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ISOLATION, CHARACTERIZATION, AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA ISOLATED FROM SEA LION (*ZALOPHUS CALIFORNIANUS*) PUPS IN NORTHWESTERN MEXICO

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?1 ABSTRACT: Bacterial infections have been documented in marine mammals for decades, and some are considered emerging pathogens with zoonotic potential. The aerobic oral (n=16) and rectal (n=17) bacterial microbiota and their antimicrobial resistance were characterized for 17 apparently healthy California sea lion pups (*Zalophus californianus*) captured with a hoop net in Farallon Island, Sinaloa, ?2 Mexico, in 2016. Bacteriologic cultures, API, and PCR were used to identify bacterial species. The *Escherichia coli* phylogenetic groups were identified by PCR, *Salmonella* serotypes were identified, and resistance to antibiotics was evaluated. Overall, 39 bacterial species were isolated, including *E. coli* and *Salmonella* spp. (35.9% each) and *Pseudomonas aeruginosa* (28.2%). For *E. coli,* UNKNOWN phylogroup was the most prevalent (57.7%), followed by the A phylogroup (37.1%). Most *Salmonella* serotypes were identified as Newport (92.8%); serotype Saintpaul was also identified (7.2%). Sea lions with bacterial cocolonization included 24.2%, from which two bacterial species were isolated, and 3% with three species. Overall, 59% of bacteria were resistant to at least one antibiotic tested, and 25.6% were extensively drug resistant. Bacteria were highly resistant to ampicillin and cefotaxime. This study demonstrates the importance of characterizing the microbiome of sea lions, and the potential effect of pathogens with antimicrobial resistance on wildlife conservation and public health.

Key words: Antimicrobial resistance, antimicrobial susceptibility, bacterial diversity, *Escherichia coli*, Mexico, *Salmonella* Newport, *Salmonella* Saintpaul, sea lions, *Zalophus californianus*.

INTRODUCTION

California sea lions (*Zalophus californianus*) are sexually dimorphic pinnipeds with polygynous behavior, distributed along the Pacific coast of North America from British Columbia, Canada, to Baja California, Mexico, and into the Gulf of California. There are five distinct genetic populations, three in the Gulf of California and two in the Pacific west off the Baja California peninsula (Szteren et al. 2006). This last geographic region is important, with 13 California sea lion breeding areas (Elorriaga-Verplancken et al. 2015). Despite documented declines of the Gulf of California populations attributed to El Niño events, global warming, and decline of prey availability between 1979 and 2016, the population appears to be stable (Masper et al. 2019).

Signs of marine ecosystem distress from nutrient loading, with potential contributions of anthropogenic global climate change, are associated with increased frequency and intensity of harmful algal blooms and associated toxicity for marine mammals and humans (Aguirre and Tabor 2004; Tabor and Aguirre 2004; Moore and Kuletz 2019). Many populations of marine vertebrates are exposed to pathogens from agricultural runoff and human

sewage, best described as pathogen pollution (Daszak et al. 2000).

The relatively recent drastic accelerated transformation of coastal ecosystems is having a large effect on sea lion populations, increasingly affected by encroachment, malnutrition, toxicants, and emerging diseases shared with domestic animals and humans (Aguirre et al. 2002; Gulland and Hall 2007; Ávalos-Téllez et al. 2014). Additionally, sea lions may serve as reservoirs of *Salmonella* spp. and shed this zoonotic bacterium in haul-out sites along the California coast (Berardi et al. 2014). The colonization of sea lions by *Salmonella* spp. could be related to fecal contamination from seabird reservoirs and human feces in wastewater discharges from boats (Gilmartin et al. 1979).

Bacterial infections have been documented in marine mammals for decades; some, such as brucellosis, are considered emerging. Simeone et al. (2015), in reviewing marine mammal reports in North America, concluded that 20% of marine mammal disease cases were associated with bacteria, and from these, 63% demonstrated clinical disease. It has been estimated that around 30% of sea lion mortality is related to bacterial infections (Stroud and Roffe 1979; Johnson et al. 2006; Ávalos-Téllez et al. 2014).

