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CHARACTERIZATION AND CLINICAL MANIFESTATIONS OF *ARCANOBACTERIUM PHOCAE* INFECTIONS IN MARINE MAMMALS STRANDED ALONG THE CENTRAL CALIFORNIA COAST

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ABSTRACT: Between 1994 and 2000, 141 *Arcanobacterium phocae* isolates were recovered from marine mammals that stranded along the central California coast (USA). *Arcanobacterium phocae* was cultured from tissue sites with abnormal discharge or evidence of inflammation in 66 California sea lions (*Zalophus californianus*), 50 Pacific harbor seals (*Phoca vitulina richardii*), 19 northern elephant seals (*Mirounga angustirostris*), five southern sea otters (*Enhydra lutris nereis*), and one common dolphin (*Delphinus delphis*). The overall prevalence of *A. phocae* among cultured stranded marine mammals was 8%. This is the first report of *A. phocae* in animals from the Pacific Ocean. Sequence analysis of a portion of the 16S ribosomal RNA gene confirmed recent isolates as *A. phocae.* Prior to phylogenetic testing and the routine use of the esculin hydrolysis and motility tests, *A. phocae* isolates may have been misidentified as *Listeria ivanovii. Arcanobacterium phocae* was commonly isolated from superficial abscesses, was often present in mixed infections, and was susceptible to all antimicrobial agents tested.

Key words: Antimicrobial susceptibility, *Arcanobacterium phocae,* bacteriology, clinical manifestations, common dolphin, marine mammal, pinniped, sea otter.

INTRODUCTION

Arcanobacterium phocae was first isolated and characterized in 1997 from common seals (*Phoca vitulina*) and gray seals (*Halichoerus grypus)* of the coastal waters around Scotland, U.K. (Ramos et al., 1997). The newly identified Gram positive bacterium was described as a non-motile, non-spore-forming, non-acid fast, coccobacillus or short rod. Phylogenetically *A. phocae* is similar to *Arcanobacterium pyogenes* (*Actinomyces pyogenes*), an important cause of pyogenic infections in animals, and, rarely, in humans, and *Arcanobacterium haemolyticum,* a cause of pharyngitis and systemic infections in humans (Mackenzie et al., 1995; Skov et al., 1998). *Arcanobacterium pluranimalium* was recently isolated from the spleen of a dead harbor porpoise (*Phocoena phocoena)* and a lung abscess in a fallow deer (*Cervus dama*) (Lawson et al., 2001). *Arcanobacterium pluranimalium* is the closest known relative to *A. phocae* in the genus, but has distinct phylogenetic and phenotypic differences.

Arcanobacterium species are commonly associated with mixed infections of wounds, but are also often cultured from mucous membranes of healthy animals and humans (Funke et al., 1997). The first reported *A. phocae* isolates were recovered in mixed cultures from seals with pneumonia and septicemia, but their pathologic significance was unknown (Ramos et al., 1997). Other *A. phocae* infections have not been described. *Arcanobacterium phocae* infections in pinnipeds may be under-reported, in part because past studies of bacterial infections in marine mammals rarely identified Gram positive rods to species level and often ignored these organisms as possible pathogens (Vedros et al., 1982; Thornton et al., 1998). Its close affinity to *Arcanobacterium* species, pathogens of humans and animals, suggests *A. phocae* may be an important cause of clin-

ical disease in marine mammals. The purposes of the study reported here were to determine the prevalence of *A. phocae* in marine mammals stranded along the central California (USA) coast between 1994 and 2000 and to describe the phenotypic and phylogenetic characteristics, antibiotic susceptibilities, and associated clinical manifestations of infection.

MATERIALS AND METHODS

A retrospective survey of medical records was performed to determine the prevalence of *A. phocae* infections and associated clinical findings in marine mammals stranded along the central California coast. The culture results from stranded animals from 1 January 1994 to 30 December 2000 were evaluated for confirmed and possibly unrecognized *A. phocae* infections based on bacterial identification methods (Quinn et al., 1994; Funke et al., 1997). Verification of *A. phocae* isolates was performed using phenotypic and phylogenetic techniques.

