

ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL ISOLATES FROM SEA OTTERS (*ENHYDRA LUTRIS*)

Deborah Brownstein,¹ Melissa A. Miller,¹ Stori C. Oates,¹ Barbara A. Byrne,² Spencer Jang,³ Michael J. Murray,⁴ Verena A. Gill,⁵ and David A. Jessup^{1,6}

¹ California Department of Fish and Game, Marine Wildlife Veterinary Care and Research Center, 1451 Shaffer Rd. Santa Cruz, California 95060, USA

² University of California-Davis, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, Davis, California 95616, USA

³ William R. Pritchard Teaching Hospital, University of California-Davis, School of Veterinary Medicine, Davis, California 95616, USA

⁴ Monterey Bay Aquarium, 886 Cannery Row, Monterey, California 93940, USA

⁵ U.S. Fish and Wildlife Service, Marine Mammals Management, 1011 East Tudor Road, Anchorage, Alaska 99503, USA

⁶ Corresponding author (email: djessup@ospr.dfg.ca.gov)

ABSTRACT: Bacterial infections are an important cause of sea otter (*Enhydra lutris*) mortality, and some of these infections may originate from terrestrial and anthropogenic sources. Antimicrobials are an important therapeutic tool for management of bacterial infections in stranded sea otters and for prevention of infection following invasive procedures in free-ranging otters. In this study, susceptibility to commonly used antimicrobials was determined for 126 isolates of 15 bacterial species or groups from necropsied, live-stranded injured or sick, and apparently healthy wild sea otters examined between 1998 and 2005. These isolates included both gram-positive and gram-negative strains of primary pathogens, opportunistic pathogens, and environmental flora, including bacterial species with proven zoonotic potential. Minimal evidence of antimicrobial resistance and no strains with unusual or clinically significant multiple-drug resistance patterns were identified. Collectively, these findings will help optimize selection of appropriate antimicrobials for treatment of bacterial diseases in sea otters and other marine species.

Key words: Antibiotic, antimicrobial susceptibility, bacteria, bacterial infections, *Enhydra lutris*, sea otter.

INTRODUCTION

Despite decades of legal protection, southern sea otter (*Enhydra lutris nereis*) population recovery has been hindered by high mortality, including deaths of prime-aged adult animals. Up to 50% of sea otter mortality has been attributed to infection by bacteria and parasites (Thomas and Cole, 1996; Kreuder et al., 2003). Potentially pathogenic enteric bacteria appear to be more prevalent along urbanized coastlines and near river mouths, suggesting that land–sea pathogen spread may be an important component of exposure to some bacterial species (Miller et al., 2009). Some aspects of sea otter biology may make them especially vulnerable to infection by bacterial contaminants in polluted runoff. Sea otters feed near shore, often within or adjacent to coastal surface water plumes (Miller et al., 2009, 2010), may rest and forage in sheltered embayments near human population centers (Jessup et

al., 2007), display intraspecific aggression that often culminates in soft tissue trauma, and consume large amounts of filter-feeding invertebrate prey that can bioconcentrate pathogens, including bacteria (Miller et al., 2006, 2010).

During sea otter conservation and research efforts antimicrobials are commonly used following invasive procedures and to treat bacterial infections. Sick, injured, and stranded otters are rehabilitated by aquaria and marine mammal rescue organizations. Bacterial infections resulting from intraspecific aggression, shark bite, gastric ulcers, and acanthocephalan peritonitis (Riedman et al., 1994; Williams and Davis, 1995; Mayer et al., 2003) are common problems that require antibacterial treatment. Prolonged stress of confinement following stranding can compromise immune function, and contact with petroleum products or conditions of captive confinement following an oil

spill may increase bacterial exposure or increase susceptibility to bacterial infection, necessitating prophylactic antimicrobial therapy. Development of resistance to antimicrobials by bacteria from various sources, including sewage, leaking septic systems, and intensive agriculture operations are a concern worldwide and also in more populated coastal areas of California (Jessup et al., 2007).

Selection of antimicrobials to treat bacterial infections is initially guided by clinician experience, type of infection (e.g., local or systemic, deep or superficial), tissues involved, exfoliative cytology, Gram stains, and later, often by culture and sensitivity. Although several bacterial genera have been associated with disease in southern sea otters (Thomas and Cole, 1996; Kreuder et al., 2003; Burek et al., 2005), the antimicrobial susceptibility of these pathogens is not well known. Primary goals of this study were to determine antimicrobial resistance patterns for some of the more common and opportunistic bacterial pathogens of sea otters and to optimize antimicrobial drug selection for clinical treatment.

MATERIALS AND METHODS

Sample collection

Bacterial samples were collected from 87 dead and 17 live-stranded or captured sea otters in California and Alaska from 1998 through 2005 (for complete listing, see Appendix provided in the online version of this article). Bacterial isolates from dead otters were collected during necropsy from animals with a postmortem interval ≤ 72 hr on ice or under refrigeration. The organ systems and specific tissues from which isolates were collected are listed in the online Appendix and Table 1. The isolates included in this study were selected opportunistically because of suspected or known pathogenicity or physical association with lesions in sea otters and do not represent what might be obtained by a random survey. Isolates from live, apparently healthy sea otters were opportunistically obtained following capture and anesthesia, as previously described (Lander et al., 2001), from swabs of the oral cavity or rectum and were selected for inclusion in this

study because of potential or known pathogenicity based on sea otter necropsy data (California Department of Fish and Game [CDFG], unpubl. data). Only isolates from sea otters with no history of antimicrobial therapy were used in this study.

