

PREVALENCES OF ZONOTIC BACTERIA AMONG SEABIRDS IN REHABILITATION CENTERS ALONG THE PACIFIC COAST OF CALIFORNIA AND WASHINGTON, USA

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ABSTRACT: Many seabirds are rehabilitated annually by wildlife rehabilitation centers along the Pacific Coast, USA. Although various strains of zoonotic bacteria have been isolated from seabirds, risks to rehabilitators at these centers have not been well documented. From November 2001 through January 2003, we determined the prevalence of detectable enteric fauna by isolation and characterization of Gram-negative bacteria from cloacal swabs taken from 26 common murres (*Uria aalge*), 49 gulls (*Larus* spp.), and 14 other seabirds treated by rehabilitators in California and Washington (USA). At least 25 bacterial species were identified, including multiple strains of *Escherichia coli*, as well as *Enterobacter cloacae*, *Citrobacter freundii*, and *Klebsiella pneumoniae*. Antibiotic resistance was found in 13 of 19 bacterial isolates tested, including *E. coli*, *K. pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Potential transfer of these bacteria poses a risk to wildlife rehabilitators and to seabirds in these centers, as well as to free-ranging birds.

Key words: Antibiotic resistance, rehabilitation centers, seabirds, zoonotic bacteria.

INTRODUCTION

Each year, thousands of seabirds affected by oil contamination, injury, or illness are brought to rehabilitation centers along the Pacific Coast of the continental USA. These centers are staffed by veterinarians, technicians, and volunteers. The birds under the care of the centers are treated and, when possible, released back to the wild. Seabirds are particularly vulnerable to the effects of oil contamination (Jessup and Leighton, 1996); in larger oil spills, hundreds of seabirds may be treated at a center in a matter of days or weeks. Two of the most abundant avian groups on the Pacific Coast of the USA are common murres (*Uria aalge*) and gulls (*Larus* spp.) (Sibley, 2000). Common murres frequently are affected by oil during marine spills (Debacker et al., 1997), and both common murres and gulls routinely come into contact with humans at rehabilitation centers on the Pacific Coast (Smith et al., 2002).

Rehabilitation workers come into close contact with these birds, their feces and other body fluids, and soiled bedding ma-

terials. Animals stressed by illness, capture, and captivity are more likely to shed potentially pathogenic bacteria than are healthy, free-ranging birds (Smith et al., 2002). Because many enteric zoonotic bacteria are transmitted by the fecal-oral route, seabirds in rehabilitation centers may serve as sources of disease agents. Conversely, seabirds also may acquire pathogenic bacteria at rehabilitation centers, and once they are released back to the wild, they could spread potential pathogens to free-ranging seabird populations (Ziegerer et al., 2002).

Zoonotic Gram-negative bacteria previously isolated from seabird species include, but are not limited to, *Salmonella* spp., *Campylobacter* spp., and *Yersinia* spp. (Kapperud and Rosef, 1983). In humans, these bacteria can cause gastroenteritis, respiratory symptoms, septicemia, and even mortality; for example, the multiple and ubiquitous strains of *Salmonella* pose a considerable public health threat and are often associated with disease outbreaks (Velge et al., 2005).

The liberal use of antibiotics in medi-

cine and animal husbandry over the course of decades has fostered the selection of resistant bacteria (Tomasz, 1994). The rise in multi-drug 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 (Williams and Heymann, 1998). Ziegerer et al. (2002) found that the number of antibiotic-resistant strains of bacteria isolated from birds at Tufts University Veterinary Clinic (Grafton, Massachusetts, USA) increased while birds were in the clinic. To better assess the risks of exposure to zoonotic bacteria by rehabilitation workers, free-living wild birds, and birds brought to rehabilitation centers, it is essential to first establish the zoonotic bacteria carried by birds in rehabilitation centers as well as any antibiotic resistance carried in these bacteria.

Our objectives were to survey seabirds in rehabilitation centers to compare prevalences of enteric species between and among groups of common seabirds, as well as to compare bacterial species richness between rehabilitation centers (with an *n* value of at least six per bird family). In addition, we investigated the potential pathogenicity to humans of selected isolates of *Escherichia coli* by testing for the presence of toxin genes. Finally, we tested antibiotic resistance in isolates that were selected to represent a variety of bacterial species as well as possible variation in strains among host species. This information was used to assess which bacteria might pose a risk to rehabilitators working with seabirds and others birds in these centers, as well as free-living wild birds.

MATERIALS AND METHODS

Seabirds were sampled at rehabilitation centers in California and Washington from November 2001 to January 2003 (Steele, 2003). Eighty-nine birds representing 16 species were sampled, with the highest numbers from the families Laridae and Alcidae (Table 1). Birds were sampled from the Humboldt Wildlife

Care Center, Arcata, California (USA; 40.72359°N, 123.86225°W), in November and December 2001; the San Francisco Bay Oiled Wildlife Care and Education Center, Cordelia, California (38.24570°N, 122.00984°W), in January, April, May, and June of 2002; the Progressive Animal Welfare Society (PAWS) Wildlife Center, Lynwood, Washington (USA; 47.62691°N, 122.12881°W), in January, April, and August 2002, and January 2003; and the Los Angeles Oiled Bird Care and Education Center, San Pedro, California (33.733894°N, 118.291425°W), in July 2002. Any birds that had been treated with antibiotics were excluded from the study.