The identification, characterization, and antimicrobial resistance (AMR) of bacteria in California sea lions and other pinniped species has been previously described, implicating bacterial infections as a primary cause of stranding in California (Johnson et al. 1998). Specific reports for the Gulf of California population relate to the presence of pathogenic *Leptospira* spp. and *Brucella* spp. (Ávalos-Téllez et al. 2014). Additionally, bacterial species of public health importance have caused high numbers of illness and death in sea turtles and marine mammals (Aguirre and Lutz 2004; Aguirre and Tabor 2004; Ávalos-Téllez et al. 2014; Zavala-Norzagaray et al. 2015).

Bacteria that colonize sea lions have shown to be resistant to antimicrobials, making treatment during rehabilitation challenging and spreading to diverse ecosystems, colonizing food, water, humans, and other marine life (Delport et al. 2015). The objective of our study was to document the oral and rectal bacterial flora in sea lion pups from Farallon de San Ignacio island reproductive rookery in the Gulf of California, Mexico, and determine their antimicrobial susceptibility.

MATERIALS AND METHODS

Study site

Farallon de San Ignacio island (25°26′11.5″ N, 109°22′45.5″ W) is considered one of the most remote volcanic islets off the coast of Sinaloa, Mexico, approximately 36 km from the port of Topolobampo (Fig. 1). It has an approximate surface of 16 ha, has a maximum altitude of 140 m above sea level, and is proximal to Farallon submarine canyon incised in the continental slope with depths surpassing 3,000 m (Amador-Buenrostro et al. 2003). The island has an annual mean temperature of 25 C and precipitation of 300 mm between June and September. It hosts a year-round colony of sea lions (Guevara-Medina et al. 2008; Samaniego-Herrera et al. 2009).

Sea lions and bacterial isolations

During September 2016, 1.5–2-mo-old sea lion pups were captured, unharmed with the use of hoop nets, for the purpose of health assessment. All pups were evaluated by a veterinarian and were classified as healthy. Morphometric data and sex of sea lion pups are shown in Supplementary Table 1. Oral (16) and rectal (17) swabs were collected, and pups were released immediately after sampling. The swabs were placed in alkaline peptone water (Thermo Fisher, Waltham, Massachusetts, USA) at pH 8.5 for *Vibrio* spp. and in buffered peptone water (Thermo Fisher) pH 7.2 for *Enterobacteriaceae* until their analysis. For *Vibrio* spp., all the oral and rectal swabs were placed in alkaline peptone water and streaked onto thiosulfate citrate bile salts sucrose agar (Becton-Dickinson, Franklin Lakes, New Jersey, USA), and CHROMagar Vibrio (CHROMagar, Paris, France). The plates were incubated overnight at 37 C. From each plate, green and yellow colonies in thiosulfate citrate bile salts sucrose agar or blue and violet colonies in CHROMagar Vibrio exhibiting diverse morphology were transferred to tryptic soy agar (TSA) with 2% NaCl agar for purity. These plates were incubated overnight at 37 C, and identification proceeded with a single isolated colony. Each colony was examined by the oxidase test and all biochemical tests described in the *Bacteriological Analytical*

