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AEROBIC BACTERIAL ISOLATIONS FROM HARBOR SEALS (*PHOCA VITULINA*) STRANDED IN WASHINGTON: 1992–2003

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Abstract: Bacterial cultures collected over 12 yr from stranded harbor seal (*Phoca vitulina*) pups and weanlings located in the North Puget Sound and San Juan Islands region of Washington were analyzed retrospectively to determine the most common pathogenic isolates and to describe their antimicrobial resistance patterns. Culture attempts ($n = 58$) from wounds, umbilici, ears, conjunctiva, nares, oral lesions, and feces yielded 134 pathogenic isolates that represented 17 genera. The majority of isolates were Gram-negative ($n = 87$; 65%) and of the tested isolates were most susceptible to amikacin ($n = 76$; 99%) and gentamicin ($n = 76$; 97%) and least susceptible to ampicillin ($n = 76$; 26%). Of the Gram-positive isolates tested ($n = 29$), all were susceptible to amoxicillin/clavulanic acid. The most frequent isolates were *Escherichia coli* (17%), β-hemolytic *Streptococcus* spp. (15%), *Enterococcus* spp. (11%), and *Pseudomonas aeruginosa* (11%), with all four exhibiting resistance to more than 50% of the antimicrobials tested. The variety of organisms isolated, the variation in either Gram-negative or Gram-positive predominance, and the multiple drug resistance patterns observed suggest that when treating stranded harbor seals, culture and sensitivity testing are warranted and that antibiotic therapy should be based on results.

Key words: Antimicrobial susceptibility, bacteria, bacterial infection, harbor seal, *Phoca vitulina*, pinniped.

INTRODUCTION

Bacterial infections are a major cause of morbidity and mortality in wild and captive pinnipeds.[4,12] They cause septicemia and are primary pathogens of the integument, respiratory, digestive, and urogenital tracts, and visual, auditory, and cardiovascular systems.[7] In harbor seals (*Phoca vitulina*), bacteria also are common secondary invaders of deep and superficial wounds from animal bites, gunshots, and boat propellers.[9] Little is known about common bacterial pathogens in free-ranging harbor seals.

Steiger et al.[11] found *Escherichia coli* and *Proteus* spp. were the most common bacterial isolates from 108 dead harbor seal pups examined in Washington. Surveys of live and dead pinnipeds stranded along the central and northern coast of California also found *E. coli* was the most common bacterial pathogen isolated.[9,12] Despite the prevalence of these bacteria, they are not always associated with disease. For example, aerobic bacterial culture performed on brains from 34 dead harbor seal pups yielded *E. coli* and *Proteus* spp. from eight and 10 samples, respectively; however only three animals had histologic evidence of septicemia or other bacterial infections.[11] Gastrointestinal disorders have been documented as a common factor in mortalities of stranded harbor seal pups; however, the role of pathogenic bacteria in these cases could not be determined.[5]

Antimicrobial resistance is a concern in human and veterinary medicine, yet little is known about antimicrobial resistance patterns of pathogenic bacteria of free-ranging harbor seals.[2,13] Previous work suggests that it is common for bacteria isolated from pinnipeds in rehabilitation to be resistant to multiple antimicrobials, but no studies have examined antimicrobial resistance patterns over time.[9] Culture and sensitivity are indicated for appropriate selection of antimicrobials for treatment of bacterial infections in stranded harbor seals, yet frequently treatment must be implemented before culture results. Thus, knowledge of antimicrobial susceptibility of harbor seal bacterial pathogens would assist clinicians when deciding which antimicrobials are best before obtaining these results. Additionally, information about antimicrobial resistance in harbor seals could help elucidate potential relationships between antimicrobial resistance in humans and in wildlife.

To determine the most common bacterial pathogens and their resistance patterns from live stranded harbor seal pups and weanlings from the North Puget Sound and San Juan Islands region of Washington, culture and sensitivity results over a 12-yr period were retrospectively examined. Additionally, we compared these pathogenic bacterial isolates to isolates cited in literature from live seals stranded in central and northern California and dead seals stranded in Washington and central California.[9,11,12]

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Results should improve selection of effective initial antimicrobial treatment for suspected bacterial infections in stranded harbor seals pending culture and sensitivity results.