Birds were identified to species and age category (juvenile or adult) (Sibley, 2000) and examined briefly for ectoparasites. Cloacal swabs were obtained by inserting a sterile Cultureswab with Cary-Blair transport medium (Becton Dickinson and Company, Sparks, Maryland, USA) into the cloaca and gently rotating the tip against the mucosa. Swabs were then immediately returned to the sleeve. Samples were kept at ambient temperature (approximately 25 C) until inoculation. Fifty-five samples were inoculated <48 hr (62%) from the time of sampling, and 34 samples were inoculated >48 hr (38%) from the time of sampling.

A fecal suspension in 1.0 ml sterile saline (0.85% NaCl) was made for each Cultureswab. The suspension was plated onto four different culture media. The initial isolation media used were MacConkey agar (Difco, Becton Dickinson and Company), Levine EMB agar (BBL, Becton Dickinson and Company), trypticase soy agar with 0.5% yeast extract (TSA/YE; Difco, Becton Dickinson and Company), and tetrathionate broth with iodine (Difco, Becton Dickinson and Company). All plates were examined after 24, 48, and 72 hr of incubation at 37 C in an aerobic chamber. After 24 hr, subcultures were made from the tetrathionate broth onto *Salmonella-Shigella* agar (BBL, Becton Dickinson and Company). Representatives of all distinct colony types were Gram stained and subcultured for purity onto a TSA/YE plate. We stored each purified isolate on TSA/YE slants at 4 C.

Each isolate was inoculated into Kligler's Iron Agar (Difco, Becton Dickinson and Company) and SIM medium (BBL, Becton Dickinson and Company) to test for hydrogen sulfide production, glucose and lactose fermentation, indole production, and motility. Capacity to grow on MacConkey agar was tested for all isolates not originally cultured from MacConkey agar. Representative Gram-negative isolates of all distinct organisms were identified using the API-20E differentiation system

TABLE 1. Birds sampled from rehabilitation centers in California and Washington (USA), November 2001 to January 2003.

Family	Species	No. of birds from each center			
		Cordelia ^a	Lynwood ^b	Arcata ^c	Total
Gaviidae	Red-throated loon (<i>Gavia stellata</i>)	1	0	0	1
	Pacific loon (<i>Gavia pacifica</i>)	1	0	0	1
Podicipedidae	Eared grebe (<i>Podiceps nigricollis</i>)	1	0	0	1
	Pied-billed grebe (<i>Podilymbus podiceps</i>)	4	0	0	4
	Western grebe (<i>Aechmophorus occidentalis</i>)	0	0	1	1
Procellariidae	Sooty shearwater (<i>Puffinus griseus</i>)	0	1	0	1
Phalacrocoracidae	Brandt's cormorant (<i>Phalacrocorax penicillatus</i>)	1	0	1	2
Anatidae	Red-breasted merganser (<i>Mergus serrator</i>)	1	0	0	1
Laridae	Ring-billed gull (<i>Larus delawarensis</i>)	1	0	0	1
	California gull (<i>Larus californicus</i>)	2	1	0	3
	Herring gull (<i>Larus argentatus</i>)	0	0	3	3
	Thayer's gull (<i>Larus thayeri</i>)	0	3	0	3
	Western gull (<i>Larus occidentalis</i>)	9	3	1	17 ^d
	Glaucous-winged gull (<i>Larus glaucescens</i>)	0	16	0	16
	Other gulls (<i>Larus</i> spp.)	0	4	2	6
Alcidae	Common murre (<i>Uria aalge</i>)	19	6	1	26
	Rhinoceros auklet (<i>Cerorhinca monocerata</i>)	2	0	0	2
Total		42	34	9	89 ^d

^a San Francisco Bay Oiled Wildlife Care & Education Center, Cordelia, California, USA (January, April, May, June 2002).

^b Progressive Animal Welfare Society Wildlife Center, Lynwood, Washington, USA (January, April, August 2002).

^c Humboldt Wildlife Care Center, Arcata, California, USA (November, December 2001).

^d Additionally, samples were collected from four Western gulls at the Los Angeles Oiled Bird Care & Education Center, San Pedro, California, USA, in July 2002.

(BioMérieux Vitek, Inc., Hazelwood, Missouri, USA). Growth at 42 C was used to confirm the identification of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Anonymous, 1992).

Initial typing of *Salmonella* spp. isolates was performed at the Veterinary Medical Teaching Hospital at the University of California in Davis, California (Smith et al., 2002). *Salmonella* spp. serotyping was performed by the National Animal Disease Laboratory (Ames, Iowa, USA) (Edwards and Ewing, 1986).