Bacterial isolation

All swabs were placed in Amies transport medium (Becton, Dickinson and Company, Sparks, Maryland, USA), refrigerated, and sent overnight to the University of California, Davis, William R. Pritchard Veterinary Medical Teaching Hospital (UCD-VMTH) for bacterial isolation and identification. To identify aerobic bacteria, swabs were streaked for isolation onto 5% defibrinated sheep blood agar, MacConkey agar, and phenylethanol agar with 5% sheep blood. Inoculated plates were incubated aerobically at 37 C with 5% CO₂ and examined at 24 hr and 48 hr postinoculation for bacterial growth. Cultures for *Salmonella* and *Vibrio* spp. were enriched for 24 hr in selenite broth or alkaline peptone water before plating onto xylose lactose tergitol 4 and thiosulfate citrate bile sucrose agar, respectively.

Gram stains, morphologic characteristics, and biochemical testing were used to initially identify the isolates as previously described (Baron and Murray, 1999). Individual colonies were subcultured onto fresh media for further characterization using selective media, additional biochemical tests, and, for a small number of isolates not readily identified by biochemical testing, 16S rRNA amplification and sequencing. *Salmonella* were serotyped at the National Veterinary Services Laboratory, Ames, Iowa, USA. Isolates were then stored at -80 C in microbank bead vials (Pro-Lab Diagnostics, Austin, Texas, USA) until being evaluated for antimicrobial drug susceptibility at UCD-VMTH.

To screen for the presence of *Campylobacter* spp., Campy-CVA agar (Hardy Diagnostics, Santa Maria, California, USA) was used. Plates were incubated under microaerophilic conditions at 37 C for 48–96 hr and any isolates were identified using standard procedures (NCCLS, 2004) and stored at -80 C in microbank bead vials until tested for antimicrobial drug susceptibility.

Antimicrobial sensitivity testing

Cryopreserved aerobic isolates were thawed, plated separately onto sheep blood agar, and incubated for 24 hr with 5% CO₂. At 24 hr, each isolate was examined for purity and

TABLE 1. Number of bacterial isolates cultured from tissues or organ systems of live and dead-stranded sea otters from California and Alaska, 1998–2005.

	Wound/ abscess	Integument	Musculo- skeletal	Nervous	Cardio- vascular	Lympho- reticular	Respiratory	Abdomen/ viscera	Gastrointestinal tract	Urogenital	Eye	Total
Gram-positive isolates												77
<i>Staphylococcus</i> spp. ^a	1		1	1	3	1	5					
<i>Streptococcus botis</i> ^a				1	6	2			2	1		
beta-hemolytic <i>Streptococci</i> ^a	4	2	1	1	6	6	7				1	
<i>Erysipelothrix</i> <i>rhusiopathiae</i> ^b				1	1	1						
<i>Corynebacterium</i> spp. ^a	1		1				7		3		1	
<i>Arcanobacterium</i> spp. ^a	3	1		1		2	2					
Gram-negative isolates												94
<i>Campylobacter</i> spp. ^b									17			
<i>Bordetella</i> <i>bronchiseptica</i> ^b							1					
<i>Escherichia coli</i> ^a	1				11		4	4	2			
<i>Salmonella</i> spp. ^b									4			
<i>Vibrio cholerae</i> ^b									3			
<i>Vibrio cholerae</i> -like spp. ^b									2			
<i>Vibrio parahaemolyticus</i> ^a				2	7	1	8	2	5	1		
<i>Photobacterium damsela</i> ^a	1		1		9		2					
<i>Pasteurella multocida</i> ^a					3		3					
Total	11	3	4	6	46	13	40	6	38	2	2	171

^a Because of the large number of isolates recovered, those strongly associated with lesions were opportunistically subsampled.

^b All available isolates were utilized.

a single colony was passed to a second sheep blood agar plate and incubated 24 hr with 5% CO₂. The purified isolate was inoculated into 2 ml brain heart infusion enrichment broth and incubated with CO₂ for 4–5 hr. Broth microdilutions were performed (Sensititre, Trek Diagnostic Systems Inc., Cleveland, Ohio, USA) to determine the minimum inhibitory concentration (MIC) required to inhibit bacterial proliferation for each tested antimicrobial. The assay was performed in accordance with protocols published by the Clinical and Laboratory Standards Institute (CLSI), formerly known as the National Committee of Clinical Laboratory Standards (NCCLS; NCCLS, 2003, 2004).

Two commercial trays containing a range of antimicrobial drugs (Trek Diagnostic Systems) were used to evaluate each bacterial isolate. The first contained a selection of antimicrobial drugs that are commonly used to treat domestic animals, as well as sea otters: amikacin, amoxicillin-clavulanic acid, ampicillin, cefazolin (first generation cephalosporin), ceftiofur (third generation cephalosporin), ceftizoxime (third generation cephalosporin), chloramphenicol, enrofloxacin, erythromycin, gentamicin, oxacillin (+2% NaCl), penicillin (4.0 µg/ml), rifampin, tetracycline, ticarcillin-clavulanic acid, and trimethoprim-sulfamethoxazole. The second tray contained antimicrobial drugs that may be used to treat exotic species and sea otters: cefotaxime (third generation cephalosporin), ceftazidime (third generation cephalosporin), ciprofloxacin, clindamycin, doxycycline, florfenicol, imipenem, piperacillin, and tylosin. Once the trays were inoculated with the bacterial broth, they were incubated 18 hr without CO₂ (except for beta-hemolytic streptococci, *Streptococcus infantarius* subsp. *coli*, and *Pasteurella multocida*, which were incubated with 5% CO₂). Antimicrobial susceptibilities were read manually using Sensitouch-Sensititre equipment (Trek Diagnostic Systems). *Campylobacter* isolates were tested for susceptibility to ciprofloxacin, doxycycline, erythromycin, and gentamicin only, using a previously described agar dilution method (McDermott et al., 2004).

