ragusa (RG). The Gela Plain hosts the largest populations of *B. oedicnemus,* with 150-200 pairs (Tinarelli *et al.* 2009). Here stone curlew nests in areas characterized by non-irrigated and open field crops (cereals, forage legumes, and artichokes, 80.9%) mixed with pasture and garrigue areas (10.7 %) (EEA, 2000) and few arboreal crops. In the Magnisi Peninsula carbonate bedrocks are covered by *Thymbra capitata* (L.) Cav. and very sparse subnitrophilous vegetation with a winter-spring cycle (Spena *et al.* 2011). The nesting area

around Ragusa is characterized by the presence of bovine farm and a rural landscape in which the vegetation mostly consists of *Oleo sylvestris-Ceratonion siliquae* Braun-Blanquet alliance. In all of the three areas cattle and, to a lesser extent, sheep and goats are present; their droppings represent a valuable food resource for the arthropod-rich fauna.

Table 1. Number of samples taken in all study areas

	GP	MP	RG	Total
Faeces	29	6	14	49
Cloacal swabs	36	8	15	59
Conjunctival swabs	35	8	15	58
Oral swabs	36	10	15	61
Total	136	32	59	227

Table 2. Number of sampled specimens in the study areas

Sites	N. adult	N. chicks	Total
GP	31	5	36
MP	7	3	10
RG	14	1	15
Total	52	9	61

The individuals were captured from ground nests, and each bird was given a complete physical examination; any signs of illness were recorded. Swabs for a bacteriological survey were collected from each bird. The oral cavity, the cloaca, and the conjunctiva were sampled with individually packed sterile microbiological swabs premoistened with sterile saline solution 09%, inserting the tip and gently rotating it against the mucosa. The swabs were subsequently inserted into tubes containing Amies transport medium (Copan Italia, Brescia, Italy) and kept in a cooler with frozen gel packs for purposes of transport for a maximum of 8 h before culture-plate inoculation, or further storage in a refrigerator at 4 °C for a maximum of another 24 h, if no earlier processing was possible due to logistical reasons. Furthermore, whenever possible, a fresh fecal sample was taken. All birds were released immediately after sampling and returned to their nests.

Bacterial Isolation and Identification

The samples were transported in conditions of refrigeration to the Microbiology Laboratory of the Department of Veterinary Sciences – University of Messina (Italy) and examined for potentially pathogens. All samples were examined for Gram - bacteria; conjunctival and oral swabs were also submitted to bacteriological examination for Gram + bacteria. Faecal samples and cloacal swabs, after an enrichment in buffered peptone water, were streaked into MacConkey Agar plates (Biolife Italiana, Milano, Italy). Conjunctival and oral swabs were cultured in nutritious broth, then streaked into MacConkey Agar plates and into Staphylococci 110 Medium plates (Biolife Italiana, Milano, Italy). Colonies demonstrating distinctive macroscopic appearance were treated as separate organisms and isolated on new plates. Isolates were subcultured in Blood Agar plates for identification by mass spectrometry MALDI-TOF (matrix assisted laser desorption/ionization - time of fligt 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 spectra were analyzed by 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). Eighty-eight strains, unidentified by MALDI-TOF mass spectrometry, after being grown on Blood Agar Base (Biolife Italiana, Milano, Italy) and diluted in physiological solution were typed at the Laboratory of Specialized Bacteriology of the Zooprophylactic Institute of Sicily, using the traditional macro test tube method (Carter 1984, Bergey 2005). The bacteria of the genus *Bacillus* spp. (POS BAT 19/Rev 0) were characterized by carbohydrates oxidation and fermentation, motility, urease, gelatinase, nitrate reduction, and Voges Proskauer (VP) tests; *Staphylococcus* and *Streptococcus* spp. (POS BAT 05 /Rev 0 and POS BAT 30/Rev 0)

were characterized by catalase, hemolysis, coagulase, oxidase, VP tests, and carbohydrate fermentation. The enterobacteria and gram-negative glucose nonfermenting bacteria (POS BAT 09 /Rev 0) were identified by OF, mobility, catalase, oxidase, urease, and triptophanase tests and utilization/ fermentation/ oxidation of carbohydrates. The serological typing of *Salmonella* spp. strains (POS BAT 04/Rev.4) was performed following the Kauffmann-White-Le Minor method in agreement with the National Salmonellosis Center of Padua, Italy (Grimont and Weill 2007).

