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Survey of antibiotic-resistant bacteria isolated from bottlenose dolphins *Tursiops truncatus* in the southeastern USA

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ABSTRACT: Contamination of coastal waters can carry pathogens and contaminants that cause diseases in humans and wildlife, and these pathogens can be transported by water to areas where they are not indigenous. Marine mammals may be indicators of potential health effects from such pathogens and toxins. Here we isolated bacterial species of relevance to humans from wild bottlenose dolphins *Tursiops truncatus* and assayed isolated bacteria for antibiotic resistance. Samples were collected during capture–release dolphin health assessments at multiple coastal and estuarine sites along the US mid-Atlantic coast and the Gulf of Mexico. These samples were transported on ice and evaluated using commercial systems and aerobic culture techniques routinely employed in clinical laboratories. The most common bacteria identified were species belonging to the genus *Vibrio*, although *Escherichia coli*, *Shewanella putrefaciens*, and *Pseudomonas fluorescens/putida* were also common. Some of the bacterial species identified have been associated with human illness, including a strain of methicillin-resistant *Staphylococcus aureus* (MRSA) identified in 1 sample. Widespread antibiotic resistance was observed among all sites, although the percentage of resistant isolates varied across sites and across time. These data provide a baseline for future comparisons of the bacteria that colonize bottlenose dolphins in the southeastern USA.

KEY WORDS: Marine mammals · MRSA · Vibrios · Coastal waters · Zoonosis · Anthropogenic impacts · Microbiology · Screening

INTRODUCTION

Contamination of coastal waters occurs from a variety of sources, including run-off and wastewater discharges (Stewart et al. 2008). This contamination typically carries microbiological pollutants, i.e. pathogens including bacteria, viruses, and protozoa, capable of causing disease in humans and other animals. Water can transport pathogens and antibiotic-resistant bacteria to areas where the microbial agents are not indigenous. In particular, the ocean is a sink for many microbes associated

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with wastes and wastewater discharges (Oates et al. 2012).

Infections are routinely implicated with morbidity and mortality of marine mammals (Stroud & Roffe 1979, Gulland & Hall 2007). Bogomolni et al. (2008) identified infectious disease as the most significant factor for mortality in marine vertebrates, based on necropsy and histopathology data. Infectious diseases were associated with 31% of the deaths reviewed, which was higher than deaths caused by trauma, dependent pups or calves unable to forage on their own, or drowning as a result of fishery by-catch. Pathologies included peritonitis, septicemia, hepatitis, aspergillosis, and bacterial enteritis, among others.

Marine mammals may provide early indications of potential health effects from contaminants in the oceans (Wells et al. 2004). Dolphins and other marine mammals have a physiology more similar to humans than other marine inhabitants (Wells et al. 2004). Their diets and physiologies have many commonalities with humans, while their exposure to the marine environment makes them more susceptible to risks from the ocean, including pathogens and toxins. Marine mammals may serve as a biological early warning system, alerting humans to risks before diseases manifest in humans (Bossart 2006, Stewart et al. 2008). Disease in these marine mammals may demonstrate health risks resulting from levels of contaminants typically found in the environment, well before health trends are identified in humans (de Swart et al. 1995).

Currently, not much is known about pathogens that colonize marine mammals in the wild. The science has improved over the past decade, with a number of studies published on potential zoonotic and host-specific pathogens (Whatmore et al. 2008, Shapiro et al. 2012, Waltzek et al. 2012). However, it is important to establish baseline data on bacterial species and traits (antibiotic resistance) that may be of relevance to humans (Ward & Lafferty 2004). These characterizations are important from a 'one-health' perspective recognizing that the health of people, animals, and our environment are inextricably linked (Kahn et al. 2008).

Estimates of human pathogens in marine mammals are limited, but species identification is needed to help identify health trends and threats. The aforementioned survey by Bogomolni et al. (2008) focused on *Brucella*, *Leptospira*, *Giardia*, and *Cryptosporidium* from marine birds and mammals in the northwest Atlantic. Prevalence of *Salmonella* strains have been studied along the western USA (Gilmartin et al. 1979, Stoddard et al. 2008), as well as in the UK (Baker et al. 1995), New Zealand (Fenwick et al. 2004), and the sub-Antarctic (Palmgren et al. 2000). A recent study of terrestrial and marine species from Monterey Bay, California (USA) identified *Campylobacter* and *Salmonella* in both terrestrial and marine animals, along with *Vibrio cholerae* and *V. parahaemolyticus* in sea otters (Oates et al. 2012).

These studies demonstrate geographic differences in the occurrence of potential pathogens. Also, data from existing studies are often derived from stranded or by-caught animals that may not be representative of the health status of their populations. Very few studies have been published cataloguing aerobic bacteria including human pathogens sampled from live marine mammals in the southeastern USA (Buck et al. 2006, Morris et al. 2011, Schaefer et al. 2011).

The goal of this study was to identify species of bacteria associated with bottlenose dolphins *Tursiops truncatus* and to evaluate these bacteria for antibiotic resistance. Fecal and blowhole specimens were collected from live dolphins sampled during capture–release operations over a 6 yr period. Coastal sites, selected to represent varying land covers in their drainage areas, were included from the mid-Atlantic USA and Gulf of Mexico. Antibiotic resistance was also compared among sites to evaluate geographic variability and anthropogenic influence. No other published studies reporting microbiology survey data from dolphins in the southeastern USA have tested for antibiotic resistance or geographic differences. These data provide a baseline for future comparisons and allow for the generation of hypotheses related to the influence of land use and other environmental factors on incidence of resistance.

MATERIALS AND METHODS

Sampling sites

Five sampling sites in the southeastern USA were investigated in this study: (1) Sarasota Bay, Florida; (2) Beaufort, North Carolina; (3) St. Joseph Bay, Florida; (4) Sapelo Island, Georgia; and (5) Brunswick, Georgia (Fig. 1). These sampling sites represent areas with variable impacts from anthropogenic activities (Table 1).