MATERIALS AND METHODS

Animals and sampling

Medical records from live stranded harbor seals (n = 318) that were admitted from 15 July 1992 through 26 September 2003 for rehabilitation at a National Oceanographic and Atmospheric Association Fisheries (NOAA Fisheries, Seattle, Washington 98115, USA)-authorized rehabilitation center (Wolf Hollow Wildlife Rehabilitation Center [WHWRC] Friday Harbor, Washington 98250, USA) were used for this study. Seals were considered stranded if found alone on beaches in high public use areas where human interference or harassment could not be mitigated or were injured from presumed fisheries interactions (NOAA Fisheries Northwest Regional Policy). Cases originated from seven counties around Puget Sound (n = 57; San Juan, Island, Skagit, Jefferson, King, Kitsap, and Whatcom) and one county along the Olympic Coast (one seal from Grays Harbor).

Of the harbor seals admitted to WHWRC for treatment, 58 (18%) had samples taken for aerobic bacterial culture and sensitivity testing. Manual restraint was used to collect all samples. Seals sampled included premature pups (lanugo coat present; n = 12), full-term pups (<2 mo; n = 33), and weanlings (2–6 mo; n = 13).[3,10] Sampling and further sensitivity testing fluctuated with available funding during certain years. No samples were collected in 1995 and 1996, and the greatest number of samples was collected in 2003 when 18 samples were taken from 11 seals. Of the 58 seals sampled, 74% were from San Juan County (n = 43).

Culture and sensitivity testing

Samples for aerobic bacterial culture were collected antemortem from sites that were reddened, swollen, hot, discharging, or were otherwise suspicious for bacterial involvement. They were not taken from tissues that seemed healthy. Swabs were taken from wounds, umbilici, ears, conjunctiva, nares, oral lesions, and feces and held in Amies transport medium without charcoal (Collection and Transport System, Beckton Dickinson and Co., Sparks, Maryland 21152, USA). Samples were refrigerated and shipped overnight to Phoenix Central Laboratory (Everett, Washington 98204, USA) for processing.

Swabs were streaked onto tryptic soy agar with 5% sheep blood, chocolate agar, phenyl ethyl alcohol agar with 5% sheep blood, MacConkey agar, thioglycollate broth or Campylobacter agar as indicated for bacterial isolation. Identification of enteric Gram-negative rods was primarily achieved using API 20E (BioMeriuex, Durham, North Carolina 27712, USA) when applicable. Nonfermenting Gram-negative rods were identified using the API 20NE panel (BioMeriuex). Gram-positive organisms were identified using conventional tests such as catalase, Gram stain, tube coagulase, pyrrolidonyl arylamidase (PYR), and triple sugar with iron tubed media.

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method using a standardized inoculum and technique with appropriate antibiotics (n = 21) for the species and organism.[1] Only antimicrobials that were consistently tested during the study period were used for this research. Antimicrobials tested included amikacin, amoxicillin/clavulanic acid, ampicillin, carbenicillin, cefotaxime, cephalothin, chloramphenicol, ciprofloxacin, enrofloxacin, erythromycin, gentamicin, marbofloxacin, piperacillin, tetracycline, ticarcillin, tobramycin, and trimethoprim sulfa.

Bacteria considered indicative of contamination or those that were considered nonpathogenic were not analyzed. Bacteria that were considered contaminants included those that did not grow in pure culture or predominance or those that could only be cultured from subculture broths. For this analysis, species were grouped by genus once contaminants and nonpathogenic bacteria were excluded. *Staphylococcus aureus* isolates were never tested with carbenicillin, because zone sizes are not published for the interpretation of the results.

RESULTS

From the samples taken, 281 bacterial isolates were obtained, of which antimicrobial sensitivity was performed on 221 (78.6%). Of these 281 bacterial isolates, 147 (54%) were excluded from analysis, because they occurred with scant (n = 41) or light (n = 70) growth, were found in subculture broths (n = 17), or were considered contaminants or nonpathogenic bacteria (n = 19). These included *Acinetobacter* spp., *Chryseobacterium* spp., *Coryneform* Gram-positive rods (other than *C. pseudotuberculosis*), *Flavobacterium* spp., *Lactobacillus* spp., *Pseudomonas* spp. (other than *P. aeruginosa*), coagulase-negative *Staphylococcus*, and α-hemolytic *Streptococcus*.