Results

Two hundred and twenty samples (96.9%) were positive for bacteria, and 7 (3.1%) were negative (2 feces; 3 cloacal swabs; 2 oral swabs). In 46 samples out of 119 tested (38.6%) coexistence of Gram + and Gram - bacteria was found (19 conjunctival swabs (19/58, 32.8%); 27 oral swabs (27/61, 44.3%).

Gram – isolation

Two hundred and fifteen strains were isolated from 227 samples. Of these, 186 belonged to 11 different genera of Enterobacteriaceae Group and 29 to 5 other families (Table 3).

Table 3. Results of bacteriological tests for Gram – detection in fecal samples (F), cloacal swabs (Cl), conjunctival swabs (C) and oral swabs (O)

Bacterial Family	Bacterial species	Number of isolates				
		F	Cl	C	O	Total
Aeromonadaceae	*Aeromonas sobria*				1	1
	Aeromonas hydrophila				1	1
Enterobacteriaceae	*Citrobacter amalonaticus*	5	4			9
Group	*Citrobacter diversus*			4	3	7
	Citrobacter farmeri	1	2			3
	Citrobacter freundii	3	3			6
	Citrobacter spp	13	20		1	34
	Enterobacter aerogenes	2	6		1	9
	Enterobacter asburiae			1	1	2
	Enterobacter cancerogenus	2	1		2	5
	Enterobacter cloacae	6	4	8	15	33
	Enterobacter kobei		3		2	5
	Enterobacter ludwigii		2			2
	Enterobacter spp				1	1
	Escherichia coli	14	5	4	2	25
	Escherichia hermannii				1	1
	Hafnia alvei	6	2	3	2	13
	Kluyvera ascorbata	1				1
	Leclercia adecarboxylata	1		8	3	12
	Proteus mirabilis	2	5			7
	Proteus vulgaris			1	1	2
	Providencia rettgeri		2			2
	Salmonella enterica ssp enterica	4				4
	Serratia liquefaciens	1				1
	Serratia rubidaea				1	1
	Shigella dysenteriae		1			1
Flavobacteriaceae	*Chryseobacterium indologenes*			3	1	4
Pseudomonadaceae	*Pseudomonas aeruginosa*	2	4		1	7
	Pseudomonas putida				1	1
	Pseudomonas stutzeri		1	7	5	13
Xanthomonadaceae	*Stenotrophomonas maltophilia*				1	1
Vibrionaceae	*Vibrio mimicus*	1				1
	Total	64	65	39	47	215

The most commonly isolated species was *Citrobacter* spp (34 strains, 15.8%), followed by *Enterobacter cloacae* (33 strains, 15.3%), *Escherichia coli* (25 strains, 11.6%), *Hafnia alvei* and *Pseudomonas stutzeri* (13 strains, 6%) and *Leclercia adecarboxylata* (12 strains, 5.6%). Potentially pathogenic species including *Salmonella enterica*, *Pseudomonas aeruginosa* and *Shigella dysenteriae* have also been identified. *Escherichia coli*, *Enterobacter cloacae* and *Hafnia alvei* were the only species detected in all 4 sampling locations (feces, cloaca, eye and beak). The 4 isolated *Salmonella* strains belonged to three different serovars of *Salmonella enterica* ssp. *enterica*: Franken (9,12 z6; z67) (two strains), Braenderup (6,7,14; e,h; e,n,z15) and Tomegbe (1,42; b; e,n,x,z15) (one strain). In most samples (138; 60.8%) a single bacterial strain was isolated; in 34 samples 2 strains (15%) and in 3 samples 3 strains (1.3%). Table 4 shows the results of bacteriological tests for sampling site.