Sarasota Bay

The Sarasota Bay study area (27° 25′ N, 82° 38′ W), extending approximately 30 km along the central

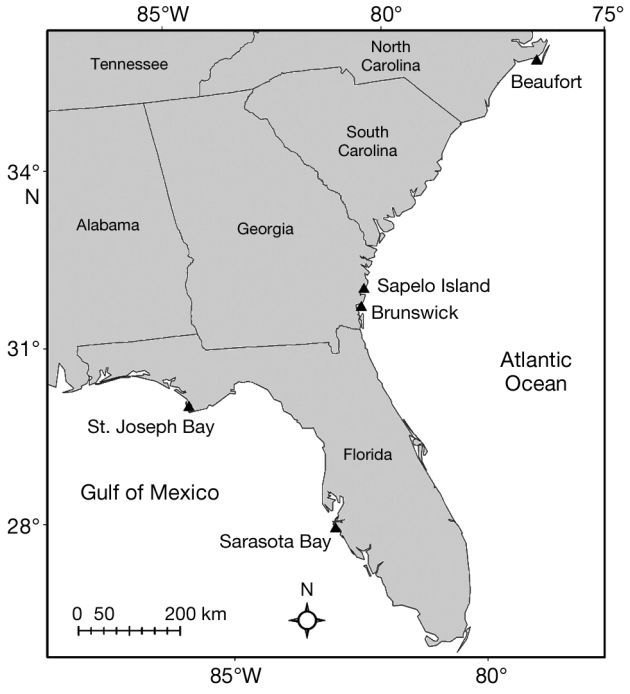

Fig. 1. Study sites in the southeastern USA, where capture–release health assessments of bottlenose dolphins were made

west coast of Florida south of Tampa, encompasses Sarasota and associated shallow bays (generally 1–4 m deep) and is bounded on the west by a series of narrow barrier islands (Wells et al. 2005). The bays are linked to the Gulf of Mexico through deeper (up to 10 m), narrow passes between the barrier islands. Subtidally the area is covered by approximately 50 km^2 of seagrass beds, while most of the shoreline in this study area is developed (Gorzelany 2003). In an effort to restore and protect Sarasota Bay, this area has been designated as a National Estuary Program (USEPA 2008). A program to study the resident community of bottlenose dolphins in this area has been ongoing since 1970, with health assessments routinely conducted since 1987 (Wells et al. 2004, Wells 2009).

Beaufort

The Beaufort study area (34° 41′ N, 76° 38′ W), located at the southern extent of the Outer Banks of eastern North Carolina, encompasses the estuarine waters surrounding Beaufort and the nearshore waters south of Shackleford Banks. The estuary drains an agricultural, forest, and pocosin watershed (Tester et al. 1995) with some development (State Port at Morehead City), while subtidally there are extensive seagrass beds (Thayer et al. 1975). Mean depth is 1 m with a tidal range of less than 2 m (Tester et al. 1995, USDOC/NOAA/NOS 2008). Parts of this study area are home to the Rachel Carson National Estuarine Research Reserve (NERR). Bottlenose dolphin health assessments were conducted in this area as part of the National Marine Fisheries Services stock assessment research to tag dolphins with satellite-linked transmitters (Waring et al. 2011).

St. Joseph Bay

The St. Joseph Bay study area (29° 48′ N, 85° 21′ W), located along the Florida panhandle near Port St. Joe, encompasses the Gulf of Mexico waters from Cape San Blas northwest to Crooked Island Sound extending 1.5 km from shore, including St. Joseph Bay and Crooked Island Sound (Balmer et al. 2008). This site, comprised of open bays and coastal waters, primarily drains wetlands and upland forest. St. Joseph Bay is unique in being the only sizable body of water along the eastern portion of the Gulf of Mexico that is not markedly influenced by the inflow of freshwater (Stewart & Gorsline 1962). This site is also home to the St. Joseph Bay Aquatic Preserve. The monitoring of bottlenose dolphins in this area began in 2004 to investigate unusual mortality events occurring between 1999 and 2006 along Florida's northern Gulf of Mexico coast in which over 300 bottlenose dolphins are known to have died (NMFS 2004, Balmer et al.

Table 1. Anthropogenic influence of study sites in the southern USA

Site	Years sampled	Anthropogenic influence	Predominant land cover in drainage area
Sarasota Bay, Florida	2004, 2005, 2006, 2009	High	Suburban development
Beaufort, North Carolina	2006	Intermediate	Agricultural and forest land covers with low density development
St. Joseph Bay, Florida	2006	Low	Wetlands and forest; not markedly influenced by freshwater runoff
Brunswick, Georgia	2009	High	Industrial development
Sapelo Island, Georgia	2009	Low	Salt marsh

2008, Waring et al. 2011). The health and status of dolphins at this site was of concern as it was impacted by 3 unusual mortality events and was the geographic focus of the event that occurred in 2004 (NMFS 2004, Balmer et al. 2008).

Brunswick

The Brunswick study area (31° 6′ N, 81° 25′ W), located along the southern coast of Georgia near Brunswick, encompasses the inshore waters from St. Simons Sound north to Altamaha Sound extending 15 km upriver of the Turtle and Altamaha Rivers (Balmer et al. 2011). This site, comprised of large sounds, rivers, and tidal creeks, is predominantly bordered by a salt marsh dominated by emergent grasses (*Spartina* and *Juncus* spp.; Kannan et al. 1997). However, multiple industrial complexes have contaminated the marsh and estuarine waters of the Turtle/Brunswick River Estuary (TBRE), resulting in high reported concentrations of metals and persistent organic pollutants in the soils, groundwater and biota of this region (Kannan et al. 1997, 1998, Maruya & Lee 1998, Maruya et al. 2001). Studies of bottlenose dolphins in the TBRE began in 2004 to evaluate the abundance, health, and survival of animals following chronic exposure to the polychlorinated biphenyl mixture Aroclor 1268 (Balmer et al. 2011).

Sapelo Island

The Sapelo study area (31° 24′ N, 81° 18′ W), located along the southern coast of Georgia approximately 30 km northeast of Brunswick, includes all inshore waters from Sapelo Sound south to Altamaha Sound extending 15 km upriver of the Sapelo River (Balmer et al. 2011). This site, comprised of large sounds, rivers, and tidal creeks, is predominantly bordered by a salt marsh dominated by emergent grasses *Spartina* sp. with numerous wooded hammocks throughout the area (Ragotzkie & Bryson 1955). Included in this site is the Sapelo Island NERR, a federally protected area established for long-term research encompassing Sapelo Island, the fourth largest and most pristine barrier island along the Georgia coast (Owen & White 2006). The monitoring of bottlenose dolphins in this study area was initiated in 2007 with the intent that dolphins in this area could serve as a reference group for comparison with dolphins inhabiting the more contaminated Brunswick study area (Balmer et al. 2011).

Sample collection

Fecal swabs and blowhole swabs were collected during wild dolphin health assessments involving capture–release of animals over a 5 yr period. Dolphins were sampled from estuarine populations that generally stay in localized areas without traveling great distances (Balmer et al. 2008, 2014, Wells 2009, Waring et al. 2011). Genetic analysis has shown distinct stock structures within various estuaries that have been sampled (Sellas et al. 2005). Additionally, some of the animals captured were tagged with radio and/or satellite-linked transmitters and showed only localized movement patterns over the duration of the tag (see Balmer et al. 2008, 2014). Although the geographic ranges of the dolphin populations are fairly well characterized, the range of the fish that might comprise their diets is not known. Capture–release efforts in Beaufort, St. Joseph Bay, Brunswick, and Sapelo were each conducted over a 2 wk period, during which time no animals were resampled. Sarasota Bay health assessments were conducted over multiple years, during which time 2 animals were resampled once. One animal (FB125) was sampled in 2006 and 2009, and another animal (FB128) was sampled in 2004 and 2009.