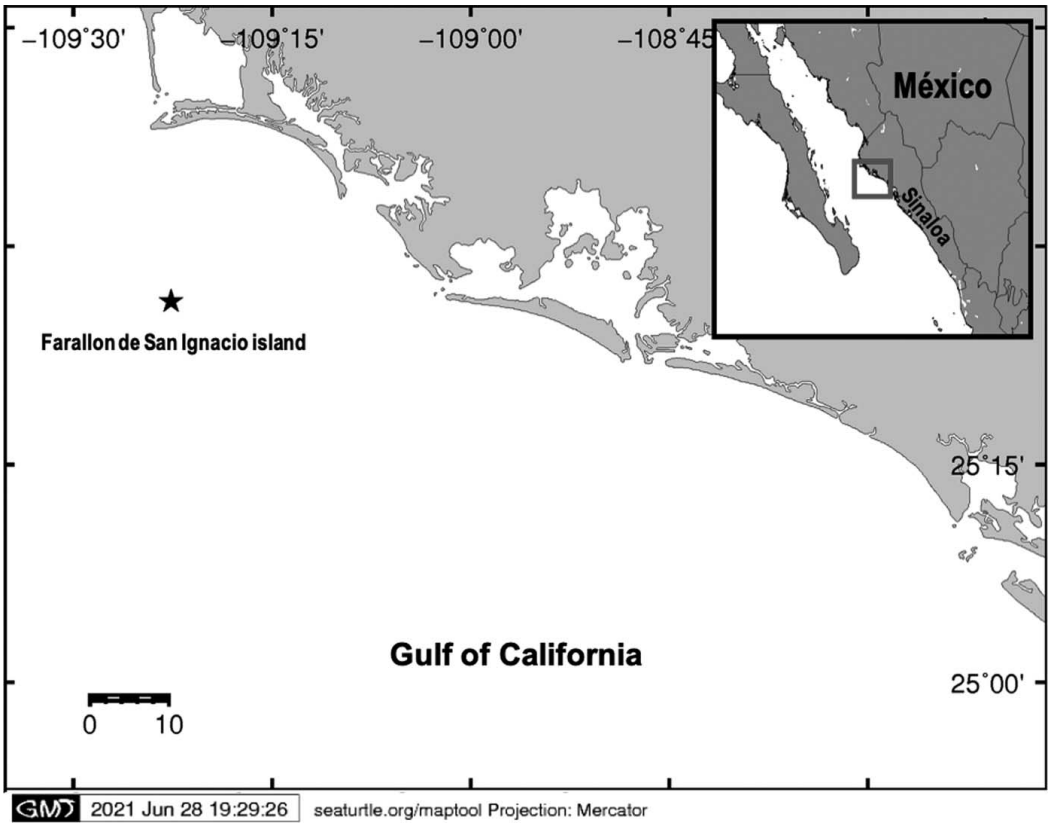

FIGURE 1. Map showing the location of the study site, Farallon de San Ignacio Island, Sinaloa, Mexico, where apparently healthy California sea lion pups (*Zalophus californianus*) were sampled in 2016.

Manual of the Food and Drug Administration for *Vibrio* spp. (Kaysner et al. 2004; Canizalez-Roman et al. 2011). For *Enterobacteriaceae*, specimens were placed in buffered peptone water and streaked onto *Salmonella-Shigella*, Hektöen, and MacConkey agar (Becton-Dickinson). The plates were incubated overnight at 37 C. The presumptive colonies were transferred to TSA agar for purity, incubated overnight at 37 C, then identified with a single isolated colony. Each colony was examined by the biochemical test for *Citrobacter freundii*, *Escherichia coli*, *Edwardsiella* spp., *Aeromonas* spp., *Plesiomonas* spp., *Morganella* or *Proteus* spp., *Salmonella* spp., *Pseudomonas* spp., *Klebsiella* spp., and *Providencia* spp., as described in the *Bacteriological Analytical Manual* of the US Food and Drug Administration (Kaysner et al. 2004; Feng et al. 2011; Andrews et al. 2022).

Molecular identification and *E. coli phylogroups*

For each sample, 10 colonies were inoculated in Luria Bertani broth medium for 18 h at 37 C to obtain genomic DNA. Bacteria pellets were taken and suspended in 200 µL of molecular biology–grade water, then the boiling method was applied (Canizalez-Roman et al. 2021); the DNA isolated was stored at –20 C. To complete the bacterial identification isolated, PCR assays were performed in a 25 µL volume consisting of 1× GoTaq green master mix (Promega, Madison, Wisconsin, USA); primers targeting conservative genes of *E. coli*, *Pseudomonas aeruginosa*l and *Salmonella* spp. (see Table 1); and 0.5 µg of purified genomic DNA template, with the remaining volume consisting of molecular biology–grade water (Fisher Scientific, Waltham, Massachusetts, USA). PCR products separated by electrophoresis in a 2% agarose gel were stained (GelRed® (Biotium, Fremont, California, USA) to allow for the visualization of DNA fragments with a digital imaging system (model E1 logia 100 imaging system; Kodak, Rochester, New York, USA). Phylogenetic groups of *E. coli* were identified following the Clermont et al. (2013) methodology.

