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Aerobic Microorganisms Identified Over a Fourteen-Month Period from the Upper Respiratory Tract of Captive Hawaiian Monk Seals (*Monachus schauinslandi*)

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Abstract

Infectious disease is a growing concern for the overall declining Hawaiian monk seal (HMS) (*Monachus schauinslandi*) population. Recently, the HMS population in the main Hawaiian Islands (MHI) is increasing, and this may result in additional rehabilitation and release events. A key aspect for population health assessment is to identify the "normal" bacteria flora (e.g., the upper respiratory tract). Our current knowledge for the HMS flora is based on microbial isolates from stranding or mortality events rather than on healthy animals. This 14-mo study includes 52 oral and 55 nasal sampling events from the two healthy resident HMSs at the Waikiki Aquarium in Honolulu, Hawaii. Extensive culturing, Gram stains, phenotypic (e.g., biochemical), and genotypic (16S rRNA sequencing) characterization were used to identify aerobic microorganisms from the upper respiratory tract. The study detected 30 species of Gram negative bacteria, 18 species of Gram positive bacteria, and two species of yeast. The "normality" of the bacterial population was established over the study time period by consistent recovery of identical bacterial species from upper respiratory tract samplings. These results may provide a baseline for normal aerobic bacterial flora in these seals. These results may also allow for comparison to other HMSs in facilities and their wild conspecifics, and have implications for diagnosis of infection in diseased animals.

Key Words: normal flora, microorganisms, Hawaiian monk seal, *Monachus schauinslandi*, bacteria, ribosomal DNA classification, marine mammals, upper respiratory tract

Introduction

The Hawaiian monk seal (HMS) (*Monachus schauinslandi*) population continues to grow in the main Hawaiian Islands (MHI), but there is a heightened concern about increased exposure to anthropogenic disease and a potentially increased need for human intervention to rehabilitate and release seals back into the wild. Currently, an estimate for the total HMS population is at only 1,161 individuals (Carretta et al., 2010). The struggling population in the Northwestern Hawaiian Islands (NWHI) is at risk for detrimental effects from potential disease outbreaks (Zessin, 2006). In 1997, a mortality event claimed almost half of the Mediterranean monk seal (*Monachus monachus*) population. It is postulated that this could have been due to a toxin from phytoplankton (Aguilar & Lowry, 2008). It is also possible that a new type of morbillivirus, identified as the monk seal morbillivirus (MSMV), may have been responsible for the mortality (Osterhaus et al., 1997). Several surveys of the wild HMS population have examined animals for possible exposure to infectious agents (Aguirre, 2001; Littnan et al., 2006; Aguirre et al., 2007). However, the authors of these surveys limited their searches to only known infectious agents and selected pathogens: *Brucella* spp., *Leptospira* spp., *Chlamydophila abortus, Toxoplasma gondii*, *Dirofilaria immitis,* various viruses (Aguirre et al., 2007), *Vibrio* spp., *Edwardsiella tarda*, *Pleisomonas shigelloides*, and *Campylobacter jejuni* (Littnan et al., 2006). These studies also focused on the prevalence of antibody titers to known pathogens and found relatively low exposure levels compared to other pinniped species.

Health assessment monitoring of the HMS population requires an understanding of the normal bacterial flora compared to potential pathogens. Some bacteria are harmless in the natural environment, but could become a threat in an immunocompromised animal (Higgins, 2000). Normal bacterial flora are necessary for a healthy existence and contribute by occupying a niche and controlling overgrowth of potentially pathogenic species that could result in infection and disease (Marsh, 2000; Iwase et al., 2010). For instance, Iwase et al. (2010) found that *Staphylococcus epidermidis*, a common nasal colonizer, can inhibit the growth and proliferation of *S. aureus*, a potential pathogen, in humans. Zoonotic disease transmission is also bidirectional and has emerged as an area of concern for interspecific contact in multispecies facilities or as a result of human handling. For example, a Dutch zoo documented transmission of *Mycobacterium pinnipedii* from Southern sea lions (*Otaria flavescens*) to six human handlers. These infections were most likely introduced through breathing in contaminated material during the cleaning process (Kiers et al., 2008). It was not the purpose of this study to examine zoonotic diseases in the HMS.