Table 4. Distribution of Gram - isolated strains from fecal samples (F), cloacal swabs (Cl), conjunctival swabs (C) and oral swabs (O) in sampling sites

	GP	MP	RG
F	5 *Citrobacter amalonaticus*	1 *Citrobacter* spp	8 *Citrobacter* spp
	1 *Citrobacter farmeri*	1 *Enterobacter cloacae*	1 *Enterobacter cancerogenus*
	3 *Citrobacter freundii*	1 *Escherichia coli*	2 *Enterobacter cloacae*
	4 *Citrobacter* spp	1 *Hafnia alvei*	7 *Escherichia coli*
	2 *Enterobacter aerogenes*	1 *Proteus mirabilis*	1 *Hafnia alvei*
	1 *Enterobacter cancerogenus*	1 *Serratia liquefaciens*	2 *Salmonella enterica*
	3 *Enterobacter cloacae*	1 *Vibrio mimicus*	
	6 *Escherichia coli*		
	4 *Hafnia alvei*		
	1 *Kluyvera ascorbata*		
	1 *Leclercia adecarboxylata*		
	2 *Pseudomonas aeruginosa*		
	1 *Proteus mirabilis*		
	2 *Salmonella enterica*		
Cl	4 *Citrobacter amalonaticus*	1 *Citrobacter* spp	1 *Citrobacter farmeri*
	1 *Citrobacter farmeri*	3 *Enterobacter cloacae*	2 *Citrobacter freundii*
	1 *Citrobacter freundii*	2 *Enterobacter kobei*	8 *Citrobacter* spp
	10 *Citrobacter* spp	1 *Escherichia coli*	1 *Enterobacter aerogenes*
	5 *Enterobacter aerogenes*	1 *Providencia rettgeri*	1 *Enterobacter cloacae*
	1 *Enterobacter cancerogenus*		1 *Enterobacter ludwigii*
	1 *Enterobacter kobei*		1 *Escherichia coli*
	1 *Enterobacter ludwigii*		1 *Providencia rettgeri*
	3 *Escherichia coli*		
	2 *Hafnia alvei*		
	5 *Proteus mirabilis*		
	3 *Pseudomonas aeruginosa*		
	1 *Pseudomonas stutzeri*		
	1 *Shigella dysenteriae*		
C	2 *Chryseobacterium indologenes*	1 *Enterobacter cloacae*	1 *Chryseobacterium indologenes*
	4 *Citrobacter diversus*	1 *Leclercia adecarboxylata*	5 *Enterobacter cloacae*
	1 *Enterobacter asburiae*	1 *Pseudomonas stutzeri*	2 *Escherichia coli*
	2 *Enterobacter cloacae*		1 *Hafnia alvei*
	2 *Escherichia coli*		2 *Leclercia adecarboxylata*
	2 *Hafnia alvei*		1 *Pseudomonas stutzeri*
	5 *Leclercia adecarboxylata*		
	1 *Proteus vulgaris*		
	5 *Pseudomonas stutzeri*		
O	1 *Aeromonas hydrophila*	1 *Chryseobacterium indologenes*	1 *Enterobacter cancerogenus*
	1 *Aeromonas sobria*	2 *Enterobacter cloacae*	5 *Enterobacter cloacae*
	3 *Citrobacter diversus*	1 *Enterobacter kobei*	1 *Escherichia hermannii*

Continued table 4. Distribution of Gram - isolated strains from fecal samples (F), cloacal swabs (Cl), conjunctival swabs (C) and oral swabs (O) in sampling sites