The fecal and blowhole areas were first wiped with a sterile gauze to prevent saltwater contamination of the samples. Samples were then collected using sterile swabs and stored in tubes containing 1 ml phosphate-buffered saline (PBS). Samples were kept on ice and shipped overnight on the day of collection to the Fort Walton Beach Medical Center (Fort Walton Beach, Florida). At the Center, samples were processed on the day following collection for isolation and characterization of bacteria.

Bacterial isolations and antibiotic sensitivity

Initial identification of bacteria was conducted using the automated MicroScan WalkAway System (Siemens Healthcare Diagnostics). This system relies on conventional and chromogenic tests for the identification of fermentative and non-fermentative Gram-negative bacilli, as well as Gram-positive bacteria including *Micrococcaceae*, *Streptococcaceae*, *Listeria monocytogenes*, and *Aerococcus*. Briefly, fecal specimens were plated for bacterial isolations on Blood, Chocolate, MacConkey, Campy and XLD, and TCBS agar. Blow hole specimens were plated on Blood, Chocolate, MacConkey, and TCBS agar. Once isolated, individual bacterial suspensions were inocu-

lated and incubated for 16 to 48 h at 35°C. Species identification was then based on hydrolysis of fluorogenic substrates, pH change indicators for substrate utilization, and production of metabolic byproducts.

Identifications of Gram-negative bacteria were further confirmed using the Analytical Profile Index (API) 20E system (bioMerieux), a kit used for the identification of *Enterobacteriaceae* and other Gram-negative bacteria. Briefly, bacterial suspensions were inoculated on a panel of 20 dehydrated reagents representing traditional biochemical assays used for identification of bacterial species. Each panel was incubated for 24 to 48 h at 35°C. Results were assigned a numerical value, the API code, which was matched with its corresponding bacterial species in the API database. Laboratory reference strains of *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* were used as positive controls for this test. Isolates were not further confirmed through genetic testing, although all species identifications were conducted using methods commonly employed in clinical laboratories.

Antimicrobial susceptibility was tested using MicroScan conventional dried media panels (Siemens Healthcare Diagnostics) with ambiguous reads confirmed using the Kirby-Bauer disk diffusion method. These tests were conducted in compliance with guidelines from the Clinical and Laboratory Standards Institute (CLSI 2006) with specific selection of antibiotics shown in Table 2. Briefly, specific suites of antibiotics were tested depending on species identification. The same set of drugs was used for a given species, regardless of sample location or collection time. Results were expressed as sensitive, intermediate, or resistant. For the purposes of this manuscript, isolates scored as intermediate were not included in calculations of resistant isolates.

Table 2. Agents used in antimicrobial susceptibility testing of bacterial isolates from bottlenose dolphins

Antibiotic	Gram-negative					Gram-positive		
	Enterobacteriaceae	*Aeromonas*	*Flavobacteriaceae; Shewanella; Pseudomonas* groups other than *P. aeruginosa*	*P. aeruginosa*	*Vibrionaceae*	*Enterococcus* spp.	*Staphylococcus* spp.	*Streptococcus* spp.
Amoxicillin/K clavulanate	X	X	–	–	X	–	X	–
Ampicillin	X	–	–	–	X	X	–	–
Ampicillin/sulbactam	X	X	–	–	X	–	–	–
Azithromycin	–	–	–	–	–	–	X	–
Aztreonam	X	X	–	–	–	–	–	–
Cefazolin	X	X	–	–	X	–	X	–
Cefepime	X	X	X	X	X	–	X	X
Cefotaxime	–	–	–	–	–	–	–	X
Cefoxitin	X	X	–	–	X	–	–	–
Ceftazidime	X	X	X	X	X	–	–	–
Ceftriaxone	X	X	X	–	–	–	X	X
Cefuroxime	X	X	–	–	X	–	–	X
Cephalothin	X	X	–	–	X	–	X	–
Ciprofloxacin	X	X	X	X	X	–	X	–
Clindamycin	–	–	–	–	–	–	X	X
Erythromycin	–	–	–	–	–	–	X	X
Gentamicin	X	X	X	X	X	X[a]	X	–
Levofloxacin	X	X	X	X	X	X	–	X
Oxacillin	–	–	–	–	–	–	X	–
Penicillin	–	–	–	–	–	X	X	X
Piperacillin/tazobactam	X	X	X	X	X	–	–	–
Tetracycline	X	X	X	–	–	X	X	X
Tobramycin	X	–	X	X	–	–	–	–
Trimethoprim/sulfamethoxazole	X	X	X	–	–	–	X	X
Vancomycin	–	–	–	–	–	X	X	X

[a]Gentamycin synergy screen

Statistical analysis

For analyzing differences in the level of antibiotic resistance among study sites, we applied a generalized linear model (GLM) with a log link function and Poisson error distribution. The fit of the model was evaluated by examining the residual deviance and Wald chi-squared statistic. Specific pairwise differences between the sites were examined using multiple pairwise GLMs and applying a sequential Bonferroni correction with an initial $\alpha = 0.05$ (Holm 1979). These methods were also used to examine differences among years for the Sarasota site, the only site with multi-year observations. Analyses were performed using Statistica 9.0 (StatSoft).

Ethics statement

This research was conducted under the authority of Scientific Research and Enhancement Permit Nos. 932-1905/MA-009526 (Brunswick and Sapelo Island), 932-1489 (St. Joseph Bay), 779-1681-00 (Beaufort), and 522-1569 and 522-1785 (Sarasota Bay) issued by the National Marine Fisheries Service. Protocols for dolphin capture–release and field sample collection in Sarasota Bay were approved by the Mote Marine Laboratory Institutional Animal Care and Use Committee; protocols for the St. Joseph Bay study were approved by the University of North Carolina, Wilmington Internal Animal Care and Use Committee; protocols for the Georgia study were approved by the NOAA Animal Care and Use Committee.

RESULTS

In total, 667 bacterial isolates were isolated and speciated (Table 3). The most common species identified belonged to the genus *Vibrio*, and included *V. alginolyticus*, *V. parahaemolyticus*, and *V. vulnificus* (Table 4). Other species that were commonly identified included *Photobacterium damselae*, *Escherichia coli*, *Shewanella putrefaciens*, *Pseudomonas fluorescens/putida*, *Acinetobacter lwoffii*, *Edwardsiella tarda*, and *Aeromonas hydrophila*. Most of these bacteria are indigenous to the marine environment. *E. coli* is not necessarily indigenous to marine environments but is known to inhabit the intestinal tracts of warm-blooded animals.

All of the bacterial species identified have been associated with clinical illness in humans, although we do not know whether these particular strains were virulent. Some of the species that were identified are designated as reportable human pathogens by the US Centers for Disease Control and Prevention (CDC), meaning that they are required to be reported when they are diagnosed by doctors or laboratories because they are considered to be of high importance in public health. Bacteria identified in this study that can be the causative agents of reportable infections include *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus* (CDC 2011). Toxigenic strains of *Streptococcus* sp., *E. coli*, and *Staphylococcus* would also be reportable, although distinguishing toxigenic from nontoxigenic strains was beyond the scope of this study.