Samples from 46 seals were included for analysis, representing 57 culture attempts. Five seals had serial cultures of the same site at two separate times

Table 1. Number of bacterial isolates by source.[a]

Bacteria Swabs taken:	Ear $n = 7$	Conjunctiva $n = 1$	Fecal $n = 2$	Nares $n = 4$[b]	Umbilicus $n = 13$	Wound $n = 22$[c]	Total
Gram-positive isolates							
Arcanobacterium spp.					1	2	3
β-hemolytic *Streptococcus*	1				3	16	20
Corynebacterium pseudotuberculosis	1			1		1	3
Enterococcus spp.	3	1		2	3	6	15
Staphylococcus spp.	2			1	1	2	6
Gram-negative isolates							
Aeromonas spp.			2	2	4	3	11
Chryseobacterium spp.		1				1	2
Citrobacter spp.				1		2	3
Escherichia coli	7			4	3	9	23
Enterobacter spp.					4	3	7
Klebsiella spp.	2			2	1	3	8
Morganella morganii					2	2	4
Pasteurella spp.		1				1	2
Proteus spp.				2		3	5
Pseudomonas aeruginosa	2		1	1	2	9	15
Serratia marcescens					1	2	3
Shewanella spp.				1		3	4
Total	18	3	3	17	25	68	134

[a] Note that due to mixed infections, the number of isolates often exceeds the number of swabs taken.
[b] One seal was sampled four times.
[c] Twenty-seven samples were taken, five wounds were sampled twice.

to evaluate antimicrobial treatment effectiveness, three seals had two different sites sampled, and one seal with a persistent infection (nasal) had the same site sampled four times. In these nine seals, bacteria isolated changed from one culture to the next, presumably because of antibiotic therapy, except in the case of two animals where culture was repeated at the same site but only one day separated the two sampling events. Relative to initiation of antibiotic therapy, samples were taken before ($n = 28$), within 12 hr ($n = 3$), within 48 hr ($n = 14$), or they were taken because animals failed to respond to treatment ($n = 12$). Antimicrobial therapy was initiated 12 to 48 hr before obtaining a culture when the area suspected of bacterial involvement was not noted on initial physical exam and animals were presumably started on antimicrobials for other conditions.

The 134 isolates analyzed in this study represented 17 genera (Table 1). Most frequently isolated were *E. coli* ($n = 23$; 17%), β-hemolytic *Streptococcus* spp. ($n = 20$, 15%), *Enterococcus* spp. ($n = 15$; 11%), and *P. aeruginosa* ($n = 15$; 11%). The majority of isolates were Gram-negative ($n = 87$; 65%), which exhibited more resistance to multiple antimicrobials than Gram-positive isolates (Table 2). Gram-negative isolates tested were most susceptible to amikacin ($n = 76$; 99%) and gentamicin ($n = 76$; 97%) and least susceptible to ampicillin ($n = 76$; 26%). Of the Gram-positive isolates tested ($n = 29$), all were susceptible to amoxicillin/clavulanic acid. Although the sample size was small, marbofloxacin, a newer flouroquinolone, was the only antibiotic found to be 100% effective against both Gram-positive ($n = 1$) and Gram-negative ($n = 7$) isolates. Antibiotics that demonstrated effectiveness against mixed bacteria (Gram-positive and Gram-negative bacteria, respectively) were gentamicin (67% and 97%), enrofloxacin (67% and 89%), and trimethoprim sulfa (88% and 66%).

Analysis of bacteria by source (Table 1) indicates that β-hemolytic *Streptococcus* spp. were the most predominant wound isolate ($n = 16$; 24%) and were cultured from 68% (15/22) of wounds. Of the wound sites cultured, 46% (10/22), 36% (8/22), and 18% (4/22) yielded mixed, exclusively Gram-positive or exclusively Gram-negative isolates, respectively.

Aerobic culture of umbilical infections yielded 46% (6/13), 31% (4/13), and 23% (3/13) of exclusively Gram-negative, mixed, and exclusively Gram-positive isolates, respectively. Of seven seals sampled for suspected external ear canal infections, *E. coli* (39%; 7/18) was the most predominant isolate. Only one seal was sampled four consecutive

Table 2. Gram-negative and Gram-positive bacterial susceptibility to antimicrobials by year.