	1 *Citrobacter* spp	1 *Pseudomonas putida*	1 *Hafnia alvei*
	1 *Enterobacter aerogenes*		1 *Pseudomonas aeruginosa*
	1 *Enterobacter asburiae*		2 *Pseudomonas stutzeri*
	1 *Enterobacter cancerogenus*		
	8 *Enterobacter cloacae*		
	1 *Enterobacter kobei*		
	1 *Enterobacter* spp		
	2 *Escherichia coli*		
	1 *Hafnia alvei*		
	3 *Leclercia adecarboxylata*		
	1 *Proteus vulgaris*		
	3 *Pseudomonas stutzeri*		
	1 *Stenotrophomonas maltophila*		
	1 *Serratia rubidea*		
Total	130 strains/136 samples	23 strains/32 samples	60 strains/59 samples

Gram + isolation

Ninety-two strains were isolated from 119 samples. Of these, 53 (57.6%) belonged to Bacillaceae Family, 30 (32.6%) to Staphylococcaceae Family and 9 (9.8%) to 3 other Families (Table 5).

Table 5. Results of bacteriological tests for Gram + detection in conjunctival (C) and oral swabs (O)

Bacterial Family	Bacterial species	Number of isolates		
		C	O	Total
Bacillaceae	*Bacillus brevis*	1	1	2
	Bacillus cereus ssp mycoides		1	1
	Bacillus fastidiosus	1		1
	Bacillus licheniformis	23	16	39
	Bacillus megaterium	5		5
	Bacillus pumilus	1		1
	Bacillus spp	1		1
	Bacillus subtilis		2	2
	Exiguobacterium acetylicum		1	1
Enterococcaceae	*Enterococcus faecalis*	1	4	5
	Enterococcus faecium	2		2
Lactobacillaceae	*Lactobacillus rhamnosus*		1	1
Paenibacillaceae	*Paenibacillus durus*	1		1
Staphylococcaceae	*Staphylococcus aureus*	1	2	3
	Staphylococcus cohnii ssp cohnii		1	1
	Staphylococcus epidermidis		1	1
	Staphylococcus gallina rum	1	1	2
	Staphylococcus hominis	2	1	3
	Staphylococcus lentus	1		1
	Staphylococcus saprophyticus		1	1
	Staphylococcus sciuri	4	11	15
	Staphylococcus warneri	1	1	2
	Staphylococcus xylosus		1	1
Total		46	46	92

The most commonly isolated species were *Bacillus licheniformis* (39 strains, 42.4%) and *Staphylococcus sciuri* (15 strains, 16.3%). Potentially pathogenic species including *Staphylococcus aureus* and *Enterococcus faecium* have also been identified. Table 6 shows the results of bacteriological tests for sampling sites.

Table 6. Distribution of Gram + isolated strains from conjunctival (C) and oral swabs (O) in sampling sites

	GP	MP	RG
C	1 *Bacillus brevis* 1 *Bacillus fastidiosus* 17 *Bacillus licheniformis* 1 *Bacillus megaterium* 1 *Bacillus pumilus* 3 *Enterococcus faecalis* 1 *Enterococcus faecium* 1 *Staphylococcus hominis* 3 *Staphylococcus sciuri* 1 *Staphylococcus warneri*	2 *Bacillus licheniformis* 1 *Enterococcus faecium* 1 *Paenibacillus durus* 1 *Staphylococcus hominis* 1 *Staphylococcus aureus* 1 *Staphylococcus hyicus* ssp *chromogenes* 1 *Staphylococcus sciuri*	1 *Bacillus* spp 4 *Bacillus licheniformis* 4 *Bacillus megaterium* 1 *Staphylococcus gallinarum* 1 *Staphylococcus lentus*
O	1 *Bacillus brevis* 1 *Bacillus cereus* ssp *mycoides* 9 *Bacillus licheniformis* 1 *Exiguobacterium acetylicum* 1 *Lactobacillus rhamnosus* 1 *Staphylococcus epidermidis* 1 *Staphylococcus gallinarum* 1 *Staphylococcus hominis* 1 *Staphylococcus saprophyticus* 8 *Staphylococcus sciuri* 1 *Staphylococcus xylosus*	2 *Bacillus licheniformis* 1 *Bacillus subtilis* 1 *Staphylococcus aureus* 1 *Staphylococcus cohni* ssp *cohni* 1 *Staphylococcus sciuri* 1 *Staphylococcus warneri*	5 *Bacillus licheniformis* 1 *Bacillus subtilis* 1 *Enterococcus faecalis* 1 *Staphylococcus aureus* 2 *Staphylococcus sciuri*
Total	56 strains/71 samples	15 strains/18 samples	21 strains/30 samples

In tables 7 and 8, the results of the bacteriological test have been reported, grouped according to the origin of the sample.