Most bacteria isolates reported from marine mammals are associated with stranded or necropsied animals (Gerber et al., 1993; Thornton et al., 1998; Parsons & Jefferson, 2000; Johnson et al., 2006; Lockwood et al., 2006) or from other body surfaces such as the genitals (Vedros et al., 1982; Johnson et al., 2006). These datasets may be skewed toward unhealthy animals and may not represent the normal flora for that particular species; however, there are only a few studies of normal bacterial flora cited in the literature. Hernandez-Castro et al. (2005) isolated aerobic bacteria from the upper respiratory tract of healthy California sea lion (*Zalophus californianus*) pups, and Vedros et al. (1982) sampled healthy adult and juvenile northern fur seals (*Callorhinus ursinus*). Juvenile Steller sea lions (*Eumetopias jubatus*) were also sampled for both resident and potentially pathogenic flora (Goldstein et al., 2007). Normal flora can also vary between species, age class, and sex (Hernandez-Castro et al., 2005; Johnson et al., 2006). Health status, nutritional state, stress, and the environment can also play a role in establishing the normal flora of an animal (Vedros et al., 1982; Thornton et al., 1998; Higgins, 2000; Buck et al., 2006).

Access to wild, endangered HMSs is limited, and efforts to reduce the amount of handling and stress are a constant challenge. For instance, Steller sea lions were shown to have a reduced white blood cell count during temporary captivity compared to free-ranging conspecifics; this was interpreted as a possible sign of reduced stress on the animal, even with continued handling (Mellish et al., 2006). While certain aspects of an animal's biology can be affected in captivity (i.e., activity levels and behavioral changes), it has been shown that studies on temporarily captive animals can be representative of wild populations (Mellish et al., 2006).

The normal healthy flora of the respiratory tract for the HMS has not been characterized. In the present study, a 14-mo survey of captive HMSs was conducted on a bimonthly basis at the Waikiki Aquarium in Honolulu, Hawaii. The objective of the present study was to establish baseline data for a listing of the normal aerobic bacterial flora in the oral and nasal cavities of HMSs in captivity. Sampling consisted of an extensive array of bacteria culture media. This was followed by both phenotypic (e.g., biochemical) and genotypic (e.g., 16S rRNA sequencing) identification in order to categorize culturable aerobic bacterial species isolated from these two anatomical sites.

Materials and Methods

Study Animals and Housing Description

Two healthy adult male resident HMSs at the Waikiki Aquarium in Honolulu, Hawaii, were sampled bimonthly in order to culture, isolate, and document the normal aerobic bacterial flora of the upper respiratory tract (i.e., oral and nasal cavities). The seals were housed in an outdoor exhibit at the Waikiki Aquarium, featuring a 302,800 L seawater pool. The pool receives continuous incoming natural seawater (265 to 492 l/min) from approximately 20 m offshore of Waikiki Beach, which is then recirculated in the tank via three large rapid-sand filters (Yates & Crow, 1995). An ozone generator is used to control water quality. Overflow water flows back out approximately 23 m offshore of Waikiki Beach. The pool is drained and thoroughly cleaned each week using freshwater and bleach.

The first male, Nuka'au (Nuka), arrived at the aquarium in 1983 from Laysan Island (NWHI) and was estimated to be between 2 to 3 y of age at the time of his arrival. During the sampling period, he was between 26 and 28 y of age. The second male, Makaonaona (Maka), arrived at the Waikiki Aquarium from the French Frigate Shoals, also in the NWHI, in 1984 as a 3-wk-old pup. His age during sampling was 22 to 23 y. Both animals were trained (by one author, LNK) to voluntarily allow sampling on a bimonthly basis.

Sample Collection

Between 8 January 2007 and 18 March 2008, throat and nasal swabs were collected (by one author, LNK) twice a month from two monk seals at the Waikiki Aquarium. An oral sample

consisted of swabbing the oropharynx area once to clear any mucus, followed by a second swab for sample collection. Routine feeding of a single fish, swallowed whole, to facilitate specimen collection was performed prior to sampling from each of the seals. Nasal samples were obtained from the nares without touching the culture swab to the exterior edge of the nostril, which could be contaminated by extraneous material. Sterile culture swabs (BBL CultureSwab™ Plus, Amies without Charcoal, Double Applicators) were used for both the oral and nasal cavity epithelial collection (Figures 1 & 2). If mucus was present, a separate swab was used to remove it from the collection site before sampling.