Antimicrobial	1992		1993		1997		1998		1999		2000		2001		2002		2003		Total	
Gram stain:	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Amikacin																				
%	100	100	100	0	100	0	100	33	100	50	100	0	100	50	91	100	100	43[a]	99[b]	
n	1	2	2	3	11	1	5	3	12	4	1	2	14	4	11	2	19	21[c]	76[d]	
Amoxicillin/clavulanic acid																				
%	100	100	100	100	9	100	0	100	42	100	0	100	36	100	55	100	47	100[a]	38[b]	
n	1	4	2	3	11	1	5	3	12	4	1	2	14	5	11	7	19	29[c]	76[d]	
Ampicillin																				
%	100	75	100	100	0	100	0	67	17	50	0	100	29	100	55	86	26	83[a]	26[b]	
n	1	4	2	3	11	1	5	3	12	4	1	2	14	5	11	7	19	29[c]	76[d]	
Carbenicillin																				
%	100			100	64	0	60		40		0		79		64		53	75[a]	60[b]	
n	1		3	3	11	1	5		10		1		14		11		19	4[c]	72[d]	
Cefotaxime																				
%	100			33	82	0	80	33	83	33	100	50	64	75	73	100	89	50[a]	79[b]	
n	1			3	11	1	5	3	12	3	1	2	14	4	11	2	18	18[c]	73[d]	
Cephalothin																				
%	100	75	100	33	18	0	0	33	50	50	0	50	36	75	36	100	37	58[a]	36[b]	
n	1	4	2	3	11	1	5	3	12	4	1	2	14	4	11	3	19	24[c]	76[d]	
Chloramphenicol																				
%	100	100	100	100	55	100	80	33	75	100	0	50	64	100	73	100	63	88[a]	67[b]	
n	1	4	2	3	11	1	5	3	12	4	1	2	14	4	11	3	19	24[c]	76[d]	
Ciprofloxacin																				
%									100			100	100	50	91		78	67[a]	91[b]	
n									1			2	14	4	11		9	6[c]	35[d]	
Enrofloxacin																				
%	100	100	100	67	100	0	80	33	83	75	100	100	86	75	91	33	89	67[a]	89[b]	
n	1	4	2	3	11	1	5	3	12	4	1	2	14	4	11	3	19	24[c]	76[d]	
Erythromycin																				
%		100		0		0		33		50		50		67		100		58[a]		
n		2		1		1		3		4		2		3		3		19[c]		
Gentamicin																				
%	100	100	100	33	100	0	100	33	100	75	100	50	93	75	91	100	100	67[a]	97[b]	
n	1	4	2	3	11	1	5	3	12	4	1	2	14	4	11	3	19	24[c]	76[d]	
Marbofloxacin																				
%																100	100	100[a]	100[b]	
n																1	7	1[c]	7[d]	

Table 2. Continued.

Antimicrobial / Gram stain:	1992		1993		1997		1998		1999		2000		2001		2002		2003		Total	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Piperacillin																				
%		100			100	64		100		100		100		93		82		79	100[a]	85[b]
n		1			3	11		5		11		1		14		11		19	3[c]	73[d]
Tetracycline																				
%		100	100	100	67	9	100	80	33	67	100	0	100	50	100	55	100	53	88[a]	51[b]
n		1	4	2	3	11	1	5	3	12	4	1	2	14	4	11	3	19	24[c]	76[d]
Ticarcillin																				
%		100			100	64		60		50		0		79		73		53	100[a]	62[b]
n		1			3	11		5		12		1		14		11		19	3[c]	74[d]
Tobramycin																				
%		100			33	91	0	100	33	100	67	100	50	100	75	91	100	89	56[a]	95[b]
n		1			3	11	1	5	3	12	3	1	2	14	4	11	2	19	18[c]	74[d]
Trimethoprim sulfa																				
%		100	100	100	100	73	100	80	100	75	75	0	100	57	75	64	67	58	88[a]	66[b]
n		1	4	2	3	11	1	5	3	12	4	1	2	14	4	11	3	19	24[c]	76[d]
Total																				
n		1	4	2	3	11	1	6	4	20	6	1	4	15	13	12	12	19	47[a]	87[b]

[a] Percentage of susceptible Gram-positive isolates.
[b] Percentage of susceptible Gram-negative isolates.
[c] Total number of Gram-positive isolates tested.
[d] Total number of Gram-negative isolates tested.

Table 3. Antimicrobial susceptibility patterns of beta-hemolytic *Streptococcus* spp.