Emerging or reemerging human pathogens were also identified. A strain of methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from a fecal swab sampled from a dolphin in Sarasota Bay in 2004 (data not shown). While *S. aureus* has been recognized as a human pathogen for over a century, MRSA has quickly evolved over the past 15 yr to incorporate new resistance traits and virulence factors (Tang & Stratton 2010, Smith & Pearson 2011). Zoonotic transmission of MRSA has been implicated in the emergence of new strains (Smith & Pearson 2011), and animal reservoirs are of concern for public health (Weese 2010).

Widespread antibiotic resistance was observed among the animals sampled. Among the 123 dolphins sampled in this study, 74% were found to have a bacterial isolate resistant to at least 1 antibiotic and 48% harbored bacteria resistant to more than 1 antibiotic. Almost half (48.5%) of the 656 isolates tested were resistant to at least 1 tested antibiotic, while 51.5% were sensitive or intermediate to all antibiotics tested (Table 5). Eleven isolates were either not tested due to lab limitations (e.g. inability to regrow isolate) or testing was not applicable. Among all isolates, 24% were resistant to only 1 antibiotic and 24% were resistant to more than 1 antibiotic. Resistance was observed to up to 13 antibiotics (Fig. 2).

The percentage of isolates showing antibiotic resistance was highest from the Sarasota and Beaufort study sites, with 54% (n = 421) and 47% (n = 55) of tested isolates showing resistance to at least 1 antibiotic, respectively (Table 5). However, isolates from all sites displayed resistance. Resistance was least common in St. Joseph Bay, where 69% (n = 81) of isolates were sensitive or intermediate to tested antibiotics. At this site, only 10% of tested isolates were resistant to multiple antibiotics.

The level of resistance, defined as the mean number of antibiotics to which isolates were resistant, differed significantly across study sites (χ^2 test, $p = 0.0000$).

Table 3. Bacterial species identified from blowhole (B) and fecal (F) samples collected during *Tursiops truncatus* dolphin capture–release health assessments at sites along the mid-Atlantic USA and Gulf of Mexico

Bacterial species	No. of isolates B	No. of isolates F	Bacterial species	No. of isolates B	No. of isolates F
Acinetobacter baumannii/haemolyticus	2	0	*Proteus mirabilis*	1	6
Acinetobacter lwoffii	18	13	*Proteus vulgaris*	2	3
Acinetobacter sp.	0	1	*Providencia rettgeri*	1	1
Aeromonas sp.	2	0	*Providencia rustigianii*	1	0
Aeromonas hydrophila	11	18	*Providencia stuartii*	0	1
Alcaligenes sp.	0	3	*Pseudomonas* sp.	2	1
Alcaligenes xylosoxidans	0	1	*Pseudomonas aeruginosa*	7	3
Bacillus sp.	2	2	*Pseudomonas fluorescens*	3	1
Bergeyella zoohelcum	2	0	*Pseudomonas fluorescens/putida*	24	11
Burkholderia cepacia	1	1	*Pseudomonas stutzeri*	10	4
Cedecea sp.	1	2	*Serratia marcesens*	1	0
Citrobacter freundii	3	1	*Shewanella putrefaciens*	19	22
Citrobacter spp.	1	0	*Staphylococcus* sp. coagulase-negative	8	3
Chryseobacterium indologenes	2	1	*Staphylococcus aureus*	0	2
Chryseobacterium spp.	0	1	*Staphylococcus capitis*	2	0
Diphtheroids	1	0	*Staphylococcus cohnii* ssp. *cohnii*	1	0
Edwardsiella tarda	7	20	*Staphylococcus epidermidis*	6	2
Elizabethkingia meningoseptica	1	0	*Staphylococcus haemolyticus*	1	0
Enterobacter aerogenes	1	0	*Staphylococcus sciuri*	1	0
Enterobacter agglomerans	3	2	*Staphylococcus warneri*	3	0
Enterobacter cloacae	3	1	*Streptococcus* sp.	0	1
Enterococcus durans	1	0	*Streptococcus hemolyticus*	3	3
Enterococcus faecalis	0	2	*Streptococcus viridans*	6	2
Escherichia coli	13	38	*Tatumella ptyseos*	4	0
Flavobacterium sp.	4	0	*Vibrio* sp.	0	2
Flavobacterium odoratum	0	1	*Vibrio alginolyticus*	53	27
Klebsiella	0	1	*Vibrio cholerae*	2	1
Klebsiella pneumoniae	8	2	*Vibrio damsela* (*Photobacterium damselae*)	29	32
Kluyvera ascorbata	0	1	*Vibrio fluvialis*	12	10
Micrococcus sp.	1	0	*Vibrio hollisae*	6	0
Morganella morganii	3	4	*Vibrio mimicus*	0	2
Pasteurella aerogenes	2	3	*Vibrio parahaemolyticus*	33	47
Pasteurella haemolytica/pneumotropica/urea	0	1	*Vibrio vulnificus*	9	9
Pasteurella multocida	1	0	*Yersinia enterocolitica*	2	1
Plesiomonas shigelloides	1	3			

Table 4. Bacterial species isolated from bottlenose dolphin samples collected during capture–release health assessments at multiple coastal and estuarine sites along the mid-Atlantic USA and Gulf of Mexico. (See Fig. 1 for site locations)

	Project total	Sarasota 2004	Sarasota 2005	Sarasota 2006	Sarasota 2009	Beaufort 2006	St. Joseph Bay 2006	Sapelo 2009	Brunswick 2009
No. of dolphins	**123**	30	17	16	11	9	16	12	12
No. of samples	**220**	57	29	30	18	16	32	19	19
Vibrio alginolyticus	**80**	10	13	16	12	3	14	4	8
Vibrio parahaemolyticus	**80**	28	14	5	3	0	5	16	9
Photobacterium damselae	**61**	17	6	6	10	6	10	2	4
Escherichia coli	**51**	20	4	9	4	3	6	2	3
Shewanella putrefaciens	**40**	8	0	6	5	2	8	7	4
Pseudomonas fluorescens/putida	**35**	6	7	14	1	0	4	0	3
Acinetobacter lwoffii	**31**	16	5	2	0	7	1	0	0
Edwardsiella tarda	**27**	6	2	6	0	5	4	0	4
Aeromonas hydrophila	**29**	4	4	4	4	0	4	4	5
Vibrio vulnificus	**18**	0	3	4	3	2	2	2	2
Other species	**215**	40	56	34	4	27	33	8	13
Total no. of isolates characterized	**667**	155	114	106	46	55	91	45	55

Table 5. Percent of tested isolates resistant to antibiotics for each study site

Site	No. of bacterial isolates	% resistant to tested antibiotics	% resistant to 1 antibiotic	% resistant to >1 antibiotic
Sarasota	421	54.4	27.3	27.1
Beaufort	55	47.3	16.4	30.9
St. Joseph Bay	81	30.9	21.0	9.9
Sapelo	45	40.0	15.6	24.4
Brunswick	54	37.0	18.5	18.5
All	656	48.5	24.1	24.4