TABLE 1. Primers used in PCR for detection of different bacteria species and *Escherichia coli* phylogroups from oral and fecal swabs taken from 17 apparently healthy California sea lion pups (*Zalophus californianus*) in northwestern Mexico in 2016.

Bacteria	Gene	Primer		Amplicon size (pb)	Reference
Pseudomonas aeruginosa	16S rDNA	PA-SS-F	GGGGGATCTTCGGACCTCA	956	Spilker et al. 2004
		PA-SS-R	TCCTTAGAGTGCCCACCCG		
Salmonella spp.	ompC	OMPC-F	ATCGCTGACTTATGCAATCG	204	Kwang et al. 1996
		OMPC-R	CGGGTTGCGTTATAGGTCTG		
Escherichia coli	16S rRNA	16 E1	GGGAGTAAAGTTAATACCTTTGCTC	584	Tsen et al. 1998
		16 E2	TTCCCGAAGGCACATTCT		
		16 E3	TTCCCGAAGGCACCAATC		
	Quadruplex	chuA-F	ATGGTACCGGACGAACCAAC	288	Clemont et al. 2013
		chuA-R	TGCCGCCAGTACCAAAGACA		
		yjaA-F	CAAACGTGAAGTGTCAGGAG	211	
		yjaA-R	AATGCGTTCCTCAACCTGTG		
		TspE4C2-F	CACTATTCGTAAGGTCATCC	152	
		TspE4C2-R	AGTTTATCGCTGCGGGTCGC		
		AceK-F	AACGCTATTCGCCAGCTTGC	400	
		ArpA1-R	TCTCCCCATACCGTACGCTA		
		ArpAgpE-F	GATTCCATCTTGTCAAAATATGCC	301	
		ArpAgpE-R	GAAAAGAAAAAGAATTCCCAAGAG		
		trpAgpC-F	AGTTTTATGCCCAGTGCGAG	219	
		trpAgpC-R	TCTGCGCCGGTCACGCCC		
		trpBA-F	CGGCGATAAAGACATCTTCAC	489	
		trpBA-R	GCAACGCGGCCTGGCGGAAG		

Salmonella serotyping

One colony of each *Salmonella* strain was subjected to slide agglutination test using *Salmonella* group O antisera. *Salmonella* isolates were further serotyped for flagellar (H) antigens by the tube agglutination test according to the Kauffmann–White–Le Minor scheme (Grimont and Weill 2007). Finally, all *Salmonella* serovars were confirmed by the Institute of Diagnosis and Epidemiological Reference (InDRE), Mexico City, Mexico, according to the antigenic formula of *Salmonella* serovars indicated in the Kauffmann–White–Le Minor scheme.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing of pathogenic isolates was performed by the Kirby-Bauer disk diffusion method (Bauer et al. 1966) following the guidelines developed by the Clinical Laboratory Standard Institute (Cockerill 2011). Suspensions of bacteria isolated from sea lions were prepared in LB at a turbidity of 0.5 according to the McFarland standard (Angulo-Zamudio et al. 2021). Then, Mueller-Hinton agar plates (BD BBL, Franklin Lakes, New Jersey, USA) were swabbed with these cultures, and antibiotic disks (BD BBL) were placed aseptically on the inoculated agar. Antibiotics tested were 1) an aminoglycoside: gentamicin; 2) quinolones and fluoroquinolones: ciprofloxacin and nalidixic acid; 3) sulfonamides and potentiated sulfonamides: sulfamethoxazole-trimethoprim; 4) tetracycline: tetracycline; 5) a beta lactam: ampicillin; 6) cephalosporins: ceftazidime and cefotaxime; and 7) a phenicol: chloramphenicol. The plates were incubated at 37 C for 18–20 h. The diameters (mm) of clear zones of growth inhibition around the antimicrobial agent disks were measured with a precision digital caliper (Absolute, Mitutoyo, Japan; Angulo-Zamudio et al. 2021). Strains from the American Type Culture Collection *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used as controls. Recommendations by Clinical Laboratory Standard Institute were used to define breakpoints of antibiotics and thus categorize the isolates as resistant, intermediate, or sensitive (White et al. 2006). Isolates that showed resistant to $\geq$3 different categories of antibiotics were classified as multidrug-resistant, and those showing resistance to $\geq$6 different categories of antibiotics were classified as extremely drug resistant (XDR; Magiorakos et al. 2012). Antibi-

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TABLE 2. Bacteria isolated from sea lion (*Zalophus californianus*) pups,[a] Farallon Island, Sinaloa, Mexico, 2016.