Table 7. Strains from the intestinal flora isolated from fecal samples and cloacal swabs

Feces		Cloacal swabs	
Bacterial species	N. strains	Bacterial species	N. strains
Escherichia coli	14	*Citrobacter* spp	20
Citrobacter spp	13	*Enterobacter aerogenes*	6
Enterobacter cloacae	6	*Escherichia coli*	5
Hafnia alvei	6	*Proteus mirabilis*	5
Citrobacter amalonaticus	5	*Citrobacter amalonaticus*	4
Salmonella enterica	4	*Enterobacter cloacae*	4
Citrobacter freundii	3	*Pseudomonas aeruginosa*	4
Enterobacter aerogenes	2	*Citrobacter freundii*	3
Enterobacter cancerogenus	2	*Enterobacter kobei*	3
Proteus mirabilis	2	*Citrobacter farmeri*	2
Pseudomonas aeruginosa	2	*Enterobacter ludwigii*	2
Citrobacter farmeri	1	*Hafnia alvei*	2
Kluyvera ascorbata	1	*Providencia rettgeri*	2
Leclercia adecarboxylata	1	*Enterobacter cancerogenus*	1
Serratia liquefaciens	1	*Shigella dysenteriae*	1
Vibrio mimicus	1	*Pseudomonas stutzeri*	1
Total	64	Total	65

Table 8. Strains isolated from oral and conjunctival swabs

Oral microflora		Conjunctival microflora	
Bacterial species	N. strains	Bacterial species	N. strains
Bacillus licheniformis	16	*Bacillus licheniformis*	23
Enterobacter cloacae	15	*Enterobacter cloacae*	8
Staphylococcus sciuri	11	*Leclercia adecarboxylata*	8
Pseudomonas stutzeri	5	*Pseudomonas stutzeri*	7
Streptococcus faecalis	4	*Bacillus megaterium*	5
Citrobacter diversus	3	*Citrobacter diversus*	4
Leclercia adecarboxylata	3	*Escherichia coli*	4
Bacillus subtilis	2	*Staphylococcus sciuri*	4
Enterobacter cancerogenus	2	*Hafnia alvei*	3
Enterobacter kobei	2	*Chryseobacterium indologenes*	3
Escherichia coli	2	*Staphylococcus hominis*	2
Hafnia alvei	2	*Enterococcus faecium*	2
Staphylococcus aureus	2	*Bacillus brevis*	1
Aeromonas sobria	1	*Bacillus fastidiosus*	1
Aeromonas hydrophila	1	*Bacillus pumilus*	1
Bacillus brevis	1	*Bacillus* spp	1
Bacillus cereus subsp *mycoides*	1	*Enterobacter asburiae*	1
Chryseobacterium indologenes	1	*Enterococcus faecalis*	1
Citrobacter spp	1	*Paenibacillus durus*	1
Enterobacter aerogenes	1	*Proteus vulgaris*	1
Enterobacter asburiae	1	*Staphylococcus aureus*	1
Enterobacter spp.	1	*Staphylococcus gallinarum*	1
Escherichia hermannii	1	*Staphylococcus lentus*	1
Exiguobacterium acetylicum	1	*Staphylococcus warneri*	1
Lactobacillus rhamnosus	1		
Proteus vulgaris	1		
Pseudomonas aeruginosa	1		
Pseudomonas putida	1		
Serratia rubidaea	1		
Staphylococcus cohnii ssp *cohnii*	1		
Staphylococcus epidermidis	1		
Staphylococcus gallinarum	1		
Staphylococcus hominis	1		
Staphylococcus saprophyticus	1		
Staphylococcus warneri	1		
Staphylococcus xylosus	1		
Stenotrophomonas maltophilia	1		
Total	93	Total	85