Establishing species-specific criteria for defining bacterial susceptibility is a difficult and expensive process. No sea otter-specific standards are available from CLSI, so criteria for MIC data interpretation for otter bacterial isolates were based on published standards (NCCLS, 2003, 2004) and results of previous in vitro and in vivo studies of humans and domestic animals (Aucoin, 2000). The standardized in vitro antimicrobial culture and susceptibility testing techniques and defined

interpretive criteria (MIC breakpoints) were used to classify sea otter-derived bacterial isolates as susceptible, intermediate, or resistant. Susceptible bacterial isolates were those in which the antimicrobial effectively killed or limited bacterial growth, suggesting that an infected animal should respond to treatment at standard dosages. Bacterial isolates classified as intermediate exhibited a lower response, suggesting that antimicrobial treatment at standard dosages would be less effective than for otters infected with susceptible strains. Resistant bacterial strains exhibited MIC values for a given antimicrobial that were unlikely to be clinically effective or reliable at standard dosages.

The MIC 90 was defined as the minimum antimicrobial concentration (in µg/ml) for which ≥90% of isolates of a given bacterial species or group was susceptible, as defined above. In our study, the MIC 90 was calculated when ≥10 bacterial isolates were available for comparison within a defined bacterial group. When there were <10 isolates in a defined bacterial group, the MIC 50 was calculated as the minimum concentration (µg/ml) for which 50% of tested isolates were susceptible to a given antimicrobial.

RESULTS

Of 171 available bacterial isolates representing 15 preselected species of known or potential sea otter pathogens (see online Appendix), 126 were selected for antimicrobial sensitivity testing using the following criteria: All available strains of some bacterial species of interest were utilized, including *Streptococcus infantarius* subsp. *coli*, *Erysipelothrix rhusiopathiae*, *Campylobacter* spp., *Bordetella bronchiseptica*, *Salmonella* spp., *Vibrio cholerae*, and *V. cholerae*-like spp. For other species, for which we had large numbers of isolates, or for multiple isolates from various tissues of the same otter, including *Staphylococcus* spp., beta-hemolytic streptococci, *Corynebacterium* spp., *Arcanobacterium* spp., *Escherichia coli*, *V. parahaemolyticus*, *Photobacterium damsela*, and *Pasteurella multocida*, we selected cases or fresh or cryopreserved isolates with a bias for those coming from pathologic lesions (Table 1). Results from this sampling regimen could differ from

TABLE 2. Minimum inhibitory concentrations (MIC) of 26 antimicrobial agents for 15 bacterial species recovered from live and dead-stranded sea otters from California and Alaska, 1998–2005.

Antimicrobial agent ^a	<i>Staphylococcus</i> spp. (n=10)		<i>Streptococcus bovis</i> (n=7) ^c		Beta-hemolytic <i>Streptococcus</i> spp. (n=13) ^d	
	MIC range	MIC 90 ^b	MIC range	MIC 50	MIC range	MIC 90
AMK	≤0.5–1.0	1.0	8.0–>32.0	32.0	2.0–32.0	>32
AMC	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2
AMP	≤0.25–1.0	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
CFZ	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0
CFT	0.12–1.0	1.0	≤0.06	≤0.06	≤0.06	≤0.06
ZOX	≤0.5–4.0	4.0	≤0.50	≤0.50	≤0.5	≤0.5
CHL	4.0–16.0	4.0	0.5–4.0	1.0	1.0–2.0	2.0
ENR	≤0.25	≤0.25	≤0.25–4.0	2.0	≤0.25–0.5	0.5
ERY	≤0.12–0.25	≤0.25	≤0.12	≤0.12	≤0.12–> 4.0	≤0.12
GEN	≤0.25	≤0.25	2.0–16.0	4.0	2.0–16.0	16.0
OXA	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0
PCN (4.0 µg)	≤0.12–1.0	0.3	≤0.12	≤0.12	≤0.12	≤0.12
RIF	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12
TET	≤0.5–4.0	≤0.5	≤0.50–>16.0	≤0.50	≤0.5–4.0	≤0.5
TIM	≤8.0–32	≤8.0	≤8.00	≤8.00	≤8.0	≤8.0
SXT	≤0.25	≤0.25	≤0.25–0.50	≤0.25	≤0.25	≤0.25
CTX	≤0.25–4.0	1.0	≤0.25	≤0.25	≤0.25	≤0.25
CAZ	≤2.0–16	8.0	≤2.0	≤2.0	≤2.0–4.0	≤2.0
CPF	≤0.5	≤0.5	1.0–4.0	2.0	≤0.5	≤0.5
CLI	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
DOX	≤0.25	≤0.25	≤0.25–8.0	≤0.25	≤0.25	≤0.25
FFC	≤0.5–2.0	1.0	0.25–0.5	0.5	0.25–4.0	1.0
IPM	≤1.0–2.0	≤1	≤1.0	≤1.0	≤1.0	≤1.0
PIP	≤1.0–4.0	≤1	≤1.0	≤1.0	≤1.0	≤1.0
TYL	≤2.5	≤2.5	≤2.5	≤2.5	≤2.5	≤2.5