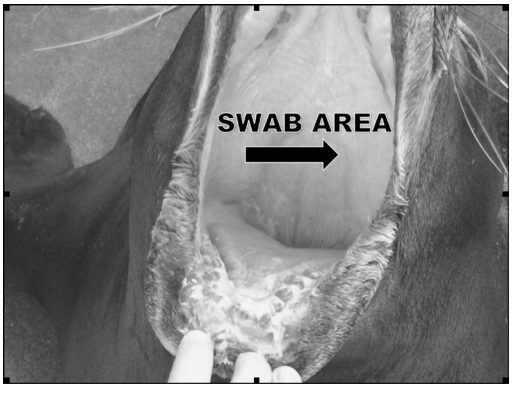

Figure 1. Swab collection area for sampling inside the oropharynx of the Hawaiian monk seals (HMSs) at the Waikiki Aquarium

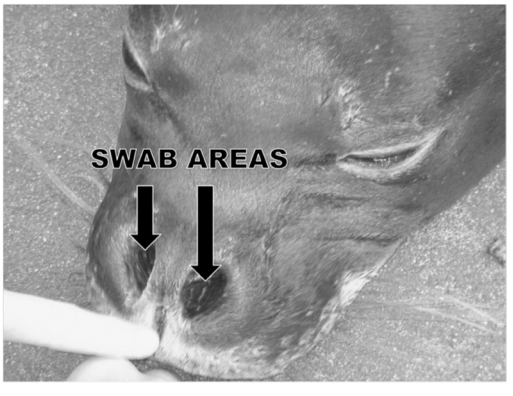

Figure 2. Swab area for sampling inside the nostrils of the HMSs at the Waikiki Aquarium; nasal swabs were collected only from open nostrils to avoid exterior contamination.

A 50 mL saltwater control was collected from the immediate surface area of the tank near the seal following each sample collection. Additionally, a control for the possible introduction of "fish flora" was obtained by culturing the fish at the same time. The food control was collected from a single night smelt fish, *Spirinchus starksi,* (suspended in 0.9% sterile physiological saline), which was used to feed the seal at the time of collection. Both samples were submitted in sterile containers and were clearly labeled prior to transport to the laboratory.

Culture Transport, Processing, and Interpretation

Cultured samples were transported on ice to Diagnostic Laboratory Services (DLS) in Honolulu within 90 min of collection. Once at the laboratory, the throat and nasal swabs were suspended in 0.9% sterile saline, and the swab was rotated to loosen and disperse the specimen into suspension. This suspension was then inoculated onto a variety of media: Trypticase Soy Agar with 5% Sheep Blood Agar (SBAP, BBL #221261), MacConkey Agar (MAC, BBL #221270), Columbia CNA Agar (CNA, BBL #221352), Thiosulfate Citrate Bile Sucrose Agar (TCBS, BBL #221872), Yersinia Selective Agar (CIN, BBL #221848), and two Chocolate Agar (CHOC, BBL #221167) plates. Incubation was performed at 35° C in CO_2, except for the CIN plate and one CHOC plate, which were incubated at 30° C in non-CO_2. Gram stains from all specimens were directly prepared using a commercial Gram staining set consisting of Gram Crystal Violet (BD #212526), Stabilized Gram Iodine (BD #212543), Gram Safranin (BD #212532), and a 50:50 Acetone-Alcohol Decolorizer (SP™ Acetone #C4300, SP™ Reagent Alcohol #C4305) and examined by light microscopy under oil immersion at 1,000X.

The water and fish (in 0.9% sterile saline) controls were shaken in their respective collection containers to loosen and disperse the specimen into suspension. A calibrated loop (1 µl) was used to inoculate the same media as was used for the HMS specimens and incubated under the same conditions. Likewise, Gram stains were prepared directly from the controls.