Prevalences (number of birds infected/number of birds examined) of bacteria isolated were compared between groups of birds with a Fisher Exact test using Number Cruncher Statistical Systems statistical software (Hintze, 2001). We used a two-way general linear model analysis of variance (ANOVA) to examine differences in species richness of bacteria isolated from common murre and gulls and between two rehabilitation centers: San Francisco Bay Oiled Wildlife Care and Education Center and the PAWS Wildlife Center. *P* values of 0.05 or less were considered significant. Species richness values were square-root transformed for normality.

Forty-eight *E. coli* isolates were selected by

their API 20E codes to represent the variability within isolates recovered from all the birds, as well as between species of birds. These were tested for the presence of the following toxin genes at the Gastroenteric Disease Center at Pennsylvania State University (University Park, Pennsylvania, USA): presence of shiga-toxin I and II (Witham et al., 1996), heat-stable toxin a, heat-stable toxin b, and heat-labile toxin (Ojeniyi et al., 1994), entero-attaching and effacing gene (EAE) (Gannon et al., 1993), and cytotoxic necrotizing factor 1 and 2 (Blanco et al., 1996). Strains positive for toxin genes were checked for the presence of alpha intimin (Reid et al., 1999) and were serotyped (Orskov et al., 1977).

Nineteen isolates from 15 birds were tested for resistance against 16 antibiotics by IDEXX Veterinary Services (Sacramento, California, USA) on an automated system (VITEK, BioMérieux Vitek, Inc.) using MIC breakpoints established by the National Committee for Clinical Laboratory Standards (Aucoin, 2000). Isolates of *E. coli*, *K. pneumoniae*, *Enterobacter cloacae*, *A. baumannii*, *Ps. aeruginosa*, and *Salmonella* spp. were tested against the antibiotics amikacin, augmentin, ampicillin, carbenicillin,

TABLE 2. Prevalences of Gram-negative bacteria from seabird families in rehabilitation centers of California and Washington (USA), November 2001 to January 2003.

Bacteria isolated	Isolates from each bird family				Total n=89
	Alcidae n=28	Laridae n=49	Podicipedidae n=6	Misc. ^a n=6	
<i>Citrobacter freundii</i>	9 (32) ^b	6 (12)	2 (33)	0 (0)	17 (19)
<i>Citrobacter youngae</i>	0 (0)	4 (8)	2 (33)	0 (0)	6 (7)
Other <i>Citrobacter</i> spp.	1 (4)	0 (0)	0 (0)	1 (17)	2 (2)
<i>Enterobacter aerogenes</i>	0 (0)	3 (6)	2 (33)	0 (0)	5 (6)
<i>Enterobacter amnigenus</i>	0 (0)	0 (0)	2 (33)	0 (0)	2 (2)
<i>Enterobacter cloacae</i>	2 (7)	3 (6)	1 (17)	2 (33)	8 (9)
<i>Enterobacter sakazakii</i>	1 (4)	0 (0)	0 (0)	0 (0)	1 (1)
<i>Escherichia coli</i>	24 (86)	47 (96)	2 (33)	6 (100)	79 (89)
<i>Escherichia fergusonii</i>	9 (32)	0 (0)	0 (0)	0 (0)	9 (10)
<i>Klebsiella oxytoca</i>	0 (0)	0 (0)	2 (33)	0 (0)	2 (2)
<i>Klebsiella pneumoniae</i>	11 (39)	5 (10)	2 (33)	1 (17)	19 (21)
<i>Klebsiella terrigena</i>	4 (14)	2 (4)	0 (0)	0 (0)	6 (7)
<i>Morganella morganii</i>	1 (4)	1 (2)	0 (0)	0 (0)	2 (2)
<i>Proteus mirabilis</i>	0 (0)	4 (8)	0 (0)	1 (17)	5 (6)
<i>Proteus penneri</i>	0 (0)	1 (2)	0 (0)	0 (0)	1 (1)
Other <i>Proteus</i> spp.	1 (4)	26 (53)	3 (50)	2 (33)	32 (36)
<i>Providencia alcalifaciens</i>	0 (0)	2 (4)	0 (0)	0 (0)	2 (2)
Other <i>Providencia</i> spp.	0 (0)	1 (2)	0 (0)	0 (0)	1 (1)
<i>Salmonella</i> serotype Newport	0 (0)	1 (2)	0 (0)	0 (0)	1 (1)
<i>Pseudomonas aeruginosa</i>	2 (7)	5 (10)	0 (0)	1 (17)	8 (9)
Other <i>Pseudomonas</i> spp.	2 (7)	0 (0)	0 (0)	0 (0)	2 (2)
<i>Acinetobacter baumannii</i>	2 (7)	5 (10)	2 (33)	1 (17)	11 (10)
Other <i>Acinetobacter</i> spp.	2 (7)	1 (2)	0 (0)	0 (0)	3 (3)
<i>Aeromonas hydrophila</i>	0 (0)	4 (8)	0 (0)	0 (0)	4 (5)
<i>Aeromonas salmonicida</i>	0 (0)	1 (2)	0 (0)	0 (0)	1 (1)
All Gram negative	27 (96)	48 (98)	5 (83)	6 (100)	86 (97)

^a From Families Gaviidae, Procellariidae, Phalacrocoracidae, and Anatidae.