Stranding location, gross and histologic findings, culture results, and primary cause of stranding were recorded for all cases. Prevalence of *A. phocae* infections was determined by dividing the number of animals from which *A. phocae* was isolated by the total number of animals cultured during the study time period. A sampled animal was only counted once, even if multiple cultures where taken from that animal during its rehabilitation.

Samples for bacterial culture were obtained from dead and live stranded marine mammals along the central California coast including southern sea otters (*Enhydra lutris nereis*), Pacific harbor seals (*Phoca vitulina richardii*), northern elephant seals (*Mirounga angustirostris*), California sea lions (*Zalophus californianus*), Steller sea lions (*Eumetopias jubatus*), northern fur seals (*Callorhinus ursinus*), Guadalupe fur seals (*Arctocephalus townsendi*), and eight species of cetaceans. All live stranded animals were transported for rehabilitation at The Marine Mammal Center (TMMC) in Sausalito, California. Animals in rehabilitation were swabbed for bacterial culture from tissue sites with abnormal discharges or evidence of inflammation. Necropsies of animals that died during rehabilitation were performed at TMMC. During 1999 and 2000, post mortem examinations were also performed on dead stranded animals at the Marine Wildlife Veterinary Care and Research Center in Santa Cruz, California. Tissue samples from lung, liver, brain, lymph node, and inflammatory lesions were routinely taken during post mortem examination for bacterial culture. All post mortem samples for bacterial isolation were collected within 12 hr of the animal's death.

Tissues and swabs in Amies transport media were held at 4 C until streaked onto trypticase soy agar with 5% sheep blood and MacConkey plates within 24 hr of collection. Samples from live animals were also streaked onto phenylethyl alcohol (PEA) plates. All plates were incubated for 24–48 hr at 37 C. The blood agar and PEA plates were incubated under 5% carbon dioxide and the MacConkey plates were incubated aerobically. Within 48 hr of incubation, colonies on sheep blood agar that were small, white, circular, and beta-hemolytic were Gram stained and a catalase test was performed. Twenty-five isolates were further biochemically characterized by motility, CAMP reaction, and API 20 Strep kit (bioMerieux Vitek, Hazelwood, Missouri, USA). *Arcanobacterium pyogenes* (ATCC 19411) and *A. haemolyticum* (ATCC 9345) were tested and included in the study for comparison.

Prior to 1999, isolates with similar colonial characteristics and morphology were not consistently tested for esculin hydrolysis and motility. The isolates were always catalase positive and CAMP positive with *Rhodococcus equi* designating them as *Listeria ivanovii* (Quinn et al., 1994). Several of these isolates were retested for motility and esculin hydrolysis and identified as *A. phocae*.

Eighteen *A. phocae* isolates were tested using a microbroth dilution assay to determine susceptibility to selected antimicrobial agents. The assay was performed as described by the manufacturer (Trek Diagnostic Systems Inc., Westlake, Ohio, USA) and in accordance with recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 1999). Inocula were prepared in Mueller Hinton broth containing 3% lysed horse blood. The minimal inhibitory concentration (MIC) was defined as the lowest antibiotic concentration that resulted in no growth of the isolate. An isolate was considered susceptible to an antibiotic if the MIC of that antibiotic was less than the susceptibility breakpoint concentration described by the NCCLS for each antimicrobial.