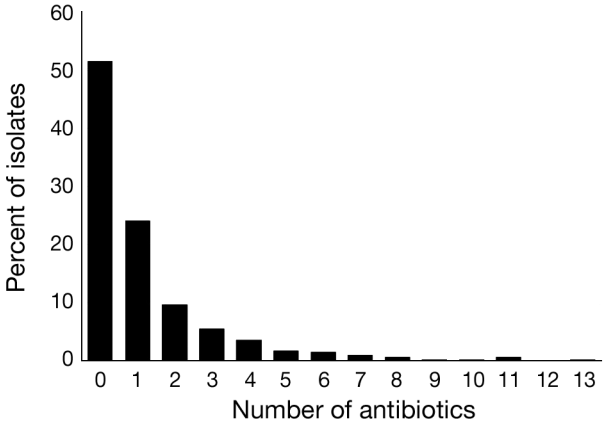

Fig. 2. Number of antibiotics to which tested bacterial isolates (n = 656) showed resistance

Pairwise comparisons of the sites using GLMs with a sequential Bonferroni correction revealed that the mean number of antibiotics to which isolates were resistant was significantly different between all sites except Sarasota and Sapelo ($p = 0.2893$), St. Joseph Bay and Brunswick ($p = 0.1362$), and Sapelo and Brunswick ($p = 0.1676$; Fig. 3, Table 6).

Resistance also appears to vary temporally, although only a limited dataset was available for this analysis. Sarasota Bay was sampled in 2004 (n = 155 isolates), 2005 (n = 114), 2006 (n = 106), and 2009 (n = 46). A significant difference ($p < 0.0001$) was detected among years, with 2005 having significantly greater levels of resistance than the other years (2005 vs. 2004: $p < 0.0001$; 2005 vs. 2006: $p = 0.0008$; 2005 vs. 2009: $p = 0.0007$; Fig. 4, Table 7). No significant differences were observed among 2004, 2006, and 2009. In 2005, there was a severe *Karenia brevis* red tide harmful algal bloom (HAB) in Sarasota Bay that may have allowed proliferation of antibiotic-resistant bacteria through drastically increased quantities of decomposing fish in the water, or perhaps through exposure to brevetoxins. Associations between antibiotic-resistant bacteria and HAB events warrant further study.

DISCUSSION

This study demonstrates widespread antibiotic resistance of bacteria isolated from marine mammals living along the southeastern USA. Sites whose surrounding land uses are associated with anthropogenic impacts were associated with higher percentages of bacteria showing antibiotic resistance. However, the relationship between human activity and antibiotic resistance in marine mammals requires additional study. Antibiotic resistance was demonstrated at all sites, suggesting that a baseline level of resistance occurs throughout coastal waters. Furthermore, the mean number of antibiotics to which isolates were resistant for each site did not show a consistent relationship with anthropogenic influence.

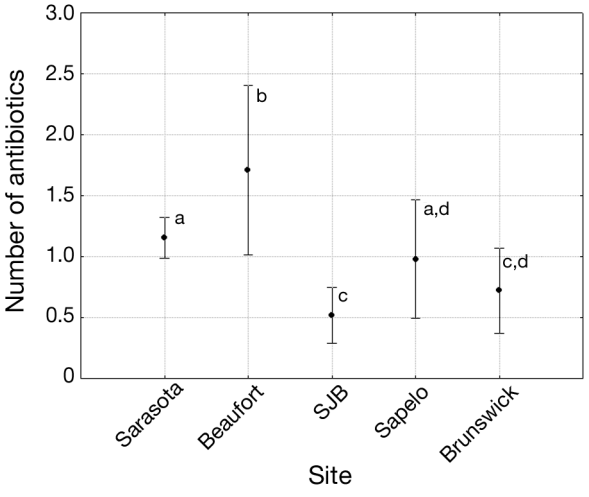

Fig. 3. Mean number of antibiotics to which bacteria were resistant by site. Letters denote significant differences as determined by pairwise generalized linear models with a sequential Bonferroni correction at an initial $\alpha = 0.05$. Sites that do not share the same letter have a significantly different level of antibiotic resistance. SJB: St. Joseph Bay

Table 6. Test results for pairwise comparison of level of antibiotic resistance between sites. Significant p-values after a sequential Bonferroni correction (initial $\alpha = 0.05$) are marked with an asterisk

	Beaufort	St. Joseph Bay	Sapelo	Brunswick
Sarasota	0.0005*	0.0014*	0.4395	0.0047*
Beaufort		<0.0001*	0.0022*	<0.0001*
St. Joseph Bay			0.0033*	0.1362
Sapelo				0.1676

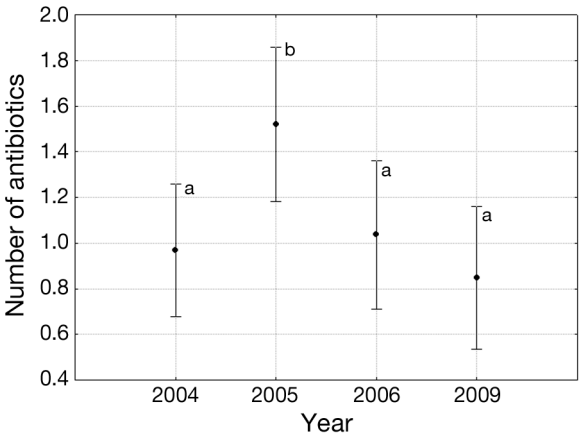

Fig. 4. Mean number of antibiotics to which isolates from Sarasota Bay were resistant for each of 4 investigated years. Letters denote significant differences as determined by pairwise generalized linear models with a sequential Bonferroni correction at an initial $\alpha = 0.05$. Years that do not share the same letter have a significantly different level of antibiotic resistance

Table 7. Test results for pairwise comparison of level of antibiotic resistance between years in Sarasota Bay. Significant p-values after a sequential Bonferroni correction (initial $\alpha = 0.05$) are marked with an asterisk