^b Number positive (%).

ceftazidime, ceftiofur, cephalothin, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, piperacillin, tetracycline, ticarcillin, tobramycin, and tribissen.

RESULTS

Gram-negative enteric bacteria were isolated from 86 of 89 birds (97%) (Table 2). A mean (\pm SD) of 2.64 (\pm 1.34) different bacterial species was isolated per bird, with a maximum of six isolated from four different birds, two Western gulls (*Larus occidentalis*) and two pied-billed grebes (*Podilymbus podiceps*) from Cordelia. The most frequently isolated species of bacteria, *E. coli*, was cultured from 79 birds. The next five most frequently isolated bacteria, in decreasing order of frequency,

were *Proteus* spp., *K. pneumoniae*, *Citrobacter freundii*, *Escherichia fergusonii*, and *A. baumannii* (Table 2). *Salmonella enterica* serotype Newport was isolated from one Western gull from Cordelia.

There were no significant differences in prevalences of any bacteria between the two most common gull species, Western gull ($n=17$) and glaucous-winged gull ($n=16$), by a Fisher Exact test ($P>0.05$). Based on a Fisher Exact test, *E. fergusonii* ($n=75$, for gulls and common murre combined, $P=0.00009$) and *K. pneumoniae* ($n=75$, $P=0.014$) were significantly higher in common murre ($n=26$) than gulls ($n=49$). Prevalence of *Proteus* spp. ($n=75$, $P=0.00001$) was significantly high-

TABLE 3. Bacterial species isolated from seabirds in rehabilitation centers and tested for resistance to antibiotics. Bacteria were isolated from seabirds in rehabilitation centers in California and Washington (USA), November 2001 to January 2003.

Bacterial isolate	Host species	Antibiotic resistance ^a														Total Resistant		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14		15	16
<i>Escherichia coli</i>	Western gull	— ^b	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
<i>Escherichia coli</i>	Western gull	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
<i>Escherichia coli</i>	Common murre	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
<i>Escherichia coli</i> type O57	Herring gull	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
<i>Escherichia coli</i>	Undifferentiated gull	—	I	I	—	—	—	R	—	—	—	—	—	—	—	—	—	3
<i>Escherichia coli</i>	Glaucous-winged gull	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
<i>Klebsiella pneumoniae</i>	Glaucous-winged gull	—	—	R	R	—	—	—	—	—	—	—	—	—	R	—	—	3
<i>Klebsiella pneumoniae</i>	Glaucous-winged gull	—	—	R	R	—	—	—	—	—	—	—	—	—	R	—	—	3
<i>Klebsiella pneumoniae</i>	Red-breasted merganser	—	—	R	R	—	—	—	—	—	—	—	—	—	R	—	—	3
<i>Klebsiella pneumoniae</i>	Pied-billed grebe	—	—	R	R	—	—	—	—	—	—	—	—	—	R	—	—	3
<i>Klebsiella pneumoniae</i>	Common murre	—	—	R	R	—	—	—	—	—	—	—	—	—	R	—	—	3
<i>Enterobacter cloacae</i>	Pacific loon	—	R	R	—	—	R	—	—	—	—	—	—	—	R	—	—	4
<i>Enterobacter cloacae</i>	Common murre	—	R	R	—	—	R	—	—	—	—	—	—	—	—	—	—	3
<i>Acinetobacter baumannii</i>	Pied-billed grebe	—	—	I	—	—	R	R	R	—	—	—	—	—	—	—	—	4
<i>Acinetobacter baumannii</i>	Pacific loon	—	—	I	—	—	R	R	R	—	—	—	—	—	—	—	—	4
<i>Salmonella</i> Newport	Western gull	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0
<i>Pseudomonas aeruginosa</i>	Glaucous-winged gull	—	R	R	—	—	R	R	R	—	—	—	—	R	—	—	R	7
<i>Pseudomonas aeruginosa</i>	Glaucous-winged gull	—	R	R	—	—	R	R	R	—	I	—	—	R	—	—	R	8
<i>Pseudomonas aeruginosa</i>	Pacific loon	—	R	R	—	—	R	R	R	—	I	—	—	R	—	—	R	8

^a 1 = amikacin; 2 = augmentin; 3 = ampicillin; 4 = carbenicillin; 5 = ceftazidime; 6 = ceftiofur; 7 = cephalothin; 8 = chloramphenicol; 9 = ciprofloxacin; 10 = enrofloxacin; 11 = gentamicin; 12 = piperacillin; 13 = tetracycline; 14 = ticarcillin; 15 = tobramycin; and 16 = tribrisen.

^b — = no resistance; I = intermediate; R = resistant.

er in gulls ($n=49$) than common murres ($n=26$). Two rhinoceros auklets (*Cerorhinca monocerata*; Alcidae) infected with *K. pneumoniae*, one of which was additionally infected with *E. fergusonii*, were not included in the previous analysis. Because of small sample sizes we did not compare prevalences (Table 2) among other families of birds or among most gull species.