All plates were incubated and examined daily for 5 d. All water and fish samples were quantified based on actual colony counts. Bacteria were identified by phenotyping using morphological and biochemical methods. The biochemical identification methods used in the study consisted of either the BioMerieux Vitek® Gram Positive Identification (GPI) or the Gram Negative Identification (GNI+) Card. Any isolate that resulted in less than 97% probability from the Vitek or could not be identified by these routine phenotypic methods was further identified using 16S rRNA whole gene sequence analysis. Sequencing was performed offsite by a contracted service (University of Hawaii at Manoa). This method consisted of amplifying a fragment of the 16S rRNA gene

DNA extracted from the bacterial isolate using the polymerase chain reaction (PCR) and P*fu* DNA polymerase. The PCR product was then purified with a Qiagen PCR purification kit and sequenced using two primer sets. Actual DNA sequencing was performed using the BigDye Terminator cycle sequencing kit (Version 3.1). The sequence was then resolved on an ABI 3730XL DNA analyzer (Applied BioSystems, Foster City, CA, USA). The full 16S rRNA gene sequences were then assembled by use of the *Segman* program (DNAStar). Sequence analysis was performed (by one author, MJB) using the *ChromasPro* program (Version 1.33; Technelysium Pty. Ltd., Eden Prairie, MN, USA), utilizing a search with the *NIH BLAST* program (www.ncbi.nlm.nih.gov/BLAST).

Bacteria that occurred in $\geq$ 10% of the specimens collected were classified as normal respiratory flora and those that occurred in < 10% of the specimens collected were considered to be possible transient respiratory flora.

Statistical Methodology

A two-tailed Fisher's exact test (*Graphpad Quickcalcs*, http://graphpad.com/quickcalcs/contingency1.cfm) was applied to 2 × 2 contingency tables to detect statistically significant differences in the occurrence of species between animals or sample sites.

Results

A total of 52 oral samples and 55 nasal samples were collected from the Waikiki Aquarium HMSs, which consisted of 30 species of Gram negative bacteria, 18 species of Gram positive bacteria, and two species of yeast. As expected, there were sporadic encounters of similar bacterial species in the water and fish food control samples. Microorganisms identified as normal flora for the anatomical site were found in greater than or equal to 10% of the samplings from the oral and nasal cavities of the HMSs at the Waikiki Aquarium (Table 1).

A new species of *Bergeyella* (gb|GU196264) was identified in this study. This *Bergeyella* sp. represented the most prevalent bacterium, occurring in 100% of Nuka's and 93% of Maka's nasal specimens. The same *Bergeyella* sp. also occurred in 81.5% of Nuka's and 76% of Maka's oral specimens. *Corynebacterium phocae* predominantly occurred in nasal samples ($p < 0.0001$), isolated in 94% of the nasal specimens (47 isolations) and compared to 6% isolation in the oral specimens (3 isolations). *Enterobacter cloacae* and *Escherichia coli* were also found predominantly in nasal specimens ($p < 0.0001$ and $p = 0.0083$, respectively). *Ornithobacterium rhinotracheale* was isolated solely from the nasal specimens of both seals ($p = 0.0256$). *Capnocytophaga cynodegmi* was only recovered from oral samples in both seals ($p = 0.0256$). *Streptococcus agalactiae* (grp B) was only isolated from Maka's nasal specimens, but it lacked statistical support to be unique to that source. Yeast (including *Candida albicans*) was only isolated from HMS Maka ($p < 0.0001$) in 12 oral and two nasal samples. No other statistically significant correlations were observed between the microorganisms, source animal, or sampling site.

Several potentially pathogenic organisms (e.g., *Gemella morbillorum* and *S. haemolyticus*) were isolated from the transient bacterial group (Table 2).

Discussion

The use of selective media, phenotypic (e.g., biochemical), and genotypic (e.g., 16s rRNA sequencing) identification, and the ability to sequentially sample healthy captive HMSs, provided the first baseline record for normal and transient upper respiratory tract bacterial flora for this species. The 48 species of bacteria and two species of yeast reveal the wide range of organisms encountered from the oral and nasal anatomical sites. These seals were exposed to a vast and diverse population of bacteria by way of their habitat, food, and routine human handling; however, this may be similar to what HMSs are exposed to in the MHI. In this study, certain microorganisms were found regularly and appeared to be a part of the normal upper respiratory flora of these animals. This data collected over a 14-mo period provides the first extensive analysis of the normal microbial flora ($\geq$ 10%) and transient microbial flora (< 10%) of captive HMSs' upper respiratory tracts.