	2005	2006	2009
2004	<0.0001*	0.5822	0.4617
2005		0.0008*	0.0007*
2006			0.2793

Widespread antibiotic resistance observed in this study is not the result of therapeutic antibiotic use (i.e. contact with human or veterinary drugs), as all specimens were collected from wild animals. It is known that resistance can be spread by horizontal gene transfer, although this has been better studied in clinical rather than marine environments (Neu 1992). It has also been demonstrated that environmental concentrations of antibiotics can inhibit growth of bacteria, exerting a selective pressure that could result in resistance (Tello et al. 2012). These bacteria may also acquire and retain resistance properties in response to other stressors (Alonso et al. 2001). High levels of antibiotic resistance have been reported in aquatic systems contaminated with heavy metals, for example (Stepanauskas et al. 2005, 2006). The observation of widespread resistance reported here is consistent with other studies of marine mammals in coastal oceans (Johnson et al. 1998, Bogomolni et al. 2008, Rose et al. 2009). For example, Rose et al. (2009) reported that 58% (n = 472) of bacterial isolates from vertebrates off the northeastern coast of the USA were resistant to at least 1 antibiotic and that 43% were resistant to more than 1 antibiotic. A higher incidence of resistance was reported for seabirds than for marine mammals, and for stranded or bycaught animals than for live animals. Among stranded pinnipeds in California, Johnson et al. (1998) reported multiple drug resistance in all but 1 of 129 tested bacterial isolates. It has been known for decades that marine bacteria are capable of producing antibiotics (Rosenfeld & Zo Bell 1947). It appears that marine microbes stage their own type of germ warfare to compete with one another, resulting in a baseline level of resistance that occurs naturally in marine environments. Naturally produced antibiotics can also serve as a carbon source in microbial catabolism (Dantas et al. 2008). The resulting antibiotic resistome is widely acknowledged and can help explain the widespread resistance observed in this study, and elsewhere (D'Costa et al. 2006, Baker-Austin et al. 2009).

Results of this study also demonstrate widespread colonization of bacteria that belong to species that could cause disease in humans. All of the most commonly identified bacterial species (Table 4) have been associated with clinical illness in humans, although this does not mean that these particular isolates could cause disease. Most of these species are opportunistic pathogens to humans, infecting immunocompromised individuals and the elderly. *Vibrio alginolyticus* is sometimes associated with ear and wound infections in humans. In the USA, 884 infections and 11 deaths were attributed to *V. alginolyticus* from 1996 to 2010 (Newton et al. 2012). This bacterium is also present in puffer fish (Tetraodontidae) and other animals, where it has been implicated in the production of tetrodotoxin, a deadly human neurotoxin (McEvoy et al. 1997). *V. parahaemolyticus* naturally inhabits marine waters and causes gastrointestinal illness in humans, usually in association with seafood consumption. Wound infections have also been reported. *Photobacterium damselae* has been associated with necrotizing wound infections in humans (Coffey et al. 1986), with at least 1 death reported (Perez-Tirse et al. 1993). *V. vulnificus* infections are often associated with wound infections or with ingestion of contaminated shellfish, particularly oysters (Jones & Oliver 2009). Worldwide, the number of people infected with this bacterium is lower compared to other vibrios. However, *V. vulnificus* is responsible for a large percentage of illnesses and deaths attributable to vibrios. Overall mortality rates for those infected with this bacterium are about 25%, with mortality rates exceeding 50% among patients who develop septicemia (Strom & Paranjpye 2000).

For most species of bacteria, including many of the species identified in this research, only certain strains are able to infect humans. Virulence factors associated with pathogenicity have been well characterized for bacteria such as *Escherichia coli* (Fleckenstein et al. 2010, Hunt 2010), while more recent research has identified specific genetic determinants of virulence among marine vibrios, including *Vibrio cholerae* (Faruque et al. 1998), *V. parahaemolyticus* (Broberg et al. 2011), and *V. vulnificus* (Oliver 2005). Environmental strains of *V. vulnificus* are predominantly virulent, with one study documenting that 90% of tested *V. vulnificus* strains isolated from marine environments were able to infect mice (Tison & Kelly 1986). We did not evaluate specific virulence factors but rather focused on species identification and antibiotic resistance. However, additional sampling of 28 dolphins from the Brunswick and Sapelo Island sites has demonstrated the occurrence of the thermostable direct hemolysin gene (*tdh*) among *V. parahaemolyticus* in dolphin fecal swabs (Moore et al. 2010). Tests for the thermostable-related hemolysin gene (*trh*), a different gene commonly associated with virulence, were negative.

Transmission of pathogens from marine environments is usually associated with consumption of contaminated shellfish or finfish, or through contact with seawater (Thompson et al. 2005). For example, the typical route of infection with *Vibrio vulnificus* in the Gulf of Mexico is humans being cut by a barnacle or oyster shell. The wound becomes infected and the patient can die within 24 to 48 h without proper antibiotic therapy (Bross et al. 2007). Exposures associated with marine mammals are not as well studied. However, there is potential for transmission of pathogens from marine mammals to humans through occupational exposures, particularly for veterinarians, researchers, or hunters who may have direct contact with the animals (Hunt et al. 2008, Moore et al. 2008). Transmission could also be a concern for people engaging in legal and illegal interactions with wild dolphins, including provisioning and swimming (Cunningham-Smith et al. 2006, Powell & Wells 2011). Finally, there is potential for pathogen transmission to those who consume meat from marine mammals (VKM 2011). These routes of transmission are limited to persons with direct and indirect contact with the animals. Zoonotic pathogens from marine mammals are not widely distributed in human populations.

The methods used in this study were limited to aerobic culture of organisms using commercial instruments and databases. This study was not intended to represent the complete microbiome of dolphins, but was designed to evaluate samples for bacterial species and traits (antibiotic resistance) of relevance to humans. Additional bacterial species were likely present in the tested samples but were not identified using the methods employed. Additionally, emerging zoonotic pathogens could have been missed. Bacterial pathogens have previously emerged from ocean ecosystems, including a marine mammalian genotype of *Brucella* that is associated with zoonotic infection (e.g. Whatmore et al. 2008).

This study also raises the question of whether antibiotic resistance can emerge from marine environments because resistance was observed in 'pristine' sites among wild animals that have not been given antibiotics. The widespread antibiotic resistance observed in this study suggests that some resistance elements likely have environmental origins and can be spread without the selective pressure of antibiotic use. Bacteria associated with marine mammals may have a functional role as antibiotic producers, or else resistance may be intrinsic in some of the bacterial species. The potential for pathogens and antibiotic resistant organisms to emerge from marine systems requires further study.

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LITERATURE CITED

Alonso A, Sánchez P, Martínez JL (2001) Environmental selection of antibiotic resistance genes. Environ Microbiol 3:1–9

Baker JR, Hall A, Hiby L, Munro R, Robinson I, Ross HM, Watkins JF (1995) Isolation of *Salmonellae* from seals from UK waters. Vet Rec 136:471–472

Baker-Austin C, McArthur JV, Lindell AH, Wright MS and others (2009) Multi-site analysis reveals widespread antibiotic resistance in the marine pathogen *Vibrio vulnificus*. Microb Ecol 57:151–159

Balmer B, Wells R, Nowacek S, Nowacek D and others (2008) Seasonal abundance and distribution patterns of common bottlenose dolphins (*Tursiops truncatus*) near St. Joseph Bay, Florida, USA. J Cetacean Res Manag 10: 157–167

Balmer BC, Schwacke LH, Wells RS, George RC and others (2011) Relationship between persistent organic pollutants (POPs) and ranging patterns in common bottlenose dolphins (*Tursiops truncatus*) from coastal Georgia, USA. Sci Total Environ 409:2094–2101