Antimicrobial	1993	1999	2001	2002	2003	Total[a]	Percentage[b]
Amikacin							
%		0	0	100			33
n		1	1	1		3	
Amoxicillin/clavulanic acid							
%	100	100	100	100	100		100
n	2	1	1	1	1	6	
Ampicillin							
%	100	100	100	100	100		100
n	2	1	1	1	1	6	
Cefotaxime							
%		0	100	100			67
n		1	1	1		3	
Cephalothin							
%	100	0	100	100	100		84
n	2	1	1	1	1	6	
Chloramphenicol							
%	100	0	100	100	100		84
n	2	1	1	1	1	6	
Ciprofloxacin							
%			100	100			100
n			1	1		2	
Enrofloxacin							
%	100	0	100	100	0		67
n	2	1	1	1	1	6	
Erythromycin							
%	100	0	100	100	100		84
n	2	1	1	1	1	6	
Gentamicin							
%	100	0	100	100	100		84
n	2	1	1	1	1	6	
Tetracycline							
%	100	0	100	100	100		84
n	2	1	1	1	1	6	
Tobramycin							
%		0	100	100			67
n		1	1	1		3	
Trimethoprim sulfa							
%	100	100	100	100	100		100
n	2	1	1	1	1	6	

[a] Total number of isolates tested.
[b] Percentage of susceptible isolates.

times for a suspected nasal infection yielding multiple, mixed isolates that changed with successive culture attempts.

Beta-hemolytic *Streptococcus* isolates were 100% sensitive to ampicillin, amoxicillin/clavulanic acid, and trimethoprim sulfa throughout the study period (Table 3). Sensitivities were not routinely indicated for β-hemolytic *Streptococcus* where penicillin or ampicillin were considered appropriate antimicrobials for treatment. Analysis of antimicrobial sensitivity of *E. coli* (Table 4) demonstrated susceptibility to aminoglycocides and fluoroquinolones throughout the study period. *Enterococcus* isolates were all susceptible to ampicillin and amoxicillin/clavulanic acid as tested, and *P. aeruginosa* isolates were 100% susceptible to amikacin, piperacillin, ticarcillin, and tobramycin. The small sample size makes it difficult to evaluate changes in antimicrobial resistance over time, but it is interesting to note that multidrug resistance is present in all four of the most common isolates (Tables 3–6).

Table 4. Antimicrobial susceptibility patterns of *Escherichia coli.*

Antimicrobial	1992	1993	1997	1999	2001	2002	2003	Total[a]	Percentage[b]
Amikacin									
%	100	100	100	100	100	100	100		100
n	1	2	2	1	5	4	5	20	
Amoxicillin/clavulanic acid									
%	100	100	0	100	80	75	60		70
n	1	2	2	1	5	4	5	20	
Ampicillin									
%	100	100	0	0	60	75	60		60
n	1	2	2	1	5	4	5	20	
Carbenicillin									
%	100		0	0	60	75	60		56
n	1		2	1	5	4	5	18	
Cefotaxime									
%	100		100	100	80	100	100		95
n	1		2	1	5	4	5	18	
Cephalothin									
%	100	100	0	100	80	75	80		75
n	1	2	2	1	5	4	5	20	
Chloramphenicol									
%	100	100	0	100	100	100	60		80
n	1	2	2	1	5	4	5	20	
Ciprofloxacin									
%					100	100	100		100
n					5	4	2	11	
Enrofloxacin									
%	100	100	100	100	100	100	100		100
n	1	2	2	1	5	4	5	20	
Gentamicin									
%	100	100	100	100	100	100	100		100
n	1	2	2	1	5	4	5	20	
Marbofloxacin									
%							100		100
n							2	2	
Piperacillin									
%	100		0	100	80	75	60		67
n	1		2	1	5	4	5	18	
Tetracycline									
%	100	100	0	100	60	75	60		65
n	1	2	2	1	5	4	5	20	
Ticarcillin									
%	100		0	0	60	75	60		56
n	1		2	1	5	4	5	18	
Tobramycin									
%	100		50	100	100	100	100		95
n	1		2	1	5	4	5	18	
Trimethoprim sulfa									
%	100	100	100	0	60	75	60		70
n	1	2	2	1	5	4	5	20	

[a] Total number of isolates tested.
[b] Percentage of susceptible isolates.