A crude lysate was prepared by suspending several bacterial colonies in 200 μl sterile molecular biology grade water (Bio Whittaker, Walkersville, Maryland, USA) and boiled for 10 min. Lysates were centrifuged for 5 min at 1,500×G, and the supernatant was used as a template for polymerase chain reaction amplification of the 16S ribosomal RNA (16S rRNA) gene. Amplification primers were complimen-

tary to terminal 16S rRNA sequences (Drancourt et al., 2000). Polymerase chain reaction products were run on an ethidium bromide-stained 1% agarose gel to check for presence of a 1.5 kb product. The 1.5 kb PCR product was purified using Microcon Centrifugal Filter Devices (Millipore Corporation, Bedford, Massachusetts, USA). Purified PCR product was resuspended in sterile water to a final concentration of 30 ng/μl as determined by spectrophotometric quantification. Samples were sequenced (Davis Sequencing, Davis, California) using automated fluorescent nucleotide sequence determination without molecular cloning. The nucleotide sequence was compared to all available sequences in the National Center for Biotechnology Information (NCBI) database with the standard nucleotide-nucleotide BLAST (blastn) program, using the default parameters.

RESULTS

One hundred and eight isolates identified as *L. ivanovii* were cultured from stranded marine mammals from 1994 to 1998 and 33 isolates of *A. phocae* were obtained in 1999 and 2000. *Listeria ivanovii* and *A. phocae* have numerous phenotypic and biochemical similarities and some tests used to differentiate these bacteria were not employed between 1994 and 1998; thus the *L. ivanovii* isolates reported from marine mammals from 1994 to 1998 may have been misidentified. Several *L. ivanovii* isolates obtained in 1998 were retested, and all were reclassified as *A. phocae*. As no *Listeria* spp. have been isolated since the esculin hydrolysis and motility tests were implemented in 1999 to distinguish between *L. ivanovii* and *A. phocae,* all isolates previously identified as *L. ivanovii* are now presumed to have been *A. phocae.*

All *A. phocae* isolates were non-motile, Gram positive coccobacilli or short rods that were beta-hemolytic on blood agar within 24 hr of inoculation. A reverse CAMP reaction resulted with *Staphylococcus aureus* and a positive CAMP reaction with *Rhodococcus equi.* The biochemical characteristics of 25 *A. phocae* isolates are summarized in Table 1. *Arcanobacterium phocae* isolates were positive for catalase (100%), α-galactosidase (96%), β-galactosidase (96%), alkaline phosphatase (92%), and glycogen (84%), while the reference strain of *A. haemolyticum* was negative on these tests. The major biochemical differences found between the *A. phocae* isolates and *A. pyogenes* were the catalase test, hippurate hydrolysis, and α-galactosidase and β-glucuronidase production.

TABLE 1. Biochemical characteristics of *Arcanobacterium phocae, A. pyogenes,* and *A. haemolyticum,* as determined by the API 20 Step[a] kit.

Characteristic	Number of strains positive/number of strains tested		
	A. phocae	*A. pyogenes*	*A. haemolyticum*
Voges-Proskauer	0/25	0/1	0/1
Hippurate	0/25	1/1	0/1
Esculin	0/25	0/1	0/1
Pyrrolidonyl arylamidase	13/25	1/1	0/1
α-Galactosidase	24/25	0/1	0/1
β-Glucuronidase	0/25	1/1	0/1
β-Galactosidase	24/25	1/1	0/1
Alkaline phosphatase	23/25	1/1	0/1
Leucine arylamidase	25/25	1/1	1/1
Arginine dihydrolase	0/25	0/1	0/1
Fermentation of:			
Ribose	24/25	1/1	1/1
L-Arabinose	0/25	0/1	0/1
Mannitol	0/25	0/1	0/1
Sorbitol	0/25	0/1	0/1
Lactose	17/25	1/1	1/1
Trehalose	1/25	1/1	0/1
Inulin	0/25	0/1	0/1
Raffinose	0/25	0/1	0/1
Starch	22/25	1/1	1/1
Glycogen	21/25	1/1	0/1

[a] bioMerieux Vitek, Hazelwood, Missouri (USA).