The most commonly isolated bacteria was *Bergeyella* sp. This new bacterial species was isolated from both oral and nasal swabs of the HMS at the Waikiki Aquarium. This new species has 86% identity to a related species, *B. zoohelcum* (gb|AY827896.1). *B. zoohelcum* is commonly isolated from canid and felid upper respiratory tracts (Montejo et al., 2001; Elliott et al., 2005; Lin et al., 2007); however, this new species has a unique 16s ribosomal RNA gene sequence. *B. zoohelcum* has been isolated from oral and nasal cultures and is considered zoonotic (Lin et al., 2007). Human infections result in cellulitis and bacteremia, often from exposure via an animal bite wound (Lin et al., 2007). *B. zoohelcum* has been found to grow well on blood agar culture media (Montejo et al., 2001; Lin et al., 2007), but it was also found to grow on SBAP in the present study.

The present study is the first record of *Capnocytophaga cynodegmi* isolated from marine

Table 1. Aerobic microorganisms observed in 10% or more of the sampling events on two resident Hawaiian monk seals (HMSs) at the Waikiki Aquarium; the microorganisms are categorized according to genus-species, Gram stain, and anatomical site. The bolded scientific name represents a species not previously isolated from marine mammals. Statistically significant non-equivalent result pairs are footnoted with their *p*-values.

Source	Gram					Maka oral		Maka nasal		Nuka oral		Nuka nasal		Total oral		Total nasal	
Number of sampling events						25		28		27		27		52		55	
Microorganism type	GNB	GNCB	GPB	GPC	Yeast	#	%	#	%	#	%	#	%	#	%	#	%
Bergeyella sp.	x					19	76	26	93	22	81	27	100	41	79	53	96
Corynebacterium phocae			x			2	8	23	82	1	4	24	89	***3	6	***47	85
Actinomyces marimammalium			x			20	80	13	46	18	67	19	70	38	73	32	58
Moraxella phenylpyruvica		x				19	76	10	36	17	63	18	67	36	69	28	51
Bisgaardia hudsonensis	x					24	96	15	54	26	96	12	44	50	96	27	49
Escherichia coli	x					0	0	3	11	1	4	7	26	**1	2	**10	18
Enterobacter cloacae	x					2	8	8	29	0	0	5	19	***2	4	***13	24
Psychrobacter sp.		x				3	12	2	7	12	44	3	11	15	29	5	9
Ornithobacterium rhinotracheale	x					0	0	3	11	0	0	2	7	*0	0	*5	9
Streptococcus marimamalium				x		3	12	1	4	0	0	1	4	3	6	2	4
Candida albicans					x	***12	48	***2	7	***0	0	***0	0	12	23	2	4
Capnocytophaga cynodegmi	x					3	12	0	0	2	7	0	0	*5	10	*0	0
Streptococcus agalactiae (grp B)				x		0	0	3	11	0	0	0	0	0	0	3	5
Vibrio alginolyticus	x					2	8	4	14	2	7	0	0	4	8	4	7

*** $p < 0.0001$ ** $p = 0.0083$ * $p = 0.025$

Note: # = number of observations, GNB = Gram negative bacillus, GNCB = Gram negative cocco-baccilus, GPB = Gram positive bacillus, and GPC = Gram positive cocci

Table 2. Transient aerobic microorganisms isolated in fewer than 10% of oral and nasal specimens from two resident HMSs at the Waikiki Aquarium; the microorganisms are categorized according to genus-species, Gram stain, and anatomical site. Bolded scientific names represent new records for marine mammals.