DISCUSSION

Although infection does not always imply disease, these data strongly suggest that *E. coli*, β-hemolytic *Streptococcus* spp., *Enterococcus* spp., and *P. aeruginosa* are common bacterial pathogens in harbor seal wounds and umbilical infections in the North Puget Sound and San Juan Islands region of Washington. In addition, and despite a small

Table 5. Antimicrobial susceptibility patterns of *Enterococcus* spp.

Antimicrobial	1997	1998	2000	2001	2002	2003	Total[a]	Percentage[b]
Amikacin								
%	0	0	0	0	0			0
n	3	1	2	1	2		9	
Amoxicillin/clavulanic acid								
%	100	100	100	100	100	100		100
n	3	1	2	1	3	3	13	
Ampicillin								
%	100	100	100	100	100	100		100
n	3	1	2	1	3	3	13	
Carbenicillin								
%	100	0						75
n	3	1					4	
Cefotaxime								
%	33	0	0	0	50			23
n	3	1	2	1	2		9	
Cephalothin								
%	33	0	0	0	50			23
n	3	1	2	1	2		9	
Chloramphenicol								
%	100	100	100	0	100			89
n	3	1	2	1	2		9	
Ciprofloxacin								
%				100	0			33
n				1	2		3	
Enrofloxacin								
%	67	0	50	100	50			45
n	3	1	2	1	2		9	
Erythromycin								
%		0	50	0	0			20
n		1	2	1	1		5	
Gentamicin								
%	33	0	50	0	50			33
n	3	1	2	1	2		9	
Piperacillin								
%	100							100
n	3						3	
Tetracycline								
%	67	100	100	100	100			89
n	3	1	2	1	2		9	
Ticarcillin								
%	100							100
n	3						3	
Tobramycin								
%	33	0	50	0	50			33
n	3	1	2	1	2		9	
Trimethoprim sulfa								
%	100	100	50	100	100			89
n	3	1	2	1	2		9	

[a] Total number of isolates tested.
[b] Percentage of susceptible isolates.

sample size, *E. coli* was the most common isolate made from the external ear ($n = 7$) and nares ($n = 4$). These data are consistent with the findings of Thornton et al.[12] where *E. coli*, *Enterococcus* spp., β-hemolytic *Streptococcus* spp., and *Pseudomonas* spp. represented 68%, 27%, 16%, and 16% of isolates, respectively, from wounds and other superficial infections in stranded harbor seals.

Table 6. Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa.*

Antimicrobial	1997	1998	1999	2001	2002	2003	Total[a]	Percentage[b]
Amikacin								
%	100	100	100	100	100	100		100
n	3	1	2	4	2	2	14	
Amoxicillin/clavulanic acid								
%	0	0	0	0	0	0		0
n	3	1	2	4	2	2	14	
Ampicillin								
%	0	0	0	0	0	0		0
n	3	1	2	4	2	2	14	
Carbenicillin								
%	100	100	0	100	0	50		65
n	3	1	2	4	2	2	14	
Cefotaxime								
%	33	0	0	0	0	0		8
n	3	1	2	4	2	2	14	
Cephalothin								
%	0	0	0	0	0	0		0
n	3	1	2	4	2	2	14	
Chloramphenicol								
%	0	0	0	0	0	0		0
n	3	1	2	4	2	2	14	
Ciprofloxacin								
%				100	100	100		100
n				4	2	1	7	
Enrofloxacin								
%	100	0	0	50	100	100		65
n	3	1	2	4	2	2	14	
Gentamicin								
%	100	100	100	75	100	100		93
n	3	1	2	4	2	2	14	
Marbofloxacin								
%						100		100
n						1	1	
Piperacillin								
%	100	100	100	100	100	100		100
n	3	1	2	4	2	2	14	
Tetracycline								
%	0	0	0	0	0	0		0
n	3	1	2	4	2	2	14	
Ticarcillin								
%	100	100	100	100	100	100		100
n	3	1	2	4	2	2	14	
Tobramycin								
%	100	100	100	100	100	100		100
n	3	1	2	4	2	2	14	
Trimethoprim sulfa								
%	0	0	0	0	0	0		0
n	3	1	2	4	2	2	14	

[a] Total number of isolates tested.
[b] Percentage of susceptible isolates.