A portion of the 16S rRNA gene of approximately 600 base pairs was amplified and sequenced. The 16S rRNA sequence obtained from the *A. phocae* isolate was found to be 98% homologous with the 16S rRNA gene of the reference *A. phocae* and 96% with *A. haemolyticum.* A second determination of a 300 base pair sequence from the *A. phocae* isolate resulted in a 1% difference among *A. phocae* (97%) and *A. haemolyticum* (96%). Sequence homology between the isolate and both *A. bernar-*

TABLE 2. Minimum inhibitory concentrations (MIC) of 17 antimicrobial agents for 18 isolates of *Arcanobacterium phocae.*

Antimicrobial	MIC (μg/ml)			
	Range	50%[a]	90%[b]	Breakpoint (μg/ml)[c]
Amikacin	≤0.5–16	2	8	≤64
Ampicillin	≤0.25	≤0.25	≤0.25	≤8
Amoxicillin/clavulanate	≤2	≤2	≤2	≤8
Cefazolin	≤2	≤2	≤2	≤32
Ceftiofur	≤0.06–0.5	0.12	0.25	≤8
Ceftizoxime	≤0.5–4	≤0.5	1	≤64
Chloramphenicol	≤0.25–1	≤0.25	0.5	≤32
Enrofloxacin	≤0.25–1	≤0.25	≤0.25	≤4
Erythromycin	≤0.12	≤0.12	≤0.12	≤8
Gentamicin	≤0.25–4	≤0.25	0.5	≤16
Oxacillin	≤2	≤2	≤2	≤4
Penicillin	≤0.03–0.12	≤0.03	≤0.03	≤4
Rifampin	≤0.12	≤0.12	≤0.12	≤4
Tetracycline	≤0.5	≤0.5	≤0.5	≤16
Ticarcillin/clavulanate	≤8	≤8	≤8	≤16
Trimethoprim-sulphamethoxazole	≤0.25	≤0.25	≤0.25	≤4

[a] Concentrations of antimicrobial at which 50% of the isolates were inhibited.
[b] Concentrations of antimicrobial at which 90% of the isolates were inhibited.
[c] Breakpoint established for susceptibility by National Committee for Clinical Laboratory Standards.

diae and *A. pluranimalium* was below 92%.

All 18 *A. phocae* isolates tested were 100% susceptible to all of the antimicrobial agents tested. Minimum inhibitory concentration (MIC) values for each antimicrobial are listed in Table 2.

From 1994 to 2000, 141 presumptive or confirmed *A. phocae* infections were identified from five species of marine mammals stranded along the central California coast. During this period, bacterial cultures were obtained from 1,760 marine mammals, including 828 California seal lions, 434 Pacific harbor seals, 304 northern elephant seals, 114 southern sea otters, and 43 cetaceans. An overall prevalence of 8.0% for *A. phocae* infection was detected across the sampled animals. The lowest prevalence of infection was in 1998 (5.3%), the highest in 2000 (13.5%) (Fig. 1). The greatest numbers of *A. phocae* isolates per species (66) were obtained from California sea lions (Table 3). However, harbor seals had the highest prevalence (11.5%) of *A. phocae* infections. *Arcanobacterium phocae* was not isolated from any of eight Steller sea lions, 34 northern fur seals, or five Guadalupe fur seals cultured in this study. From the 43 cetaceans that were examined, only one *A. phocae* isolate was recovered. This isolate was obtained from the lung of a dead, frozen-thawed common dolphin (*Delphinus delphis*) from Monterey Bay (California) that died due to severe pulmonary abscesses. Four of the five isolates from sea otters were cultured between 1999 and 2000. Increased numbers of bacterial cultures were performed

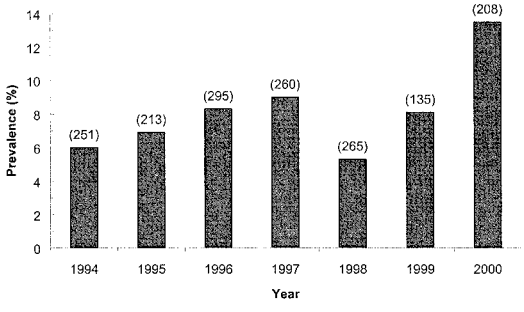

FIGURE 1. Prevalence of *Arcanobacterium phocae* in cultures from marine mammals performed from 1994 to 2000. (*n*)=number of animals cultured.