Bacteria isolated	Anatomical isolation site, *n* (%)		Total isolates, *n* (%)
	Oral	Rectal	
n	14 (35.9)	25 (64.1)	39
Escherichia coli	1 (7.1)	13 (52.0)	14 (35.9)
Salmonella spp.	6 (42.9)	8 (32.0)	14 (35.9)
Pseudomonas aeruginosa	7 (50.0)	4 (16.0)	11 (28.2)

[a] Samples from sea lions: oral *n*=16; rectal *n*=17.

otics were selected on the basis of their use to treat human infections with gram-negative bacteria and represent different classes of antimicrobial agents that are available to treat these infections in Mexico.

Statistical analysis

The chi-square test was used to evaluated statistical differences. Associations between nominal variables were analyzed with Fisher's exact test, the chi square test, or both. Statistical significance was determined as $P \leq 0.05$; analyses were made with the SPSS Statistics program version 20 (IBM, New York, New York, USA).

RESULTS

Bacterial distribution isolated from sea lions

From the 33 swabs, we isolated 39 different bacteria (Table 2) from oral (14/39, 35.9%) and rectal swabs (25/39, 64.1%). Three bacterial species were isolated (Table 2): *E. coli* (14/39, 35.9%), *Salmonella* spp. (14/39, 35.9%), and *P. aeruginosa* (11/39, 28.2%). Most *E. coli* and *Salmonella* spp. were found in rectal samples, whereas *P. aeruginosa* was isolated mainly from oral samples.

E. coli phylogroups and *Salmonella* serotypes

Of 14 *E. coli* strains, the phylogroup UNKNOWN was the most prevalent (8/14, 57%), following by A (5/14, 37%); the least prevalent was B1 (1/14, 7%). Phylogroups B2, C, D, E, F, CLADE I, and CLADE II were not identified. Most (13/14) of the *Salmonella* strains isolated were identified as serotype Newport; eight of these were isolated from rectal samples and five from oral samples. One *Salmonella* strain isolated from an oral sample was serotype Saintpaul (Table 3).

TABLE 3. Distribution of *Escherichia coli* phylogroups and *Salmonella* serotypes isolated from sea lion (*Zalophus californianus*) pups,[a] Farallon Island, Sinaloa, Mexico, 2016.

	Anatomical isolation site, *n* (%)		Total, *n* (%)
	Oral	Rectal	
E. coli phylogroups[b]			
n	1 (7.2)	13 (92.8)	14
A	1 (100)	4 (30.7)	5 (37.1)
B1	0 (0.0)	1 (7.7)	1 (7.2)
Unknown	0 (0.0)	8 (61.5)	8 (57.1)
Salmonella serotypes			
n	8 (57.1)	6 (42.9)	14
Newport	8 (100)	5 (83.3)	13 (92.8)
Saintpaul	0 (0.0)	1 (16.7)	1 (7.2)

[a] Samples from sea lions: oral *n*=16; rectal *n*=17.

[b] The phylogroups B2. C, D, E, F, CLADE I, and CLADE II were not found.

Cocolonization of bacterial species

Sea lions were colonized with more than one bacterial species; rectal and oral samples were identified with bacterial cocolonization (Table 3). No bacteria were isolated, specifically in oral samples, in 4 of 33 (12.1%) swabs. One bacterial species was isolated from 20 of 33 (60.6%) samples, whereas 8 of 33 (24.2%) samples presented with two bacterial species of isolates, mostly from rectal samples. Three combinations of two bacterial species were identified: 1) *E. coli* and *P. aeruginosa*, 2) *E. coli* and *Salmonella* spp., and 3) *Salmonella* spp. and *P. aeruginosa* (Table 3). One sea lion pup had a combination of three bacterial species, isolated from a rectal swab, providing 3% of all specimens collected (Table 4).