Enteric microflora

Of the 16 species isolated from fecal samples and the 16 isolated from cloacal swabs, only 11 are in common. Strains of *Salmonella* spp were only detected in faecal samples. The number of strains of *Escherichia coli* isolated in the faeces was higher than in the cloacal swabs.

Oral and conjunctival microflora

Thirty-seven different bacterial species were isolated in the oral cavity. The most commonly isolated species was *Bacillus licheniformis* (16 strains, 17.2%). Twenty-four different bacterial species were isolated in the conjunctival sac, the most common of which was *Bacillus licheniformis* (23 strains, 27.1%).

Discussion

Detection of potentially pathogenic bacteria

Little data is available about the prevalence of potentially pathogen bacterial species in healthy wild birds, and even fewer in birds belonging to the Burhinidae. To our knowledge,

this is the first study to determine the presence of potentially pathogenic bacteria in stone curlew living in semi-natural habitat. Some of the detected bacterial species can be considered potentially pathogenic not only for wild species but also for domestic animals and for humans. The presence of microorganisms typically associated with avian disease such as *Aeromonas hydrophila*, *Salmonella* spp, *Pseudomonas aeruginosa* and *Escherichia coli* in apparently healthy individuals indicates that wild birds of the examined species harbour potentially pathogenic subclinical microorganisms. Strains of *Salmonella enterica*, *Shigella dysenteriae* and *Escherichia coli* were isolated from the individuals inhabiting nest n. 22 (GP), who displayed an abraded tail. This can be indicative of a morbid state, caused by these pathogenic enterobacteria, which can cause severe discomfort in the cloacal region (Montesinos 2016). *Salmonella* spp. is a worldwide-distributed pathogen which constitutes a potential risk for public health. This microorganism is considered a true multi-host pathogen with a long environmental persistence (Murray 1991). *Salmonella* spp have been isolated from numerous free-ranging avian species, including psittacine, gallinaceous birds, waterfowl, and raptors (Hudson *et al.* 2000). The prevalence of infection ranges from 1.9% in Falconiformes to 8.7% in ring-billed gulls (Mikaelian *et al.* 1997). In agreement with previous research, we found a prevalence of 6.6% (4/61 individuals examined). In previous studies on wild birds, no strains of *Salmonella* spp and *Escherichia coli* have been isolated (Foti *et al.* 2017). This result can partially be explained by the diet of the birds included in these surveys. *Salmonella* spp and *Escherichia coli* are most commonly found in omnivorous and carnivorous birds (Bangert *et al.* 1988), whereas graminivorous birds, such as many passerines, have much lower prevalence (Brittingham *et al.* 1988, Steele *et al.* 2005). Brittingham *et al.* (1988) showed that in a population of passerines *Escherichia coli* was isolated only in the specimens picking seeds out of the horse manure. Altogether, our results suggest that stone curlew from agro-pastoral areas are being colonized with commensal or potentially pathogenic bacteria from agricultural or human sources; the prevalence of bacteria is probably influenced by environmental and alimentary factors. The presence of numerous bacteria belonging to the genera *Bacillus* spp and *Enterobacter* spp, especially in samples taken from the conjunctiva and the oral cavity, suggest that they derive from environmental contamination. *Bacillus licheniformis*, the most commonly isolated species, is widely distributed in the environment as a facultative anaerobic microorganism (Ludwig *et al.* 2009). In fact, although *Bacillus* spp are commonly considered soil organisms, they are increasingly found in hospitalized patients and appear sufficiently virulent to behave as pathogens/opportunistic pathogens for humans (Celandroni *et al.* 2016). While opportunistic infections with *B. lichenlformis* are rare in humans, bovine infections are fairly common, and the bacillus has been repeatedly reported to be responsible for placentitis with subsequent abortion in pregnant cows (Agerholm *et al.* 1995). Other isolated bacteria seem to be saprophytic water and soil organisms that rarely act as human and animal pathogens: *Aeromonas sobria* and *A. hydrophila* are ubiquitous, waterborne microorganisms that have often been implicated as the causative agents of clinical illnesses in humans (Lai *et al.* 2007), in both cold-blooded and warm-blooded animals (Janda and Abbott 2010) but especially in birds (Glunder and Siegmann 1989). Janda and Abbott (2010) state that animals are an ever-present reservoir for the introduction and exchange of *Aeromonas* species in the environmental microbial world. *Chryseobacterium indologenes* is a rod organism found in soil and plants. Although this bacterium only rarely causes human disease, it is sometimes found in food and water sources, usually in hospitals as a nosocomial