TABLE 3. Prevalence of *Arcanobacterium phocae* isolated from live and dead stranded marine mammals from 1994 to 2000 in California.

Number of *A. phocae* isolates	California sea lion (*n*=828)	Harbor seal (*n*=434)	Northern elephant seal (*n*=304)	Sea otter (*n*=114)	Cetacea (*n*=43)
Antemortem	23	39	15	0	0
Postmortem	43	11	4	5	1[a]
Total	66	50	19	5	1
Prevalence (%)	8.0	11.5	6.3	4.4	2.3

[a] Common dolphin.

on fresh dead sea otter carcasses after 1998.

Frequencies of infections from antemortem culture of sites of inflammation or discharge are summarized in Table 4. Of 77 *A. phocae* isolates obtained from live animals, 39 were from harbor seals, 23 were from California sea lions, and 15 were from northern elephant seals. *Arcanobacterium phocae* was most frequently isolated from abscesses (57.1% of total isolates), which were predominantly superficial or umbilical abscesses. Most of the superficial (16) and all of the umbilical (12), periorbital (1), and periodontal (2) abscesses containing *A. phocae* were found in harbor seals.

In animals examined postmortem, *A. phocae* was regularly isolated from multiple sites from the same animal. Results of bacterial cultures for tissues from animals examined at necropsy are summarized in Table 5. A total of 84 tissue or swab samples from 15 different sites was infected with *A. phocae.* Isolates were most commonly obtained from superficial abscesses, wounds, and exudates. Internal organs were rarely the only sites of infection. Of nine *A. phocae* isolates obtained from liver tissue, only two were not also obtained from other sites in the same animal. One exception was brain: of nine *A. phocae* isolates obtained from brain only four were also cultured from other sites.

Arcanobacterium phocae isolates from these marine mammals were often present as part of a mixed bacterial infection. *Arcanobacterium phocae* was isolated in pure culture in only 13 of 141 (9.2 %) cultures. Pure cultures of *A. phocae* were obtained

TABLE 4. Frequency of *Arcanobacterium phocae* cultured from sites of inflammation in live stranded marine mammals.

Culture site	California sea lion (*n*=828)	Harbor seal (*n*=434)	Northern elephant seal (*n*=304)	Total from site (=77)	Percent of total number of isolates
Abscess—superficial	10	16	3	29	38
Abscess—umbilicus	—	12	—	12	16
Abscess—periodontal	—	1	—	1	1
Abscess—periorbital	—	2	—	2	3
Bullet wound	—	1	—	1	1
External ear canal	—	4	—	4	5
Nasal discharge	2	—	1	3	4
Skin disease	—	—	3	3	4
Ocular discharge	—	—	1	1	1
Osteomyelitis	4	1	1	6	8
Shark bite	4	1	1	6	8
Surgical incision	1	—	1	2	3
Tracheal discharge	1	—	—	1	1
Wound/laceration	1	1	4	6	8

TABLE 5. Frequency of *Arcanobacterium phocae* isolated from sites of inflammation and tissue samples taken during postmortem examination of stranded marine mammals.

Culture site and tissue	California sea lion (n=828)	Northern Harbor seal (n=434)	Northern elephant seal (n=304)	Sea otter (n=114)	Common dolphin (n=1)	Total from site (=84)	Percent of total number of isolates
Abscess—subcutis	12	5	—	1	—	18	21
Abscess—periodontal	—	—	1	—	—	1	1
Brain	3	3	1	2	—	9	11
Bullet wound	1	—	—	—	—	1	1
Dermal wound	—	—	—	1	—	1	1
Liver	6	1	1	—	—	8	10
Lung	9	2	2	—	1	14	17
Kidney	1	—	—	—	—	1	1
Lymph node	3	—	1	—	—	4	5
Spleen	1	—	—	1	—	2	2
Bone	1	—	—	—	—	1	1
Peritoneal fluid	5	3	—	—	—	8	10
Pleural fluid	3	—	—	—	—	3	4
Shark bite	4	1	—	1	—	6	6.0
Umbilicus	—	4	—	—	—	4	5
Tonsil	1	—	1	—	—	1	1
Pharyngeal discharge	1	—	—	—	—	1	1
Tumor	2	—	—	—	—	2	2