Antimicrobial resistance of bacterial species

Antimicrobial resistance information of the bacterial species isolated is summarized in Table 5. Overall, bacteria demonstrated resis-

TABLE 4. Cocolonization of bacteria species isolated from sea lion (*Zalophus californianus*) pups, Farallon Island, Sinaloa, Mexico, 2016.

No. bacteria species isolated	Bacterial species cocolonization			Anatomical isolation site, *n* (%)		Total bacterial combination, *n* (%)
				Oral, 16 (48.4)	Rectal, 17 (51.6)	33
0	—	—	—	4 (25.0)	0 (0.0)	4 (12.1)
1	*Escherichia coli*	—	—	0 (0.0)	6 (35.3)	20 (60.6)
	Salmonella spp.	—	—	6 (37.5)	2 (11.8)	
	P. aeruginosa	—	—	4 (25)	2 (11.8)	
2	*E. coli*	*P. aeruginosa*	—	0 (0.0)	1 (5.9)	8 (24.2)
	E. coli	*Salmonella* spp.	—	1 (4.0)	5 (29.4)	
	Salmonella spp.	*P. aeruginosa*	—	1 (4.0)	0 (0.0)	
3	*E. coli*	*P. aeruginosa*	*Salmonella* spp.	0 (0.0)	1 (5.9)	1 (3.0)

tance most commonly to cefotaxime (38.5%); ampicillin (30.7%); nalidixic acid, sulfamethoxazole-trimethoprim, and tetracycline (25.6%, each); and chloramphenicol (23%), with fewer resistant to gentamicin and ceftazidime (17.9% and 5.1%, respectively). All bacteria were sensitive to ciprofloxacin. A total of 59% of bacteria were resistant to at least one antimicrobial, and 25.6% were XDR. *Pseudomonas aeruginosa* was the most resistant by antimicrobial class and, by category ($P<0.05$), 100% were resistant to at least one antimicrobial and 81.8% were XDR. In contrast, 35.7% of *E. coli* isolates were resistant to at least one antibiotic and only 2.6% were XDR. For *Salmonella* spp., 50% were resistant to at least one antimicrobial agent and the other 50% were sensitive (Table 5).

DISCUSSION

The three bacteria species that we isolated from sea lions may be opportunistic pathogens for sea lions, especially in stress situations such as during rehabilitation (e.g., Petrauskas et al. 2006; Berardi et al. 2014; Chatterton et al. 2020).

Salmonellae have the ability to survive at salinities as high as 3.5% (Petrin et al. 2022), which permits colonization of sea animals; they are commonly found in marine organisms including cetaceans, pinnipeds, reptiles, fish, and shellfish (Minette 1986). *Salmonella* spp. are classified as opportunistic pathogens. Salmonellae have been detected with low prevalence in subclinical opportunistic infections in healthy sea lions; however, when the immune system is compromised, such as during rehabilitation or in captivity, they may become pathogenic. For example, Thornton et al. (1998) isolated *Salmonella* spp. from the lower gastrointestinal tract of in 10% of 865 stranded sea lions along the US Californian coast suffering from lesions, including abscesses, pneumonia, pleuritis, septicemia, and gastroenteritis. Also, Calle et al. (1995) described a case of enteric salmonellosis in a captive walrus (*Odobenus rosmarus divergens*). Regarding the two salmonellae we isolated, *Salmonella* Newport has been previously reported in pinnipeds (Gilmartin et al. 1979a, b), including 20 of 50 (40%) California sea lion pups and 22 of 90 (24%) Northern fur seals (*Callorhinus ursinus*) on San Miguel Island (located in California channel, Pacific Ocean). Fenwick et al. (2004), identified four *Salmonella* serotypes from sea lions (*Phocarctos hookeri*), including *Salmonella* Newport (Fenwick et al. 2004). The *Salmonella* Saintpaul serotype has not been previously identified in sea lions; however, it was isolated from elephant seal pups (*Mirounga angustirostris*; Carrasco et al. 2011). The presence of both *Salmonella* serotypes in sea lions could be

TABLE 5. Antimicrobial resistance of bacterial strains isolated sea lion (*Zalophus californianus*) pups, Farallon Island, Sinaloa, Mexico, 2016.