Mean (\pm SD) species richness values of bacteria found in gulls and common murres combined by rehabilitation centers were 2.79 (\pm 1.03) at Cordelia (gulls, $n=12$; common murres, $n=19$) and 2.29 (\pm 1.04) at Lynwood (gulls, $n=27$; common murres, $n=6$). Mean species richness of bacteria for gulls in both centers combined was 2.58 (\pm 1.46), and for common murres it was 2.69 (\pm 1.01). Based on a general

linear-model ANOVA, these differences were not significant ($df=62$, $P=0.09$).

The EAE gene was present in six of 48 *E. coli* isolates tested from gulls and murres, and the alpha intimin gene was present in three isolates originally recovered from a common murre and Western gull from Cordelia and an unidentified species of gull from Lynwood. One type O57 was isolated from a herring gull (*L. argentatus*) in Arcata, and the other five *E. coli* isolates did not conform to known serotypes. The remaining two isolates with the EAE gene were recovered from a glaucous-winged gull (*L. glaucescens*) and a Western gull from Lynwood.

Antibiotic resistance was confirmed in 13 of 19 selected bacterial isolates (Table 3). All isolates with resistance to the tested antibiotics had resistance to multiple an-

tibiotics (range, 3–8 antibiotics). There was no resistance to the 16 antibiotics in five of six isolates of *E. coli*, including the isolate typed as O57, and the *Salmonella* Newport isolate. Resistance to ampicillin was most prevalent (53%), followed by resistance to ceftiofur (37%), cephalothin (32%), and augmentin (26%) (Table 3). Sample sizes were too small to compare resistance between bird species or rehabilitation centers.

DISCUSSION

Escherichia coli was isolated most frequently from seabirds in this study. This might be explained at least partially by diet, which may be an important determinant of gut flora (Bangert et al., 1988). Seabirds are considered carnivorous (piscivorous) or omnivorous. *Escherichia coli* was the most common species found in surveys of omnivorous birds as well as carnivorous birds (Bangert et al., 1988), whereas granivorous birds, such as many passerines, had much lower prevalences of *E. coli* (Glunder, 1981; Brittingham et al., 1988).

The frequency of *Salmonella* spp. isolation in this study (1.1%; Table 2) was lower than that observed among seabirds elsewhere (Butterfield et al., 1983). In other studies birds were sampled at or near sites with greater potential for bacterial contamination, such as sewage outfalls or landfills (Kapperud and Rosef, 1983), or sampling was limited to gulls, which have been long implicated as carriers of *Salmonella* (Fenlon, 1983). The ready availability of human waste disposal sites fosters transmission of enteric bacteria to gulls (Fricker, 1984).

Salmonella Newport was previously reported in a gull (*Larus* sp.) (Fenlon, 1983), a common loon (*Gavia immer*) (White and Forrester, 1979), and in California sea lions (*Zalophus californianus*) (Smith et al., 2002). It is commonly isolated from human sewage and environmental samples (Fenlon, 1983) and is the third most common *Salmonella* spp. serotype isolated

from humans in the United States (Zansky et al., 2002). During the period extending from 1997 to 2001, the number of confirmed human infections of *Salmonella* serotype Newport reported to the Centers for Disease Control and Prevention increased from 5% to 10% of all *Salmonella* spp. infections (Zansky et al., 2002).

Escherichia fergusonii and *K. pneumoniae* occurred significantly more often in common murres (Alcidae) than in gulls (Laridae) (Table 2). Common murres are colonial birds and, in rehabilitation centers, are caged with other common murres to decrease their stress level while in captivity (Stoskopf and Kennedy-Stoskopf, 1986). Based on the high prevalence of *E. fergusonii* and *K. pneumoniae* we observed in murres, we question if the practice of caging them together may facilitate an increased transmission of these potential pathogens.

Although most common murres samples were collected at one center (Cordelia) and most gull samples were collected from another center (Lynwood), there were no significant differences in species richness of bacteria isolated between the centers. There were differences in how rapidly the samples were shipped after collection. More gull samples were evaluated from Cultureswabs ≥ 48 hr after the sample was taken (67% of gull samples; 24% of murre samples). Many microorganisms readily maintain viability in Cultureswabs from 24 hr to 48 hr, but viability decreased after 48 hr (Smith and Jackson, 2001). In this study, recovery of *Proteus* spp. was significantly greater from samples inoculated more than 48 hr after sampling, which may indicate bacterial overgrowth in these samples and may explain the higher prevalence found in gulls compared to common murres (Table 2).

Of the six *E. coli* that carried the EAE gene, one was identified as a type O57, a strain found in swine (Fratamico et al., 2004). The remaining five could not be typed, possibly because the serotypes used for comparisons were primarily from hu-

mans and other mammals, and avian *E. coli* strains generally do not readily conform to types recognized in mammals (Gerlach, 1986). Of these five, three also carried the alpha intimin genes, indicating potential pathogenicity. The presence of both EAE and alpha intimin places them in a class of *E. coli* strains known as enteropathogenic *E. coli* (EPEC), which is linked to human illness (Nataro and Kaper, 1998). In addition to the normal fecal-oral route of transmission, EPEC also may be transmitted by dust particles (Nataro and Kaper, 1998).