transinfection (Hsueh *et al.* 1996, Chen *et al.* 2013); *Stenotrophomonas maltophilia* is an environmental microrganism living in aqueous habitats, considered an emerging global potential pathogen. The increasing incidence of nosocomial and community-acquired *S. maltophilia* infections is of particular concern for immunocompromised individuals, as this bacterial pathogen is associated with a particularly high mortality rate (Brooke 2012). The same concern is raised by the presence of *Exiguobacterium acetylicum*, which in 2007 was reported to be responsible for hospital-acquired infection (Keynan *et al.* 2007). Due to the eating habits of stone curlew, the detection of a strain of *Vibrio mimicus* in an individual living in the Magnisi Peninsula was particularly surprising, as this species is usually isolated in waters and shellfish and is normally associated with waterbirds. The infection could be related to the presence of *V. mimicus* in the waterways of the Peninsula that are used by stone curlew to drink. *V. mimicus* is pathogenic for humans, in which it can cause serious episodes of cholera-like diarrhea and otitis (Davis *et al.* 1981, Chowdhury *et al.* 1987, Shi *et al.* 1998). Finally, the comparison between isolates from feces and cloacal swabs suggests that the latter are not a completely reliable sampling method to analyze the intestinal microbial flora because it can underestimate the presence of potentially pathogenic bacteria such as *Salmonella* spp and *Escherichia coli*.

Epidemiological considerations

Several studies show that wild birds can acquire pathogenic bacteria by feeding on raw sewage and garbage, and can spread these agents to humans directly or by contaminating commercial poultry operations (Abulreesh *et al.* 2007, Radhouni *et al.* 2012). Wild birds can also acquire pathogenic bacteria from farms and spread them along migration routes (Reed *et al.* 2003). This form of environmental contamination increases the risk of infection, with water supplies being the most likely channel of transmission (Abulreesh *et al.* 2007, Pindi *et al.* 2013, Vittecoq *et al.* 2016). In GP, MP and RG livestock on many farms rely on small ponds, streams and other untreated water sources for at least part of their drinking water. In the investigated areas, large numbers of stone curlew roosting on or near water may contribute to its contamination and to the spread of disease to other animals. The detection of potentially pathogenic bacteria such as *S. enterica*, *V. mimicus*, *Aeromonas* spp and *S. aureus* in stone curlew shows that these birds can play an essential role in the ecology and circulation of these microorganisms. Our results highlight the importance of taking more effective measures for the preservation of wild birds in their breeding areas, also taking into account the possibility, demonstrated in the past, that some avian pathogens can be activated during the breeding season of their hosts by sex hormones (Haberkorn 1968, Hubálek 2004).

Conclusion

Since the microbiome of each species is strongly influenced by its life habits (Brittingham *et al.* 1988), *B. oedicnemus* can be considered a good indicator of environmental contamination by potentially pathogenic bacteria, deriving from human activities and above all from breeding farms. It can, therefore, be regarded as sentinel species to be used as an environmental health indicator.

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