	Total bacteria species, *n* (%), 39	Bacteria isolated, *n* (%)		
		Escherichia coli, 14	*Salmonella* spp., 14	*Pseudomonas aeruginosa*, 11
Class and antimicrobial				
Aminoglycoside				
Gentamicin	7 (17.9)	3 (21.4)	2 (14.3)	2 (18.2)
Quinolones and fluoroquinolones				
Ciprofloxacin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nalidixic acid	10 (25.6)	1 (7.1)	0 (0.0)	9 (81.8)*
Sulfonamides and potentiated sulfonamides				
Sulfamethoxazole-trimethoprim	10 (25.6)	1 (7.1)	0 (0.0)	9 (81.8)*
Tetracyclines				
Tetracycline	10 (25.6)	1 (7.1)	0 (0.0)	9 (81.8)*
Beta lactams				
Ampicillin	12 (30.7)	1 (7.1)	2 (14.3)	9 (81.8)*
Cephalosporins				
Ceftazidime	2 (5.1)	0 (0.0)	2 (14.3)	0 (0.0)
Cefotaxime	15 (38.5)	3 (21.4)	3 (21.4)	9 (81.8)*
Phenicols				
Chloramphenicol	9 (23.0)	0 (0.0)	0 (0.0)	9 (81.8)
Category[a]				
Susceptible	16 (41.0)	9 (64.3)	7 (50.0)	0 (0.0)
Resistant to any antibiotic	23 (59.0)	5 (35.7)	7 (50.0)	11 (100)
MDR	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
XDR	10 (25.6)	1 (2.6)	0 (0.0)	9 (81.8)*
No. of antimicrobials resistant to				
0	16 (41.0)	9 (64.3)	7 (50.0)	0 (0.0)
1	11 (28.2)	4 (28.6)	5 (35.7)	2 (18.1)
2	2 (5.1)	0 (0.0)	2 (14.3)	0 (0.0)
3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
5	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
6	10 (25.6)	1 (7.1)	0 (0.0)	9 (81.8)

[a] MDR = multidrug resistance to three or more different categories of antibiotics; XDR = extensively drug-resistant to six or more different categories of antibiotics.

* $P<0.05$. *P* values were calculated with Fisher's exact test.

associated with fecal contamination from seabirds (Moré et al. 2017). Wastewater discharges from large cruise ships and small vessels with human feces could also influence the colonization of *Salmonella* serotypes (Gilmartin et al. 1979).

The high prevalence of *Salmonella* Newport indicates that it may be endemic in this specific sea lion population. For example, *Salmonella* Bovismorbificans was isolated in 32 of 37 (86%) stranded grey seal pups (*Halichoerus grypus*), which was indistinguishable from this organism isolated from Scottish cattle in the same region (Baily et al. 2016). Cross-species transmission among humans, livestock, domestic dogs, and terrestrial wildlife has previously been documented (Drozdz et al. 2021), and *Salmonella enterica* was isolated from both New Zealand sea lions and feral pigs (*Sus scrofa*) in the Auckland Islands (Fenwick et al. 2004).

Escherichia coli is a common bacterium that colonizes many species, including humans; however, it is rare in sea lions. It was identified for the first time in apparently healthy sea lions, in the lower section and terminal end of the small intestine and the entire large intestine (Oppenheimer and Kelly 1952). It has also been isolated from dead sea lions; however, the presence of some of these isolates was probably due to postmortem invasion from the gastrointestinal tract (Oppenheimer and Kelly 1952). The presence of *E. coli* in sea lions is still controversial. This bacterium has been identified in healthy animals and in animals with edema disease related to *E. coli* serotype O147:K:H52 (Diamond et al. 1980). Additionally, *E. coli* has been associated with endocarditis (Diamond et al. 1980). The presence of *E. coli* in the oceans may be related to spillover from contaminated stored marine fish or may be introduced by human fecal contamination (Canizalez-Roman et al. 2019).