Total number sampling events							52		55	
	Gram						Oral		Nasal	
Microorganism observed in sample	GNB	GNCB	GNC	GPB	GPC	Yeast	#	%	#	%
Acinetobacter sp.	x						1	1.9	0	0.0
Acinetobacter venetianus	x						1	1.9	0	0.0
Bacillus cereus				x			1	1.9	1	1.8
Brevibacterium equis				x			1	1.9	0	0.0
Corynebacterium phocae strain *2*				x			0	0.0	1	1.8
Corynebacterium sp.				x			0	0.0	3	5.5
Gemella morbillorum					x		2	3.8	0	0.0
Haemophilus felis	x						0	0.0	1	1.8
Haemophilus parasuis	x						1	1.9	0	0.0
Microbacterium oxydans				x			0	0.0	1	1.8
Microbacterium sp.				x			0	0.0	1	1.8
Neisseria sp.			x				1	1.9	0	0.0
Pantoea agglomerans	x						1	1.9	2	3.6
Pantoea ananatis	x						0	0.0	2	3.6
Pantoea stewartii	x						0	0.0	3	5.5
Plesiomonas shigelloides	x						2	3.8	1	1.8
Pseudomonas aeruginosa	x						0	0.0	1	1.8
Pseudomonas alcaligenes	x						0	0.0	1	1.8
Pseudomonas stutzeri	x						2	3.8	0	0.0
Psychrobacter menintidis		x					0	0.0	1	1.8
Shewanella putrefaciens	x						1	1.9	1	1.8
Simonsiella steedae	x						3	5.8	0	0.0
Staphylococcus auricularis					x		0	0.0	1	1.8
Staphylococcus epidermidis					x		1	1.9	1	1.8
Staphylococcus haemolyticus					x		0	0.0	1	1.8
Staphylococcus hominis					x		1	1.9	2	3.6
Staphylococcus sp. (not *S. aureus*)					x		1	1.9	0	0.0
Stenotrophomonas maltophila	x						1	1.9	1	1.8
Streptococcus canis					x		1	1.9	0	0.0
Suttonella (Cardiobacterium) ornithocola	x						0	0.0	3	5.5
Vibrio corallyticus	x						0	0.0	2	3.6
Vibrio harveyii	x						1	1.9	1	1.8
Vibrio shilonii	x						1	1.9	0	0.0
Vibrio vulnificus	x						0	0.0	1	1.8
Yeast (not *C. albicans*)						x	2	3.8	0	0.0

= number of observations, GNB = Gram negative bacillus, GNCB = Gram negative coccobaccilus, GNC = Gram negative cocci, GPB = Gram positive bacillus, and GPC = Gram positive cocci

mammals. Several commonly isolated bacteria in the Waikiki Aquarium HMSs have been reported in marine mammals and in pinniped upper respiratory tracts. However, novel microbial findings were also elucidated with this extensive study and data presented in the current work. *Bisgaardia hudsonensis* was isolated from 77 specimens in the present study. It was also isolated from the nares and prepuce of juvenile Steller sea lions (Goldstein et al., 2007). *Corynebacterium phocae, E. coli,* β-hemolytic *Streptococcus* spp. (grp B), and *Vibrio alginolyticus* have been previously isolated from the respiratory system of marine mammals (Higgins, 2000). *Actinomyces marimammalium* was isolated from multiple organs of a pneumonia-infected hooded seal (*Cystophora cristata*) and small intestine of a grey seal (*Halichoerus grypus*) (Hoyles et al., 2001). *Moraxella phenylpyruvica* was isolated from the nasal cavity of healthy California sea lion pups

(Hernandez-Castro et al., 2005). *Moraxella* spp. have also been isolated from the respiratory tract as well as other organ systems of pinnipeds (Vedros et al., 1982; Thornton et al., 1998; Johnson et al., 2006). *Psychrobacter* isolates have been cultured from sources such as sea water, fish gills, poultry skin, and processed foods, with some species having an affinity for cold Antarctic temperatures (Romanenko et al., 2002; Bozal et al., 2003). *Psychrobacter* spp. have been isolated from the nares and genitals of otariids (Johnson et al., 2006; Goldstein et al., 2007), and *P. lutiphocae* was isolated from the feces of a seal, displaying presence in phocids as well (Yassin & Busse, 2009). *Streptococcus marimammalium* was isolated from the lungs of a harbor seal (Lawson et al., 2005).