from three umbilical abscesses and a flipper abscess in harbor seal pups; two brain lesions and a superficial abscess in sea otters; two brain lesions, a superficial abscess, lung, and a bone infection in California sea lions; and from ocular discharge in an elephant seal. Three to four different bacterial species were often present in mixed culture with *A. phocae*. *Escherichia coli* (42 isolates) and beta-hemolytic *Streptococcus* spp. (40 isolates) were the two bacteria most commonly present in mixed culture; with *A. phocae*. The number of *E. coli* colonies was usually low when present in mixed culture, whereas beta-hemolytic *Streptococcus* spp. growth was usually equivalent to the level of *A. phocae* growth. Other bacteria commonly isolated in mixed culture with *A. phocae* included *Enterococcus* spp. (26 isolates), *Proteus* spp. (17 isolates), *Staphylococcus aureus* (18 isolates), *Streptococcus viridans* (16 isolates), *Pseudomonas* spp. (11 isolates), *Corynebacterium* spp. (nine isolates) and *Klebsiella* spp. (eight isolates).

DISCUSSION

This is the first report of *A. phocae* isolation from California sea lions, northern elephant seals, southern sea otters, and a common dolphin, and the first report of the presence of *A. phocae* in animals from the Pacific Ocean. *Arcanobacterium phocae* was a significant pathogen of stranded California marine mammals and was commonly isolated from abscesses and other inflamed tissues.

Arcanobacterium phocae and *L. ivanovii* are similar in phenotypic and biochemical characteristics. Each appears as small white circular colonies surrounded by a zone of strong beta-hemolysis on blood agar and are Gram positive short-rods or coccobacilli that occur singly or in pairs. Both bacteria are catalase positive and have the same reactions with the CAMP test. The initial identification of *L. ivanovii* was based on these tests and not until *A. phocae* was described by Ramos et al. (1997) did our misidentification become apparent. With additional testing of motility and esculin hydrolysis, *L. ivanovii* can be distinguished from *A. phocae*. Phylogenetic analysis was performed on the *A. phocae* isolates cultured from California marine mammals to verify their identity. Our isolates showed close phenotypic and

genotypic affinity to *A. phocae* and *A. hemolyticum* (Ramos et al., 1997). The retrospective study of microbiological records revealed that *A. phocae* was probably cultured from stranded marine mammals in California as early as 1994, but likely had been misidentified as *L. ivanovii* due to limitations in available phenotypic tests. Isolates that were reported as *L. ivanovii* from the same group of sampled animals were included in the retrospective study (Thornton et al., 1998).

The prevalence of *A. phocae* infections in stranded marine mammals in California increased from 1994 to 2000. Differences in the number of reported infections may be due to increased effort dedicated to bacteriologic screening of necropsy samples and infected wounds. However, preventative measures such as clipping umbilical hair and flushing regularly with antiseptic solution were also implemented to prevent common infections such as omphalitis in harbor seal pups. There were no *A. phocae* cases in harbor seals in 1999 and only three cases in 2000.

Prior to the extensive diagnostic analysis of sea otter carcasses initiated in 1998, tissues from only 12 otters had been sampled, and only one *A. phocae* isolate recovered (8.3%). As more bacterial cultures were performed the frequency of *A. phocae* infections detected in sea otters increased. Few live-stranded cetaceans were treated clinically during the study, and dead-stranded cetaceans were often severely autolyzed. This is the first report of *A. phocae* from a cetacean, but because of the small number of animals examined and the poor quality of most samples, the significance of *A. phocae* in cetaceans is unknown.