^a AMK = amikacin; AMC = amoxicillin-clavulanic acid; AMP = ampicillin; CFZ = ceftazolin; CFT = ceftiofur; ZOX = ceftiozime; CHL = chloramphenicol; ENR = enrofloxacin; ERY = erythromycin; GEN = gentamicin; OXA = oxacillin (+2% NaCl); PCN (4.0 µg) = penicillin (4 µg); RIF = rifampin; TET = tetracycline; TIM = ticarcillin-clavulanic acid; SXT = trimethoprim-sulfamethoxazole; CTX = cefotaxime; CAZ = ceftazidime; CPF = ciprofloxacin; CLI = clindamycin; DOX = doxycycline; FFC = florfenicol; IPM = imipenem; PIP = piperacillin; TYL = tylosin.

^b MIC values (µg/ml) that are intermediate resistant or resistant, based on CLSI guidelines, are bolded.

^c n=5 for CFT, CTX, CAZ, CPF, CLI, DOX, FFC, IPM, PIP, TYL.

^d n=12 for AMK, AMP, CFZ, CFT, ZOX, CHL, ENR, ERY, GEN, PCN (4.0 µg), RIF, TET, SXT.

^e n=9 for AMK, AMC, AMP, CFZ, CFT, ZOX, ENR, ERY, GEN, OXA, PCN (4.0 µg), RIF, TET, TIM, SXT.

^f n=5 for CTX, CAZ, CPF, CLI, FFC, IPM, PIP, TYL.

^g n=14 for PCN (4.0 µg).

^h ND = Not done.

those obtained through randomized strain isolation and selection methods. Due to cost constraints, only one representative isolate of each bacterial species was evaluated via MIC testing for each sea otter; so concurrent infection by multiple strains of the same bacterium with differing antimicrobial drug sensitivities could have been underrecognized.

Most isolates were recovered from wounds, abscesses, and major organ sys-

tems (Table 1) with 73% obtained from the cardiovascular, respiratory, and gastrointestinal systems. Most isolates (88%) exhibited resistance to “inappropriate” antimicrobials, defined as antibiotics with therapeutic ranges and mechanisms that do not include the bacterium of interest and will not be discussed further, but 2% of isolates exhibited resistance to antimicrobials that would be deemed appropriate for clinical treatment. Table 2 summa-

TABLE 2. Extended.

<i>Erysipelothrix rhusiopathiae</i> (n=3)		<i>Corynebacterium</i> spp. (n=10) ^e		<i>Arcanobacterium</i> spp. (n=7) ^f		<i>Campylobacter</i> spp. (n=17)	
MIC range	MIC 50	MIC range	MIC 90	MIC range	MIC 50	MIC range ^h	MIC 90 ^h
>32.0	>32.0	≤0.5–8.0	8	2.0–8.0	4.0	ND	ND
≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	ND	ND
≤0.25–0.5	≤0.25	≤0.25–0.5	≤0.25	≤0.25	≤0.25	ND	ND
≤2.0–16.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	ND	ND
≤0.06–> 8.0	0.25	≤0.06–2.0	2	≤0.06–0.25	0.1	ND	ND
≤0.5–32	≤0.5	≤0.5–2.0	2	≤0.5–1.0	≤0.5	ND	ND
4.0–8.0	4.0	≤0.25–2.0	2	≤0.25–1.0	≤0.25	ND	ND
0.25–4.0	≤0.25	≤0.25	≤0.25	≤0.25–0.5	≤0.25	ND	ND
≤0.12–1.0	≤0.12	≤0.12	≤0.12	≤0.12–1.0	≤0.12	0.125–1.0	1.0
16.0–>16.0	>16	≤0.25–1.0	1	≤0.25–1.0	0.5	0.125–1.0	0.5
≤2.0–> 4.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	ND	ND
≤0.12–0.5	≤0.12	0.12–0.25	0.25	ND	ND	ND	ND
>4.0	>4.0	≤0.12	≤0.12	≤0.12	≤0.12	ND	ND
≤0.5–>16	1.0	≤0.5	≤0.5	≤0.5	≤0.5	ND	ND
≤8.0	≤8.0	≤8.0	≤8.0	≤8.0	≤8.0	ND	ND
≤0.25–0.5	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	ND	ND
≤0.25–8.0	≤0.25	0.25–1.0	0.5	≤0.25	≤0.25	ND	ND
≤2.0–16	≤2.0	≤2.0–8.0	8	≤2.0–4.0	≤2.0	ND	ND
≤0.5–4.0	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	0.06–4.0	1.0
≤0.25–> 2.0	≤0.25	≤0.25–2.0	2	≤0.25–>2.0	≤0.25	ND	ND
≤0.25–8.0	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	< 0.06–0.125	0.1
1.0–2.0	2.0	≤0.12–1.0	1	≤0.12–0.25	≤0.12	ND	ND
≤1.0–2.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	ND	ND
≤1.0–4.0	≤1.0	≤1.0–4.0	4	≤1.0	≤1.0	ND	ND
≤2.5	≤2.5	≤2.5	≤2.5	≤2.5–10.0	≤2.5	ND	ND

rizes the susceptibility of each bacterial species or group to the tested antimicrobials.