The HMSs at the Waikiki Aquarium live in an enclosed habitat, even though a total water turnover is encountered at least three times daily. They are exposed to their own fecal matter, oral and nasal mucus, environmental materials (e.g., bird droppings, insects, and other fauna), and windborne debris. A study at the Asa Zoological Park in Japan sampled the water, feces, and nares of a variety of mammals, reptiles, birds, and water sources; their results revealed 232 Gram negative bacterial isolates (Ahmed et al., 2007). The most common species isolated in their study was *E. coli*, appearing in 52.6% of samplings, and *Enterobacter cloacae*, which was found in 4.7% of the samplings (Ahmed et al., 2007). Therefore, it is not unexpected that the HMSs' oral and nasal specimens frequently contained *E. coli* and *E. cloacae* because they are a normal part of their gastrointestinal and urogenital system (Vedros et al., 1982; Thornton et al., 1998; Higgins, 2000; Johnson et al., 2006; Goldstein et al., 2007). These enteric bacteria have also been isolated in California sea lion pup nares (Hernandez-Castro et al., 2005). In another study conducted with rehabilitated northern elephant seals, higher instances of antimicrobial resistant strains of *E. coli* were found after time spent in a rehabilitation facility compared to in free-ranging animals (Stoddard et al., 2009).

Ornithobacterium rhinotracheale has been found to be a pathogenic threat to broiler chickens worldwide, causing infectious lesions on chickens and other poultry and resulting in removal of the infected bird before possible human consumption (Veen et al., 2000). The HMS habitat at the Waikiki Aquarium is an outdoor exhibit, with exposure to the elements and transient local fauna. Several bird species have been observed to reside and nest in the large shade-providing trees of the exhibit, serving as a possible source of *O. rhinotracheale* to the seals. *Ornithobacterium* spp. have also been isolated from the nares and prepuce of Steller sea lions, both free-ranging and in temporary captivity (Goldstein et al., 2007).

Candida spp., especially *C. albicans*, infections are the most common fungal (yeast) infection in marine mammals. *Candida* spp. mainly affects those animals in captive facilities and has been attributed to antibiotic usage and treatment of water (Higgins, 2000). Infections are identified by inflammation of the mucous membranes such as mouth, eyes, vagina, and rectum; however, they can also present as skin lesions and alopecia (Higgins, 2000). Yeasts are also commonly isolated from cetaceans such as bottlenose dolphins and killer whales (Gaydos et al., 2004; Buck et al., 2006). A study on captive marine mammals found yeast in 23 to 62% of water samples (Buck, 1980).

The present work isolated *E. coli, V. alginolyticus,* and *Plesiomonas shigelloides* in agreement with those described in the HMSs found in the wild (Littnan et al., 2006). Other isolates from wild HMSs included *Edwardsiella tarda* and *V. parahemolyticus* (Littnan et al., 2006), which were periodically isolated in the control water samples at the Waikiki Aquarium. However, they were never cultured from the HMSs' specimens. This study contained 47 species previously not reported in an extensive literature search from HMSs (Aguirre, 2001; Littnan et al., 2006; Aguirre et al., 2007) and 23 species not previously reported from marine mammals (Vedros et al., 1982; Thornton et al., 1998; Higgins, 2000; Hernandez-Castro et al., 2005; Johnson et al., 2006; Lockwood et al., 2006; Goldstein et al., 2007).

The present study conducted over a 14-mo period provides strong evidence for the diverse aerobic microbial flora present in the oral and nasal sites of the HMSs in captivity. Through the use of an extensive array of culture media, phenotypic (e.g., biochemical), and genotypic (e.g., 16S rRNA sequencing) identification, this study allowed for a thorough search and documentation to species of upper respiratory flora for HMSs.

In summary, this dataset provides a clear baseline describing the normal aerobic upper respiratory microbial flora of two Hawaiian monk seals in captivity at the Waikiki Aquarium over a 14-mo period. The results represent both normal and transient microbes found in HMSs in captivity. This work has also identified the first finding of a novel *Bergeyella* species, which was revealed as a common isolate in the HMSs in the present study. This study and its findings provide a rapid health assessment for the HMS, assist with the interpretation of respiratory cultures, and may serve as a guide in the use of therapy for seals showing signs of respiratory disease. Further study is needed to corroborate these findings with the microbial flora present in HMSs in the wild.

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