Arcanobacterium phocae was most commonly isolated from superficial pyogenic infections and exudates with a mixed population of bacteria. However, *A. phocae* was also isolated from deep-seated and systemic infections such as osteomyelitis and brain abscesses. Many of the infections appeared to have a portal of entry associated with a break in the skin such as bite and bullet wounds, surgical incision sites, or traumatic wounds. When *A. phocae* was cultured from internal organs, an associated superficial infection was usually involved. Isolation of *A. phocae* from internal organs could indicate systemic infection, but might also be due to postmortem bacterial proliferation. Liver and lung tissue were commonly submitted for bacterial culture at necropsy, resulting in the large number of positive cultures from these tissues, when compared to other internal organs.

For several sea otter cases, systemic infection originating from a superficial wound was clearly documented at necropsy. In some cases bacteria appeared to spread via the vascular or lymphatic system, and also locally along natural tissue planes. For example, a pure culture of *A. phocae* was obtained from brain tissue of a sea otter with severe suppurative meningoencephalitis. Gross and histopathologic examination revealed that the bacteria had extended from a facial abscess (a presumptive bite wound) along the external sheath of the maxillary branch of the trigeminal nerve directly to the brain. Histopathologic examination of brain tissue confirmed severe meningoencephalitis with large numbers of intralesional small bacterial rods.

The clinical profile of *A. phocae* infections in marine mammals, as described in this study, appears similar to that of *A. hemolyticum* infections in humans. *Arcanobacterium hemolyticum* is primarily associated with pharyngitis and superficial wound infections in humans, but has also been isolated from humans with osteomyelitis, endocarditis, and pulmonary and brain abscesses (Mackenzie et al., 1995; Funke et al., 1997; Dobinsky et al., 1999). Systemic and deep-seated infections are reported primarily from immunosuppressed patients with pre-existing disease, such as diabetes or malignant neoplasia (Skov et al., 1998). *Arcanobacterium hemolyticum* is often isolated in association

with beta-hemolytic streptococci, possibly indicating pathogenic synergy between the two species (Dobinsky et al., 1999).

Arcanobacterium hemolyticum isolated from humans is usually highly susceptible to penicillin, macrolides, aminoglycocides, and most other antimicrobials (Funke et al., 1997). Broad-spectrum beta-lactams, clindamycin, and erythromycin are recommended for treating *A. hemolyticum* infections. Beta-lactam antibiotics should also be useful in treating *A. phocae* infections in marine mammals. The eighteen isolates tested for antimicrobial MIC were all highly susceptible to penicillin and all of the other antimicrobials tested. When choosing an antibiotic to treat *A. phocae* in marine mammals, site of infection and antimicrobial susceptibility of other bacteria in a mixed culture should also be considered.

The natural host of *A. phocae* is unknown. The bacterium has been cultured from nasal, fecal, and vaginal swabs taken from wild-caught apparently healthy harbor seals in California (F. M. D. Gulland, unpubl. data), and may be similar to other *Arcanobacterium* spp. that are recognized as normal flora of mucous membranes in animals and humans (Funke et al., 1997). *Arcanobacterium phocae* was often cultured from infected bite wounds, including those from conspecifics, as well as superficial wounds, suggesting that *A. phocae* is part of the normal oral or skin flora of marine mammals that may serve as an opportunistic pathogen.

In conclusion, *A. phocae* is a common pathogen of wound infections that is occasionally associated with systemic infections in stranded marine mammals in California. The bacterium may have been misidentified in past studies and overlooked as a pathogen. *Arcanobacterium phocae* is most likely an opportunistic pathogen, causing severe infection in animals with wounds or other pre-existing disease. *Arcanobacterium phocae* is susceptible to beta-lactam antibiotics, but other types of antibiotic treatment may be necessary depending on the type of bacteria present in mixed culture with *A. phocae* and the site of infection.

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