Gram-positive bacteria (*Staphylococcus* spp., beta-hemolytic streptococci, *S. infantarius* subsp. *coli*, *E. rhusiopathiae*, *Corynebacterium* spp., and *Arcanobacterium* spp.) accounted for 40% of tested isolates. Staphylococcal isolates were comprised of *S. aureus* (n=3), *S. delphinus* (n=2), *Staphylococcus* sp. (n=2), and coagulase-negative *Staphylococcus* (n=3) and were obtained from heart blood or wounds or other lesions (Table 1). *Streptococcus* spp., including *S. infantarius* subsp. *coli* and beta-hemolytic streptococci were the predominant gram-positive bacteria cultured from sea otters and were isolated from abscesses, joints, heart blood, heart valves, lymph nodes, lung, abdominal fluid, or kidneys at necropsy

(Table 1). In general, gram-positive bacterial isolates were susceptible to most antimicrobials with gram-positive spectra. Nevertheless, staphylococci were resistant to penicillin and florfenicol, *S. infantarius* subsp. *coli* were generally resistant to amikacin, enrofloxacin, and ciprofloxacin, and beta-hemolytic streptococci were resistant to amikacin with intermediate resistance to gentamicin and florfenicol (Table 2). The gram-positive bacteria that showed resistance to the greatest number of antimicrobials were the *E. rhusiopathiae* isolates (Table 2). In contrast, sea otter *Arcanobacterium* spp. isolates demonstrated no antimicrobial resistance.

Gram-negative bacteria (*Campylobacter* spp., *B. bronchiseptica*, *E. coli*, *Salmonella* spp., *V. cholerae* and *V. cholerae*-like spp., *V. parahaemolyticus*, *P. damsela*, and *P. multocida*) accounted for 60% of

TABLE 2. Extended.

<i>Bordetella bronchiseptica</i> (n=1)	<i>Escherichia coli</i> (n=17)		<i>Salmonella</i> spp. (n=4)		<i>Vibrio cholerae</i> (n=3)		
	MIC 50	MIC range	MIC 90	MIC range	MIC 50	MIC range	MIC 50
8	<0.5–4.0	≤4.0	1.0–2.0	1.0	2.0–4.0	2.0	2.0
≤2.0	≤ 2.0 –> 16.0	> 16.0	≤2.0	≤2.0	4.0	4.0	4.0
8	2.0 –> 16.0	> 16.0	0.5–1.0	1.0	2.0	2.0	2.0
> 16.0	≤ 2.0 –> 16.0	> 16.0	≤2.0–4.0	≤2.0	4.0–8.0	8.0	8.0
> 8.0	0.12–8.0	1.0	0.25–0.5	0.5	≤0.06	0.06	0.06
> 32.0	≤0.5–16.0	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
2	4.0–8.0	8.0	4.0	4.0	1.0	1.0	1.0
0.5	≤0.25–>8.0	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
4	> 4.0	> 4.0	> 4.0	> 4.0	4.0	4.0	4.0
4	≤0.25–1.0	1.0	≤0.25–0.5	≤0.25	0.5	0.5	0.5
> 4.0	>4.0	>4.0	> 4.0	> 4.0	> 4	> 4	> 4
> 4.0	> 4.0	> 4.0	> 4.0	> 4.0	2.0–4.0	4.0	4.0
4	≥ 4.0	> 4.0	> 4.0	> 4.0	0.5–1.0	0.5	0.5
≤0.50	1.0 –> 16.0	> 16.0	1.0–2.0	1.0	≤0.5	≤0.5	≤0.5
≤8.0	≤ 8.0 – 64.0	32.0	≤8.0	≤8.0	≤8.0	≤8.0	≤8.0
≤0.25	≤0.25–>4.0	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
> 32.0	≤0.25–8.0	1.0	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
4	≤2–16.0	≤2	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0
≤0.50	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
> 2.0	> 2.0	> 2.0	> 2.0	> 2.0	2.0 –> 2.0	> 2	> 2
≤0.25	0.5 –> 16.0	> 16.0	1.0–2.0	2.0	≤0.25	≤0.25	≤0.25
1	2.0 – 16.0	8.0	1.0 – 2.0	1.0	0.5	0.5	0.5
2	≤1.0–8.0	4.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0
≤1.0	≤ 1.0 – 64.0	64.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0
> 20.0	> 20.0	> 20.0	> 20.0	> 20.0	> 20.0	> 20.0	> 20.0

tested isolates. Seventeen *E. coli* (15 nonhemolytic and two hemolytic) from southern sea otters were identified and tested, but results for both hemolytic and nonhemolytic *E. coli* were combined for analysis and inclusion in Table 2. All gram-negative isolates excluding *Campylobacter* spp. were resistant to four or more antimicrobials (Table 2).

Vibrionaceae (n=32) and *Enterobacteriaceae* (n=21) comprised 69.7% of gram-negative isolates and the majority (38.6%) of gram-negative strains were recovered from heart blood or the cardiovascular system (Table 1). All *Vibrionaceae* and *Enterobacteriaceae* were resistant to erythromycin, penicillin, clindamycin, and tylosin. Erythromycin and tylosin were also ineffective against *P. multocida* (Table 2). *Escherichia coli* and *B. bron-*

chiseptica were resistant to the greatest number of antimicrobials (Table 2).

DISCUSSION

Unique characteristics of the life history and biology of sea otters make them ideal sentinels for monitoring health of the near shore marine ecosystem of Pacific coastal North America (Jessup et al., 2007; Miller et al., 2009). These include their preference to feed along the shoreline, reliance on near shore-feeding marine invertebrates as prey, and regular use of comparatively small home ranges. These attributes facilitate identification of location, frequency, and severity of biological and chemical pollution at the land-sea interface (Jessup et al., 2007; Miller et al., 2009).