Species we isolated that are known or suspected human pathogens include *K. pneumoniae* (Ko et al., 2002), *Ps. aeruginosa* (Hsueh et al., 2002), *Aeromonas* spp. (Altwegg and Geiss, 1989), *E. fergusonii* (Funke et al., 1993), *Enterobacter* spp. (Sanders and Sanders, 1997), *A. baumannii* (Bergogne-Bérézin and Towner, 1996), *Proteus* spp., *Providencia* spp., and *Morganella morganii* (O'Hara et al., 2000). Several of these, including *Ps. aeruginosa*, often are associated with nosocomial infections (Hsueh et al., 2002).

Ten of 16 antibiotics tested had at least one bacterial isolate with resistance to it (Table 3). Antibiotic resistance in bacteria has been found in other studies at rehabilitation centers (Smith et al., 2002; Ziegerer et al., 2002) as well as in studies of free-ranging birds (White and Forrester, 1979; Nascimento et al., 2003). Resistance to ampicillin (53%), a commonly used antibiotic, is consistent with results obtained from research conducted at other sites (Nascimento et al., 2003). *Pseudomonas aeruginosa*, *K. pneumoniae*, *Acinetobacter* spp., and *E. coli* have evolved in recent years into important nosocomial pathogens because of their multi-drug resistance (Jones, 2001). Among the isolates tested, *Ps. aeruginosa* was resistant to the most antibiotics.

Humans and seabirds come into close contact in wildlife rehabilitation centers. The transfer of zoonotic bacterial pathogens from bird to human, human to

bird, and bird to bird represents risks for human and seabird health that can largely be prevented. Considering that many enteric bacteria are spread primarily via the fecal-oral route (Flammer, 1999), the transfer of enteric bacteria can effectively be reduced with proper hygiene, husbandry, and disinfection. The efficacy of simple measures, such as hand washing, is well documented (Pittet et al., 2000). Surfaces such as countertops and door-knobs, as well as objects used in patient care, such as blankets and sponges, are easily overlooked in cleaning and may harbor bacteria and should be disinfected regularly. In addition, housing birds individually may help to avoid transfer of novel pathogens to susceptible birds.

The pathogenicity of many of these bacteria to seabirds is poorly understood (Gerlach, 1986), although morbidity and mortality have been observed (Hall et al., 1977; Brand et al., 1988). Because an animal's susceptibility to bacteria may be influenced by a number of factors, including the physiologic and psychological stresses involved in rehabilitation (Thornton et al., 1998), measures to minimize stress during the rehabilitation process should also be emphasized.

This study focused on captive birds in rehabilitation centers and on their caregivers, not on free-ranging seabirds. However, reducing transmission of pathogenic bacteria among seabirds in rehabilitation centers would reduce the potential risk to free-ranging seabirds whenever rehabilitated birds are released back into wild populations.