Balmer BC, Wells RS, Schwacke LH, Schwacke JH and others (2014) Integrating multiple techniques to identify stock boundaries of common bottlenose dolphins (*Tursiops truncatus*). Aquat Conserv (in press)

Bogomolni AL, Gast RJ, Ellis JC, Dennett M, Pugliares KR, Lentell BJ, Moore MJ (2008) Victims or vectors: a survey of marine vertebrate zoonoses from coastal waters of the Northwest Atlantic. Dis Aquat Org 81:13–38

Bossart GD (2006) Marine mammals as sentinel species for oceans and human health. Oceanography 19:134–137

Broberg CA, Calder TJ, Orth K (2011) *Vibrio parahaemolyticus* cell biology and pathogenicity determinants. Microbes Infect 13:992–1001

Bross MH, Soch K, Morales R, Mitchell RB (2007) *Vibrio vulnificus* infection: diagnosis and treatment. Am Fam Physician 76:539–544

Buck JD, Wells RS, Rhinehart HL, Hansen LJ (2006) Aerobic microorganisms associated with free-ranging bottlenose dolphins in coastal Gulf of Mexico and Atlantic Ocean waters. J Wildl Dis 42:536–544

CDC (US Centers for Disease Control and Prevention) (2011) Summary of notifiable diseases: United States, 2009. MMWR Morb Mortal Wkly Rep 58:1–100

CLSI (Clinical and Laboratory Standards Institute) (2006) Approved standard M2-A9. Performance standards for antimicrobial disk susceptibility tests. Clinical and Laboratory Standards Institute, Wayne, PA

Coffey JA Jr, Harris RL, Rutledge ML, Bradshaw MW, Williams TW Jr (1986) *Vibrio damsela*: another potentially virulent marine vibrio. J Infect Dis 153:800–802

Cunningham-Smith P, Colbert DE, Wells RS, Speakman T (2006) Evaluation of human interactions with a provisioned wild bottlenose dolphin (*Tursiops truncatus*) near Sarasota Bay, Florida, and efforts to curtail the interactions. Aquat Mamm 32:346–356

D'Costa VM, McGrann KM, Hughes DW, Wright GD (2006) Sampling the antibiotic resistome. Science 311:374–377

Dantas G, Sommer MO, Oluwasegun RD, Church GM (2008) Bacteria subsisting on antibiotics. Science 320:100–103

de Swart RL, Harder T, Ross P, Vos H, Osterhaus A (1995) Morbilliviruses and morbillivirus diseases of marine mammals. Infect Agents Dis 4:125–130

Faruque SM, Albert MJ, Mekalanos JJ (1998) Epidemiology, genetics, and ecology of toxigenic *Vibrio cholerae*. Microbiol Mol Biol Rev 62:1301–1314

Fenwick SG, Duignan PJ, Nicol CM, Leyland MJ, Hunter JE (2004) A comparison of *Salmonella* serotypes isolated from New Zealand sea lions and feral pigs on the Auckland Islands by pulsed-field gel electrophoresis. J Wildl Dis 40:566–570

Fleckenstein JM, Hardwidge PR, Munson GP, Rasko DA, Sommerfelt H, Steinsland H (2010) Molecular mechanisms of enterotoxigenic *Escherichia coli* infection. Microbes Infect 12:89–98

Gilmartin WG, Vainik PM, Neill VM (1979) *Salmonellae* in feral pinnipeds off the Southern California coast. J Wildl Dis 15:511–514

Gorzelany J (2003) Sarasota county manatee protection plan. Tech Rep 874. Mote Marine Laboratory, Sarasota, FL

Gulland FMD, Hall AJ (2007) Is marine mammal health deteriorating? Trends in the global reporting of marine mammal disease. EcoHealth 4:135–150

Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Stat 6:65–70

Hunt JM (2010) Shiga toxin-producing *Escherichia coli* (STEC). Clin Lab Med 30:21–45

Hunt TD, Ziccardi MH, Gulland FMD, Yochem PK, Hird DW, Rowles T, Mazet JAK (2008) Health risks for marine mammal workers. Dis Aquat Org 81:81–92

Johnson SP, Nolan S, Gulland FM (1998) Antimicrobial susceptibility of bacteria isolated from pinnipeds stranded in central and northern California. J Zoo Wildl Med 29: 288–294

Jones MK, Oliver JD (2009) *Vibrio vulnificus*: disease and pathogenesis. Infect Immun 77:1723–1733

Kahn LH, Kaplan B, Monath TP, Steele JH (2008) Teaching 'one medicine, one health'. Am J Med 121:169–170

Kannan K, Maruya KA, Tanabe S (1997) Distribution and characterization of polychlorinated biphenyl congeners in soil and sediments from a Superfund site contaminated with Aroclor 1268. Environ Sci Technol 31: 1483–1488

Kannan K, Nakata H, Stafford R, Masson G, Tanabe S, Giesy JP (1998) Bioaccumulation and toxic potential of extremely hydrophobic polychlorinated biphenyl congeners in biota collected at a Superfund site contaminated with Aroclor 1268. Environ Sci Technol 32:1214–1221

Maruya K, Lee R (1998) Aroclor 1268 and toxaphene in fish from a southeastern U.S. estuary. Environ Sci Technol 32: 1069–1075

Maruya KA, Walters TL, Manning RO (2001) Residues of toxaphene in finfish and shellfish from Terry and Dupree Creeks, Georgia, USA. Estuaries 24:585–596

McEvoy EG, Rogers A, Gibson R (1997) Preliminary investigation of *Vibrio alginolyticus*-like bacteria associated with marine nemerteans. Hydrobiologia 365:287–291

Moore MJ, Gast RJ, Bogomolni AL (2008) Marine vertebrate zoonoses: an overview of the DAO Special Issue. Dis Aquat Org 81:1–3

Moore JG, Dickerson JWJ, Schwacke LH, Rowles TK, Stewart JR (2010) *Vibrio* concentrations in bottlenose dolphins from Sapelo Island and Brunswick, GA. International *Vibrios* in the Environment Conference, Biloxi, MS

Morris PJ, Johnson WR, Pisani J, Bossart GD, Adams J, Reif JS, Fair PA (2011) Isolation of culturable microorganisms from free-ranging bottlenose dolphins (*Tursiops truncatus*) from the southeastern United States. Vet Microbiol 148:440–447

Neu HC (1992) The crisis in antibiotic resistance. Science 257:1064–1073

Newton A, Kendall M, Vugia DJ, Henao OL, Mahon BE (2012) Increasing rates of vibriosis in the United States, 1996–2010: review of surveillance data from 2 systems. Clin Infect Dis 54(Suppl 5):S391–S395

NMFS (National Marine Fisheries Service) (2004) Interim report on the bottlenose dolphin (*Tursiops truncatus*) unusual mortality event along the panhandle of Florida March–April 2004. Available at http://www.nmfs.noaa.gov/pr/pdfs/health/ume_bottlenose_2004.pdf