TABLE 2. Extended.

<i>Vibrio cholerae</i> -like spp. (n=2)		<i>Vibrio parahaemolyticus</i> (n=15) [§]		<i>Photobacterium damsela</i> (n=12)		<i>Pasteurella multocida</i> (n=5)	
MIC range	MIC 50	MIC range	MIC 90	MIC range	MIC 90	MIC range	MIC 50
2.0	2.0	≤0.5–4.0	4	≤0.5–8.0	4.0	4.0–8.0	4.0
4.0	4.0	≤2	≤2	≤2.0–>16.0	≤2.0	≤2.0	≤2.0
2.0	2.0	≤0.25–16.0	8	≤2.0–>16.0	>16	≤0.25	≤0.25
4.0	4.0	≤2.0–16.0	16	≤2.0–>16.0	16.0	≤2.0	≤2.0
0.06	0.06	0.12–0.5	0.5	≤0.06–0.25	≤0.06	≤0.06	≤0.06
≤0.5	≤0.5	≤0.25–32.0	0.5	≤0.5	≤0.5	≤0.5	≤0.5
0.5–1.0	0.5	0.5–4	1	≤0.25–>16.0	1.0	≤0.25–0.5	0.5
≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
1.0–2.0	1.0	≤0.12–>4.0	4	2.0–>4.0	4.0	1.0–4.0	2.0
0.5	0.5	≤0.25–2.0	1	≤0.25–1.0	1.0	1.0–2.0	2.0
>4.0	>4.0	<2.0–>4.0	>4	>4.0	>4.0	≤2.0–>4.0	≤2.0
4	4.0	>4	>4	2.0–>4.0	>4.0	≤0.12	≤0.12
0.5	0.5	≤0.12–1.0	1	0.5–>4.0	2.0	0.25–1.0	0.3
≤0.5	≤0.5	≤0.5–1.0	0.5	≤0.5–>16.0	≤0.5	≤0.5	≤0.5
≤8.0	≤8.0	≤8	≤8	≤8.0	≤8.0	≤0.8	≤0.8
≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
≤0.25	≤0.25	≤0.25–1.0	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
≤2.0	≤2.0	≤2–8.0	≤2	≤2.0	≤2.0	≤2.0	≤2.0
≤0.5	≤0.5	≤0.50–1.0	≤0.50	≤0.5	≤0.5	≤0.5	≤0.5
2.0–>2.0	2.0	≤0.25–>2.0	>2	0.5–>2.0	>2.0	>2.0	>2.0
≤0.25	≤0.25	≤0.25–4.0	1	≤0.25–>16.0	≤0.25	≤0.25–0.5	≤0.25
0.25–0.5	0.25	≤0.12–1.0	0.5	≤0.12–16.0	0.5	≤0.12–0.25	≤0.12
≤1.0	≤1.0	≤1.0	≤1	≤1.0	≤1.0	≤1.0	≤1.0
≤1.0	≤1.0	1.0–8.0	4	≤1.0–4.0	4.0	≤1.0	≤1.0
10.0–>20.0	10.0	≤2.5–>20.0	>20.0	≥20.0	20.0	20.0	20.0

Most municipal sewage generated along coastal California is released into the ocean after treatment, but primary and secondary treatment fails to kill many pathogenic bacteria (Rao et al., 1986). Sewage effluent contains antimicrobial residues that may incubate with bacteria under conditions that could allow emergence of antimicrobial-resistant strains (Murray et al., 1984). Old or inadequate infrastructure, seasonally heavy precipitation, and accidents result in uncontrolled releases of sewage yearly. Residences in rural portions of coastal California rely on private septic systems and some boats discharge sewage directly into the ocean. Best management practices meant to limit release of livestock and poultry fecal bacteria into watercourses are voluntary and not widely applied. Untreated feces from pets and wildlife are periodically

flushed into the ocean with storm runoff and together with human and livestock feces, may serve as sources of bacterial and protozoal pathogens (Sercu et al., 2009). Johnson et al. (1998) and Stoddard et al. (2009) provided evidence that antimicrobial-resistant bacteria can develop in marine wildlife rehabilitation facilities. Thus, multiple sources of pathogenic and opportunistic bacteria with varying levels of resistance to antimicrobials may enter the ocean, particularly in more developed areas of California.

Gram-positive bacteria

Sea otters inflict bite wounds on each other during territorial confrontations and mating and receive skin lacerations and puncture wounds from handling crustacean and echinoderm prey. Gram-positive bacteria commonly colonize these

wounds. The 10 *Staphylococcus* isolates in this study were most commonly obtained from wounds and were susceptible to most antimicrobials, except penicillins including ampicillin and carbenicillin, as previously reported for staphylococcal isolates from California sea lions (*Zalophus californianus*), elephant seals (*Mirounga angustirostris*), and harbor seals (*Phoca vitulina*; Johnson et al., 1998). No staphylococci were resistant to oxacillin; hence no methicillin-resistant strains (MRS) were identified in this study.

Three of the beta-hemolytic streptococci isolates were identified as *S. phocae* group G, an organism that has also been isolated from California sea lions (Johnson et al., 2006) and southern sea otters (Imai et al., 2009). Staphylococci and streptococci are normal flora of the skin and oral cavity of carnivores (Hirsch and Biberstein, 2004b) and may opportunistically contaminate bite wounds (Goldstein, 1992), spread systemically, and colonize heart valves (Kvart and Häggström, 2000).