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LITERATURE CITED

- ALTWEGG, M., AND H. K. GEISS. 1989. *Aeromonas* as a human pathogen. *Critical Reviews in Microbiology* 16: 253–286.
- ANONYMOUS. 1992. 20E Analytical profile index: Enterobacteriaceae and other Gram-negative bacteria. 10th Edition. BioMérieux Vitek, Hazelwood, Missouri, 327 pp.
- AUCOIN, D. 2000. Target: The antimicrobial reference guide to effective treatment. 2nd Edition. North American Compendium, Inc., Port Huron, Michigan, 158 pp.
- BANGERT, R. L., A. C. S. WARD, E. H. STAUBER, B. R. CHO, AND P. R. WIDDERS. 1988. A survey of the aerobic bacteria in the feces of captive raptors. *Avian Diseases* 32: 53–62.
- BERGOGNE-BÉREZIN, E., AND K. J. TOWNER. 1996. *Acinetobacter* spp. as nosocomial pathogens: Microbiological, clinical, and epidemiological features. *Clinical Microbiology Reviews* 9: 148–165.
- BLANCO, M., J. E. BLANCO, J. BLANCO, M. P. ALONSO, C. BALSALOBRE, M. MOURINO, C. MADRID, AND A. JUAREZ. 1996. Polymerase chain reaction for detection of *Escherichia coli* strains producing cytotoxic necrotizing factor type 1 and type 2 (CNF-1 and CNF-2). *Journal of Microbiological Methods* 26: 95–101.
- BRAND, C. J., R. M. WINDINGSTAD, L. M. SIEGFRIED, R. M. DUNCAN, AND R. M. COOK. 1988. Avian morbidity and mortality from botulism, aspergillosis, and salmonellosis at Jamaica Bay Wildlife Refuge, New York, USA. *Colonial Waterbirds* 11: 284–292.
- BRITTINGHAM, M. C., S. A. TEMPLE, AND R. M. DUNCAN. 1988. A survey of the prevalence of selected bacteria in wild birds. *Journal of Wildlife Diseases* 24: 299–307.
- BUTTERFIELD, J., J. C. COULSON, S. V. KEARSEY, P. MONAGHAN, J. H. MCCOY, AND G. E. SPAIN. 1983. The herring gull *Larus argentatus* as a carrier of *Salmonella*. *Journal of Hygiene (London)* 91: 429–436.
- DEBACKER, V., L. HOLSBEEK, G. TAPIA, S. GOBERT, C. R. JOIRIS, T. JAUNIAUX, F. COIGNOUL, AND J. M. BOUQUEGNEAU. 1997. Ecotoxicological and pathological studies of common guillemots *Uria aalge* beached on the Belgian coast during six successive wintering periods (1989–90 to 1994–95). *Diseases of Aquatic Organisms* 29: 159–168.
- EDWARDS, P. R., AND W. EWING. 1986. *Salmonella*. In *Identification of Enterobacteriaceae*. 4th Edition, P. R. Edwards and W. Ewing (eds.). Elsevier Press, New York, New York, pp. 181–340.
- FENLON, D. R. 1983. A comparison of *Salmonella* serotypes found in the faeces of gulls feeding at a sewage works with serotypes present in the sewage. *Journal of Hygiene, Cambridge* 91: 47–52.
- FLAMMER, K. 1999. Zoonoses acquired from birds. In *Zoo & wild animal medicine: Current therapy*. 4th Edition, M. E. Fowler (ed.). W. B. Saunders Co., Philadelphia, Pennsylvania, pp. 151–156.
- FRATAMICO, P. M., L. K. BAGI, E. J. BUSH, AND B. T. SOLOW. 2004. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* in swine feces recovered in the National Animal Health Monitoring System's Swine 2000 study. *Applied and Environmental Microbiology* 70: 7173–7178.
- FRICKER, C. R. 1984. A note on *Salmonella* excretion in the black headed gull (*Larus ribibundus*) feeding at sewage treatment works. *Journal of Applied Bacteriology* 56: 499–502.
- FUNKE, G., A. HANY, AND M. ALTWEGG. 1993. Isolation of *Escherichia fergusonii* from four different sites in a patient with pancreatic carcinoma and cholangiosepsis. *Journal of Clinical Microbiology* 31: 2201–2203.
- GANNON, V. P. J., M. RASHED, R. K. KING, AND E. J. G. THOMAS. 1993. Detection and characterization of the EAE gene of Shiga-like toxin producing *Escherichia coli* using polymerase chain reaction. *Journal of Clinical Microbiology* 31: 1268–1274.
- GERLACH, H. 1986. Bacterial diseases. In *Clinical avian medicine and surgery*, G. J. Harrison and L. R. Harrison (eds.). W. B. Saunders Co., Philadelphia, Pennsylvania, pp. 434–453.
- GLUNDER, G. 1981. Occurrence of Enterobacteriaceae in feces of granivorous passeriform birds. *Avian Diseases* 25: 195–198.
- HALL, R. F., D. G. WALDHALM, W. A. MEINERSHAGEN, AND D. A. DUBOSE. 1977. Isolation of *Salmonella* spp. from dead gulls (*Larus californicus* and *Larus delawarensis*) from an Idaho irrigation reservoir. *Avian Diseases* 21: 452–454.