Johnson et al.[9] found *E. coli* was the most frequent organism isolated from all culture and sensitivity sites collected from animals stranded along the central and northern California coast. Thornton et al.[12] also found *E. coli* isolates present in inflammatory lesions as well as lungs, livers, and brains of seals that died while in rehabilitation. A literature review of bacteria isolated from marine mam-

mals found that *E. coli* has been isolated from the integumentary, respiratory, digestive, and genitourinary systems and from abscesses and that it was associated with septicemia.[7] Our findings are consistent with this previous work as well as with work by Steiger et al.[11] who isolated *E. coli* from 24% (8/34) of samples taken from dead stranded harbor seals in the inland waters of Washington.

In contrast to work by Steiger et al.,[11] our research isolated *Proteus* spp. in only 4% (5/134) of isolates, whereas their cultures yielded *Proteus* spp. in 29% (10/34) of isolates. They postulated that the high numbers of *Proteus* isolated could have been due to post-mortem changes or sampling contamination and could not be a confirmed cause of morbidity.[11]

Enterobacteriaceae (*Citrobacter* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Serratia* spp.) represented 56% of Gram-negative isolates in this study. In harbor seals, *E. coli* and other Enterobacteriaceae are not considered primary pathogens but instead opportunistic secondary invaders of wounds.[9] Aerobic bacterial culture performed on brains from 34 dead pups yielded *E. coli* and *Proteus* spp. from eight and 10 samples, respectively; however, only three animals had post-mortem signs of septicemia or other bacterial infections.[11] Further investigation is warranted to discover the role of *E. coli* in causing morbidity and mortality and the pathogenic impact of different types of *E. coli* in harbor seals.

Beta-hemolytic *Streptococcus* was the most common Gram-positive isolate and was a frequent wound isolate. In a literature review, Higgins[7] found that in seals from Europe *Streptococcus* spp. was the most common bacteria. Higgins also found that β-hemolytic *Streptococcus* was associated with abscesses, septicemia, and bronchopneumonia in harbor porpoises (*Phocoena phocoena*).[7] In California, however, *Streptococcus* spp. was not isolated frequently in live stranded harbor seals, and β-hemolytic *Streptococcus* was only found in 16% (9/56) of wounds, ocular and urethral discharges, and umbilical infections.[9,12] Beta-hemolytic *Streptococcus* was isolated from brain and liver tissue of dead harbor seals from Washington and California.[11,12]

In 1998, *Listeria ivanovii* made up 30% (17/56) of bacterial isolates cultured from harbor seal superficial abscesses, wounds, and umbilici.[12] Recent evidence suggests, however, that these isolates were probably misidentified and most likely were *Arcanobacterium pyogenes*, now classified as *A. phocae*.[8] Although not identified to species, we did isolate *Arcanobacterium* from two wounds and one umbilical infection. Culture and identification methodology cannot explain this difference in frequency of isolation, which could be due to differences in sample size or actual differences in prevalence.

Results of this study confirm previous work suggesting that it is common for bacteria isolated from pinnipeds to be resistant to multiple antimicrobials. Similar to previous work on antimicrobial resistance in bacteria isolated from harbor seals, *E. coli* was our most common isolate and demonstrated frequent resistance to the penicillins, sulphonamides, and cephalosporins.[9] In our study, gentamicin and enrofloxacin were 100% effective in treating *E. coli* infections ($n = 20$; Table 4), but previous studies found them to be 84% ($n = 37$) and 79% ($n = 29$) effective, respectively. In contrast to previous reports, we found amoxicillin/clavulanic acid and trimethoprim sulfa to be 70% effective overall against *E. coli*, compared with 49% ($n = 37$) and 32% ($n = 37$), respectively.[9] Isolates for the four most common bacteria were analyzed over time, but sample size each year was not large enough to determine whether antimicrobial resistance was increasing over time (Tables 3–6).

Based on data presented, broad-spectrum antibiotics should be considered for initial antimicrobial therapy in stranded harbor seals where bacterial involvement is suspected. The variety of mixed organisms cultured, and the observed multiple drug resistance, suggest that culture and sensitivity testing are warranted and that antibiotic therapy should be modified based on culture results. In addition, continued monitoring of bacterial culture and sensitivity results from stranded harbor seals should eventually yield meaningful comparisons between antimicrobial resistance patterns from harbor seals, humans, and domestic animals.[6]

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