The *S. infantarius* subsp. *coli* isolates we evaluated were from California ($n=2$) and Alaska ($n=5$). This organism has been recognized recently as a significant cause of fatal valvular endocarditis and sepsis in northern sea otters (*Enhydra lutris kenyoni*; Burek et al., 2005) in Alaska, but is a considerably less common cause of death in southern (California) sea otters (CDFG, unpubl. data). Although infections in otters are often fatal, improved recognition and expedited therapy could reduce case fatality rates. Field strains of streptococci are often resistant to fluoroquinolones and aminoglycosides (Aucoin, 2000). Our *S. infantarius* subsp. *coli* isolates were considered intermediate in their resistance to ciprofloxacin, enrofloxacin, and amikacin, but none were resistant to penicillin or ampicillin, two antimicrobials often chosen for initial therapy of sea otters (M. Murray, pers. comm.).

Erysipelothrix rhusiopathiae is a facultative pathogen of cetaceans and pinnipeds, causing septicemia, endocarditis,

and skin abscesses (Wood and Shuman, 1981; Suer and Vedros, 1988). It is a zoonotic pathogen linked to occupational or recreational exposure to marine mammals or fish (Reboli and Farrar, 1989). Penicillins and most cephalosporins are considered effective for treating *Erysipelothrix* infections in humans (Reboli and Farrar, 1989) and sensitivity testing of our isolates suggests they could be effective treatment choices for otters. Collectively, the three *E. rhusiopathiae* isolates were resistant to 19 of 26 (73%) antimicrobials, including some cephalosporins. This information should help facilitate antimicrobial drug selection for sea otters, other marine mammals, and humans with suspected or culture-confirmed *E. rhusiopathiae* infections.

Corynebacteria have been isolated at necropsy from sea otters with infected wounds (M. Miller, pers. comm.) but their role as primary pathogens is not well established. Relatively recently their importance in human disease has been appreciated (CDC-NARMS, 2007). Of the nine corynebacteria isolated from sea otters, two were identified as *C. ulcerans*, a bacterial pathogen of the skin and internal organ systems of livestock. The remaining seven isolates were grouped as *Corynebacterium* sp. Sea otter-origin *Corynebacteria* spp. were susceptible to all antimicrobials.

The seven isolates of *Arcanobacterium* spp., including four *A. phocae*, were obtained from a variety of tissues and are an opportunistic pathogen of marine mammals, including sea otters (Johnson et al., 2003). All otter *Arcanobacterium* spp. isolates were susceptible to all antimicrobials tested.

Based on our preliminary data, it appears that, even in the absence of specific antimicrobial sensitivity information, most gram-positive bacterial infections in sea otters can be effectively treated with a combination of higher doses of penicillins and enrofloxacin or with third generation cephalosporins.

Gram-negative bacteria

Escherichia coli can cause intestinal and genitourinary tract infections in humans, domestic animals, and wildlife (Sussman, 1985; Wilson et al., 1988). Antimicrobial resistance among enteric bacteria, which may originate from environmental contamination by human and animal feces, is a serious clinical problem in human and veterinary medicine (Niemi et al., 1983; Dwight et al., 2004). Stoddard et al. (2008) demonstrated an association between increased exposure to runoff and a higher probability of antimicrobial-resistant *E. coli* infections in stranded elephant seals in California. Amoxicillin-clavulanic acid is considered a good choice for treatment of *E. coli* infections in small animals, with a 75–90% probability of in vitro efficacy (Aucoin, 2000). However, we isolated several *E. coli* strains from sea otters that fell outside the MIC 90 and were classified as resistant, including a hemolytic *E. coli* strain.

We found no evidence for multidrug-resistant strains of *E. coli* in sea otters and did not isolate *E. coli* O157:H7. The later is of interest because the Salinas River, which drains a region where *E. coli* O157:H7-contaminated spinach was detected in September 2006 (CDC, 2006), empties into Monterey Bay in an area heavily used by sea otters. These results are consistent with prior surveys for *E. coli* O157:H7 in sea otters and marine invertebrates from central California (Miller et al., 2006, 2009).

Salmonella spp. are a major cause of foodborne illness in humans and have been associated with enteritis, cholecystitis, abscesses, pneumonia, and septicemia (Gilmartin et al., 1979; Howard et al., 1983). *Salmonella* spp. from sea otters were resistant to erythromycin, oxacillin, penicillin, rifampin, clindamycin, and tylosin. Antimicrobial therapy is only recommended for severe systemic and enteric disease, with chloramphenicol, ampicillin, cefotaxime, ceftriaxone, and ciprofloxacin (Price et al., 2000). Investi-

gators found a low prevalence of *Salmonella* infection in sea otters from central California and a possible association between enteric *Salmonella* infection and seabird and/or pinniped exposure (Miller et al., 2009).

Humans may serve as asymptomatic reservoirs for *V. cholerae* and it is commonly isolated from sediments in estuaries. Emergence of human disease often follows warm weather or mass-flooding events such as typhoons (Hales et al., 1999; Speelman et al., 2000). All three sea otter *V. cholerae* isolates were obtained from feces. Some toxigenic *V. cholerae* strains cause acute intestinal illness and watery diarrhea in humans, which can lead to dehydration and death if not treated promptly. Toxin production was not assessed for sea otter *V. cholerae* isolates due to cost constraints. Tetracycline is the treatment of choice for humans, but trimethoprim-sulfamethoxazole, erythromycin, doxycycline, chloramphenicol, and furazolidone (WHO, 2000) can also be used. These same antimicrobials were effective against sea otter *V. cholerae* isolates in vitro, except for erythromycin, which showed intermediate susceptibility.