- HINTZE, J. 2001. NCSS and PASS. Number Cruncher Statistical Systems software. Kaysville, Utah.
- HSUEH, P., M. CHEN, C. SUN, W. CHEN, H. PAN, L. YANG, S. CHANG, S. HO, C. LEE, W. HSIEH, AND K. LUH. 2002. Antimicrobial drug resistance in pathogens causing nosocomial infections at a University Hospital in Taiwan, 1981–1999. *Emerging Infectious Diseases* 8: 63–68.
- JESSUP, D. A., AND F. A. LEIGHTON. 1996. Oil pollution and petroleum toxicity to wildlife. In *Non-infectious diseases in wildlife*, G. Hoff, A. Fairbrother, and L. Locke (eds.). Iowa State University Press, Ames, Iowa, pp. 141–156.
- JONES, R. N. 2001. Resistance patterns among nosocomial pathogens. *Chest* 119: 397S–404S.
- KAPPERUD, G., AND O. ROSEF. 1983. Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. in Norway. *Applied and Environmental Microbiology* 45: 375–380.
- KO, W., D. L. PATTERSON, A. J. SAGNIMENI, D. S. HANSEN, A. VON GOTTBURG, S. MOHAPATRA, J. M. CASELLAS, H. GOOSSENS, L. MULAZIMOGLU, G. TRENHOLME, K. P. KLUGMAN, J. G. MCCORMACK, AND V. L. YU. 2002. Community-acquired *Klebsiella pneumoniae* bacteremia: Global differences in clinical patterns. *Emerging Infectious Diseases* 8: 160–166.
- NASCIMENTO, A. M. A., L. CURSINO, H. GONÇALVES-DORNELAS, A. REIS, E. CHARTONE-SOUZA, AND M. Â. MARINI. 2003. Antibiotic-resistant Gram-negative bacteria in birds from the Brazilian Atlantic forest. *Condor* 105: 358–361.
- NATARO, J. P., AND J. B. KAPER. 1998. Diarrheagenic *Escherichia coli*. *Clinical Microbiological Review* 11: 142–201.
- O'HARA, C. M., F. W. BREENER, AND J. M. MILLER. 2000. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clinical Microbiology Reviews* 13: 534–546.
- OJENIYI, B., P. AHRENS, AND A. MEYLING. 1994. Detection of fimbrial and toxin genes in *Escherichia coli* and their prevalence in piglets with diarrhea. The application of colony hybridization assay, polymerase chain reaction and phenotypic assays. *Journal of Veterinary Medicine B* 41: 49–59.
- ORSKOV, I., F. ORSKOV, B. JANN, AND K. JANN. 1977. Serology, chemistry and genetics of O and K antigens of *Escherichia coli*. *Bacteriological Reviews* 41: 667–710.
- PITTET, D., S. HUGONNET, S. HARBARTH, P. MOUROUGA, V. SAUVAN, S. TOUVENEAU, AND T. V. PERNER. 2000. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. *Lancet* 356: 1307–1312.
- REID, S. D., D. J. BETTING, AND T. S. WHITAM. 1999. Molecular detection and identification of intimin alleles in pathogenic *E. coli* by multiplex PCR. *Journal of Clinical Microbiology* 37: 2719–2722.
- SANDERS, W., JR., AND C. C. SANDERS. 1997. *Enterobacter* spp.: Pathogens poised to flourish at the turn of the century. *Clinical Microbiology Reviews* 10: 220–241.
- SIBLEY, D. A. 2000. The Sibley guide to birds. Alfred A. Knopf, New York, New York, 544 pp.
- SMITH, E. G., AND T. L. JACKSON. 2001. Comparison of a new liquid Stuart aerobic swab transport system with two currently available systems. In *Abstracts of the 101st Annual Meeting of the American Society for Microbiology*. Orlando, Florida, Abstract C-53.
- SMITH, W. A., J. A. MAZET, AND D. C. HIRSH. 2002. *Salmonella* in California wildlife species: Prevalence in rehabilitation centers and characterization of isolates. *Journal of Zoo and Wildlife Medicine* 33: 228–235.
- STEELE, C. M. 2003. Prevalences of zoonotic disease agents among seabirds in rehabilitation centers along the Pacific coast. Master's Thesis, Humboldt State University, Arcata, California, 37 pp.
- STOSKOPF, M. K., AND S. KENNEDY-STOSKOPF. 1986. Aquatic birds. In *Zoo and wild animal medicine*, M. E. Fowler (ed.). W. B. Saunders Company, Philadelphia, Pennsylvania, pp. 293–313.
- THORNTON, S. M., S. NOLAN, AND F. M. D. GULLAND. 1998. Bacterial isolates from California sea lions (*Zalophus californianus*), harbor seals (*Phoca vitulina*), and northern elephant seals (*Mirounga angustirostris*) admitted to a rehabilitation center along the central California coast, 1994–1995. *Journal of Zoo and Wildlife Medicine* 29: 171–176.
- TOMASZ, A. 1994. Multiple-antibiotic-resistant pathogenic bacteria. *New England Journal of Medicine* 330: 1247–1251.
- VELGE, P., A. CLOECKAERT, AND P. BARROW. 2005. Emergence of *Salmonella* epidemics: The problems related to *Salmonella enterica* serotype Enteritidis and multiple antibiotic resistance in other major serotypes. *Veterinary Research* 36: 267–288.
- WHITE, F. H., AND D. J. FORRESTER. 1979. Antimicrobial resistant *Salmonella* spp. isolated from double-crested cormorants (*Phalacrocorax auritus*) and common loons (*Gavia immer*) in Florida. *Journal of Wildlife Diseases* 15: 235–237.
- WILLIAMS, R. J., AND D. L. HEYMANN. 1998. Containment of antibiotic resistance. *Science* 279: 1153–1154.
- WITHAM, P. K., C. T. YAMASHIRO, K. J. LIVAK, AND

- C. A. BATT. 1996. A PCR-based assay for the detection of *Escherichia coli* Shiga-like toxin gene in ground beef. *Applied Environmental Microbiology* 62: 1347–1353.
- ZANSKY, S., B. WALLACE, D. SCHOONMAKER-BOPP, P. SMITH, F. RAMSEY, J. PAINTER, A. GUPTA, P. KALLURI, AND S. NOVIELLO. 2002. Outbreak of multi-drug resistant *Salmonella* Newport—United States, January–April 2002. *Morbidity and Mortality Weekly Report* 51: 545–548.
- ZIEGERER, K., F. S. TSENG, AND M. A. POKRAS. 2002. Impact of antibiotic use in a wildlife rehabilitation clinic. *Wildlife Rehabilitation* 20: 69–83.

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