Escherichia coli phylogroup B2 or D has been isolated from sea lions (*Neophoca cinerea*) from Australian colonies in Seal Bay, Kangaroo Island, and San Esteban Island in the Gulf of California and the Pacific Ocean in Mexico. Both phylogroups are associated with high colonization capacity and virulence (Fulham et al. 2018). In contrast, we found high prevalence of *E. coli* phylogroup UNKNOWN, followed by A and B1. These phylogroups are not associated with virulent *E. coli*. However, a recent report indicates that *E. coli* can carry supplementary virulence genes, which could increase virulence of nondiarrheagenic *E. coli* (Angulo-Zamudio et al. 2021). Stoddard et al. (2009) determined that antimicrobial drug use increased resistance of commensal gastrointestinal *E. coli* of wild northern elephant seals treated during rehabilitation. Appropriate use of antimicrobial therapy in captive situations is important to reduce the risk of AMR bacteria developing.

An epizootic in Galapagos sea lions (*Z. c. wollebaeki*) caused by *P. aeruginosa* characterized by suppurative, cutaneous nodules has been described (Rand 1975). *Pseudomonas* spp. were also isolated from superficial abscesses, wounds, ocular and urethral discharges, lungs, and liver in California sea lions during rehabilitation (Thornton et al. 1998). Also, *Mycobacterium marinum* and *P. aeruginosa* were isolated from a California sea lion with chronic sinusitis in a zoo in New Zealand (Chatterton et al. 2020). During this study, *P. aeruginosa* was found mainly in the oral cavity, suggesting than this bacterium could be part of the normal microbiota of the upper respiratory tract; however, further studies are required to determine the pathophysiology of *P. aeruginosa* in sea lions.

We found that California sea lion pups were cocolonized with two or three different bacterial species. Similar results have been reported previously for viruses and protozoa. Multiple enteric coinfections in sea lions involving eukaryotic viruses, bacteriophages, astroviruses, and siphoviruses have been reported (Li et al. 2011). In another study, three California sea lions were coinfected with two different intestinal coccidian parasites, types A and B (Carlson-Bremer et al. 2012). Stress and immunosuppression may deteriorate the state of health in captivity, and bacteriologic surveys may be essential to characterize which microorganisms are part of normal microbiota and which may play an opportunistic pathogenic role (Thornton et al. 1998).

Another important aspect of this study was the investigation of AMR. Hernández-Castro et al. (2020) found that *E. coli* isolated from sea lions was resistant to ampicillin/sulbactam, trimethoprim/sulfamethoxazole, cefoxitin, and amoxicillin/clavulanate. These resistance trends match the results of our study, in which we demonstrated resistance to eight of nine antimicrobials tested. Stoddard et al. (2009) documented that 77.8% of northern elephant seals released from rehabilitation had AMR *E. coli* compared with 38.4% of seals at admission. In another study, gram-negative and gram-positive bacteria including *Salmonella* spp., *E. coli* and *Klebsiella* spp., and *Proteus* spp. were isolated from stranded pinnipeds, and all bacteria were multidrug-

?6

resistant (Johnson et al. 1998). In contrast, bacteria isolated in the current study were identified as more resistant because only XDR phenotype bacteria were isolated.

The use of antibiotics during rehabilitation plays a central role in increased levels of AMR bacteria in marine animals (Smith et al. 2002). Additionally, horizontal transfer of antibiotic resistance genes among bacteria by conjugation of plasmids, transduction by bacteriophages, and natural transformation by extracellular DNA may play an important role in the increase of resistance to antimicrobials (Huddleston 2014; Juhas 2015). High antimicrobial resistance may limit the ability to treat bacterial infections and may increase the incidence of infectious diseases in marine mammals; moreover, resistance may potentially be transferred to microbiota of other species (McEwen and Fedorka-Cray 2002). The presence of three bacterial species in the oral and rectal cavities represent a potential problem for sea lions because these bacteria may become pathogenic during stressful situations and in immunosuppressed individuals. Furthermore, sea lions share beaches and coastal waters with humans and often rest on manmade structures; these bacteria represent a potential zoonotic risk during direct and indirect human–sea lion interactions.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-21-00183.

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