Vibrio parahaemolyticus, like *V. cholerae*, can be found in estuarine habitat and can cause gastrointestinal illness and skin infections in humans (Abbot et al., 2007) and treatment is similar (NCID-CDC, 2005). All sea otter *V. parahaemolyticus* isolates were susceptible to tetracycline and ciprofloxacin, but intermediate resistance to ampicillin (MIC 90 at 8.0 µg/ml) and partial resistance to penicillins, oxacillin, clindamycin and tylosin was observed.

Photobacterium damsela may cause severe necrotizing wound infections and sepsis following seawater exposure in humans (Coffey et al., 1986), and rapid selection and application of an effective antimicrobial is important. The majority of *P. damsela* isolates from sea otters (67%) were obtained from heart blood with a few

from joint or lung tissue. *Photobacterium damsela* is not a common pathogen of small animals and its in vitro susceptibility to antimicrobials is not well understood. In humans, *P. damsela* is reported to be susceptible to penicillin, tetracycline, and chloramphenicol (Coffey et al., 1986). In our study, *P. damsela* isolates from sea otters were susceptible to tetracycline and chloramphenicol, but were resistant to penicillins in vitro.

Pasteurella multocida is a bacterial pathogen of pet and agricultural animals (Hirsch and Biberstein, 2004a). It can be a facultative pathogen of the upper respiratory tract and can be transmitted via bite wounds. Penicillins are not commonly used to treat *P. multocida* in small animals, although the sea otter isolates were all susceptible to penicillin. Cephalosporins or enrofloxacin would also be good initial treatment choices.

Campylobacter spp. are responsible for millions of cases of food-borne illness in humans each year (Butler, 2001) and appear to be relatively common in marine and estuarine environments along the California coast (Abeyta et al., 1995). Of 17 *Campylobacter* isolates from sea otters, two were identified as *C. lari*, three were identified as *C. coli*, and the remainder were classified as *Campylobacter* spp. Interpretive criteria for antimicrobial susceptibility in animals have not yet been published for *Campylobacter* spp.; therefore, breakpoints for antimicrobial inhibitory values are extrapolated from human isolates. Ninety percent of the *Campylobacter* spp. isolates tested were sensitive to the four antimicrobials investigated (ciprofloxacin, doxycycline, gentamicin, and erythromycin), but one was resistant to ciprofloxacin (MIC=4.0 µg/ml).

One *B. bronchiseptica* isolate was included in this study due to its clinical importance as the cause of fatal bronchopneumonia in a sea otter (Staveley et al., 2003). The sea otter *B. bronchiseptica* isolate was susceptible to cefazolin, ceftiofur, ceftizoxime, oxacillin, penicillin, ri-

fampin, cefotaxime, clindamycin, tylosin, and erythromycin.

Some gram-negative bacteria isolated from sea otters may be opportunistic pathogens and further studies are currently underway to explore associations between specific lesions and bacterial strains and to compare isolates obtained from sea otters with those from other terrestrial and marine sources. Generally, for the range of gram-negative bacteria isolated from sea otters, enrofloxacin, ciprofloxacin, ampicillin-clavulanic acid, third generation cephalosporins, and, in some cases, tetracycline or doxycycline, appear to be reasonable choices for antimicrobial therapy.

We identified a range of pathogenic and opportunistic bacteria infecting sea otters in the near-shore marine environment and provide data on antimicrobial resistance. As bacterial resistance is variable, prolonged clinical treatment should be guided by culture and sensitivity testing. Based on prior reports (Smith et al., 1978; Baker et al., 1998; Brew et al., 1999), several bacteria that infect marine mammals can cause necrotizing dermatitis, systemic disease, and death in humans (Hunt et al., 2008) and the information provided should be considered by physicians.

Antimicrobials are important for clinical care of sea otters as they often present with severe injuries and local or systemic infections. Sea otter fur is critical for thermoregulation and hair coat integrity cannot be compromised, so surgical sites cannot be shaved prior to topical disinfection for surgery. Otters handled as part of conservation and research efforts that require flipper tagging, biopsy, telemetry implant surgery, and venipuncture, may be given prophylactic antimicrobials to reduce the risk of subsequent infection. Careful consideration of the most common potential pathogens, their likely resistance pattern, and the pharmacokinetics of the drug in the host animal is critical.

Sea otters are relatively large and

aggressive carnivores and repeated handling may be dangerous for clinical care staff, be stressful for both parties, and have negative health consequences for otters. They have an exceptionally keen sense of smell and taste, precluding placement of most chemotherapeutics within food items, regardless of attempts to mask the scent or flavor. To date, our clinical experience and the findings presented here support use of longer-acting procaine and benzathine penicillin compounds and the fluoroquinolones for initial treatment. These choices have some negative effects; repeated intramuscular injections are associated with discomfort and lameness. Initial experience with recently released injectable, broad spectrum, prolonged activity cephalosporins have been encouraging and evaluating the efficacy of these newer drugs against some of the more common opportunistic pathogens of sea otters in vitro would be beneficial.

Development of antimicrobial resistance is a topic of intense public and governmental scrutiny (Cuny et al., 2010) and a serious concern of zoological and wildlife veterinarians. Evidence increasingly suggests that inappropriate antimicrobial use may cause an increased incidence of resistant bacteria (Goossens et al., 2005). However, we found very little evidence for this in sea otters from California and Alaska between 1998 and 2005.

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