Oates SC, Miller MA, Byrne BA, Chouicha N and others (2012) Epidemiology and potential land-sea transfer of enteric bacteria from terrestrial to marine species in the Monterey Bay Region of California. J Wildl Dis 48: 654–668

Oliver JD (2005) *Vibrio vulnificus*. In: Belkin S, Colwell RR (eds) Oceans and health: pathogens in the marine environment. Springer Science, New York, NY, p 253–276

Owen B, White S (2006) NERRS 10th anniversary report on the system-wide monitoring program. Data applications: 1995–2005. NOAA National Estuarine Research Reserve System, Silver Spring, MD

Palmgren H, McCafferty D, Aspan A, Broman T and others (2000) *Salmonella* in sub-Antarctica: low heterogeneity in *Salmonella* serotypes in South Georgian seals and birds. Epidemiol Infect 125:257–262

Perez-Tirse J, Levine JF, Mecca M (1993) *Vibrio damsela*. A cause of fulminant septicemia. Arch Intern Med 153: 1838–1840

Powell JR, Wells RS (2011) Recreational fishing depredation and associated behaviors involving common bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. Mar Mamm Sci 27:111–129

Ragotzkie RA, Bryson RA (1955) Hydrography of the Duplin River, Sapelo Island, Georgia. Bull Mar Sci 5:297–314

Rose JM, Gast RJ, Bogomolni A, Ellis JC, Lentell BJ, Touhey K, Moore M (2009) Occurrence and patterns of antibiotic resistance in vertebrates off the Northeastern United States coast. FEMS Microbiol Ecol 67:421–431

Rosenfeld WD, Zo Bell CE (1947) Antibiotic production by marine microorganisms. J Bacteriol 54:393–398

Schaefer AM, Bossart GD, Mazzoil M, Fair PA, Reif JS (2011) Risk factors for colonization of *E. coli* in Atlantic bottlenose dolphins (*Tursiops truncatus*) in the Indian River Lagoon, Florida. J Environ Public Health 2011:597073

Sellas AB, Wells RS, Rosel PE (2005) Mitochondrial and nuclear DNA analyses reveal fine scale geographic structure in bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico. Conserv Genet 6:715–728

Shapiro K, Miller M, Mazet J (2012) Temporal association between land-based runoff events and California sea otter (*Enhydra lutris nereis*) protozoal mortalities. J Wildl Dis 48:394–404

Smith TC, Pearson N (2011) The emergence of *Staphylococcus aureus* ST398. Vector Borne Zoonotic Dis 11:327–339

Stepanauskas R, Glenn TC, Jagoe CH, Tuckfield RC, Lindell AH, McArthur JV (2005) Elevated microbial tolerance to metals and antibiotics in metal-contaminated industrial environments. Environ Sci Technol 39:3671–3678

Stepanauskas R, Glenn TC, Jagoe CH, Tuckfield RC, Lindell AH, King CJ, McArthur JV (2006) Co-selection for microbial resistance to metals and antibiotics in freshwater microcosms. Environ Microbiol 8:1510–1514

Stewart JR, Gast RJ, Fujioka RS, Solo-Gabriele HM and others (2008) The coastal environment and human health: microbial indicators, pathogens, sentinels and reservoirs. Environ Health 7(Suppl 2):S3

Stewart RA, Gorsline DS (1962) Recent sedimentary history of St. Joseph Bay, Florida. Sedimentology 1:256–286

Stoddard RA, DeLong RL, Byrne BA, Jang S, Gulland FMD (2008) Prevalence and characterization of *Salmonella* spp. among marine animals in the Channel Islands, California. Dis Aquat Org 81:5–11

Strom MS, Paranjpye RN (2000) Epidemiology and pathogenesis of *Vibrio vulnificus*. Microbes Infect 2:177–188

Stroud RK, Roffe TJ (1979) Causes of death in marine mammals stranded along the Oregon coast. J Wildl Dis 15: 91–97

Tang YW, Stratton CW (2010) *Staphylococcus aureus*: an old pathogen with new weapons. Clin Lab Med 30:179–208

Tello A, Austin B, Telfer TC (2012) Selective pressure of antibiotic pollution on bacteria of importance to public health. Environ Health Perspect 120:1100–1106

Tester PA, Geesey ME, Guo C, Paerl HW, Millie DF (1995) Evaluating phytoplankton dynamics in the Newport River estuary (North Carolina, USA) by HPLC-derived pigment profiles. Mar Ecol Prog Ser 124:237–245

Thayer GW, Wolfe DA, Williams RB (1975) The impact of man on seagrass systems. Am Sci 63:288–296

Thompson JR, Marcelino LA, Polz MF (2005) Diversity, sources, and detection of human bacterial pathogens in the marine environment. In: Belkin S, Colwell RR (eds) Oceans and health: pathogens in the marine environment. Springer, New York, NY, p 29–68

Tison DL, Kelly MT (1986) Virulence of *Vibrio vulnificus* strains from marine environments. Appl Environ Microbiol 51:1004–1006

USDOC/NOAA/NOS (US Department of Commerce/National Oceanic and Atmospheric Administration/National Ocean Service)(2008) United States Coast Pilot 4, Atlantic Coast: Cape Henry to Key West. Office of Coast Survey, NOAA, Silver Spring, MD

USEPA (US Environmental Protection Agency) (2008) National Estuary Program 2004–2006 Implementation Review Report. USEPA, Washington, DC

VKM (Vitenskapskomiteen for mattrygghet) (2011) Human pathogens in marine mammal meat. Norwegian Scientific Committee for Food Safety, Oslo

Waltzek TB, Cortes-Hinojosa G, Wellehan JF, Gray GC (2012) Marine mammal zoonoses: a review of disease manifestations. Zoonoses Public Health

Ward JR, Lafferty KD (2004) The elusive baseline of marine disease: Are diseases in ocean ecosystems increasing? PLoS Biol 2:e120

Waring GT, Josephson E, Maze-Foley K, Rosel PE (eds) (2011) U.S. Atlantic and Gulf of Mexico marine mammal stock assessments–2010. NOAA Tech Memo NMFS NE 219. National Marine Fisheries Service, Woods Hole, MA. Available at www.nefsc.noaa.gov/nefsc/publications/

Weese JS (2010) Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J 51:233–244

Wells RS (2009) Learning from nature: bottlenose dolphin care and husbandry. Zoo Biol 28:635–651

Wells RS, Rhinehart HL, Hansen LJ, Sweeney JC and others (2004) Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. EcoHealth 1:246–254

Wells RS, Tornero V, Borrell A, Aguilar A and others (2005) Integrating life-history and reproductive success data to examine potential relationships with organochlorine compounds for bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. Sci Total Environ 349:106–119

Whatmore AM, Dawson C, Groussaud P, Koylass MS and others (2008) Marine mammal *Brucella* genotype associated with zoonotic infection. Emerg Infect Dis 14:517

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