Antibiotic Susceptibility Patterns of Bacteria Isolated from Cetaceans Stranded in the Philippines

Marie Christine M. Obusan,[1,2] Lemnuel V. Aragones,[2]
Windell L. Rivera[1,3] and Maria Auxilia T. Siringan[3]

[1]*Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, 1101*
E-mail: mmobusan@up.edu.ph
[2]*Institute of Environmental Science and Meteorology, College of Science,*
University of the Philippines, Diliman, Quezon City, 1101
[3]*Natural Sciences Research Institute, College of Science, University of the Philippines, Diliman, Quezon City, 1101*

Abstract

As sentinels, cetaceans provide the link between ocean and human health by indicating the emergence of disease threats, pathogenic microorganisms, and antimicrobial resistance. Cetaceans that stranded in the Philippines from January 2012 to March 2013 were screened for antibiotic resistance. The susceptibility patterns of *Achromobacter xylosidans*, *Acinetobacter* spp., *Aeromonas* spp., *Burkholderia cepacia*, *Enterococcus faecalis*, *Enterococcus* sp., *Moraxella* sp., *Proteus mirabilis*, *Providencia stuartii*, *Rhizobium radiobacter*, *Serratia marcescens*, *Sphingomonas* sp., *Staphylococcus epidermidis*, and *Vibrio* spp. isolated from nine cetaceans representing the species *Globicephala macrorhynchus*, *Kogia sima*, *Kogia breviceps*, *Stenella attenuata*, and *Steno bredanensis* were determined using a selection of antibiotics. More than half of these isolates showed either single or multiple resistances to the antibiotics tested. Development of antibiotic resistance in a rough-toothed dolphin (*S. bredanensis*) was observed after the administration of antibiotics during the course of rehabilitation. The findings of the study can serve as a basis for providing medical intervention during management and rehabilitation of stranded cetaceans, and have implications relevant to zoonotic transmission of potentially pathogenic or antibiotic resistant bacteria from cetaceans to other marine species and humans. Investigating stranded cetaceans for the occurrence of bacteria and antibiotic resistance is one way of monitoring the health of their counterparts in the wild, offering insights as to the possible involvement of bacterial infections in local stranding events.

Key Words: antibiotic resistance, bacteria, stranding events, cetaceans, Philippines

Introduction

The recognition of One Health brings together interdisciplinary approaches to explore the interconnections among the health of humans, animals, and environments (Gibbs, 2014). Marine mammals play a crucial role in understanding these interconnections, acting as sentinels for indicating the emergence or resurgence of diseases, presence of potentially pathogenic microorganisms, and antimicrobial resistance in the marine environment. Although considered prime sentinels of human health, marine mammal species still suffer from lack of information on their microbiota and associated diseases (Moore, 2008; Bossart, 2011). This is said to be the consequence of limited data on the dynamics of infectious agents in marine ecosystems, lack of marine mammal health monitoring, and historical focus on domestic rather than wildlife health, with an emphasis on farm animal economy rather than conservation (Acevedo-Whitehouse et al., 2003; Gulland & Hall, 2007).

The Philippines is home to a diverse array of 30 marine mammal species, 28 of which are cetaceans (Aragones, 2013). Their stranding events (both single- and multi-animal) have an increasing trend in recent years, which may be related to human interactions including fisheries interactions, species abundance, and improved survey effort (Aragones et al., 2010, 2017; Obusan et al., 2016). While it is often difficult to ascertain the specific cause of a stranding event, investigating conditions relevant to diseases and infections may help in delimiting the probable factors causing the event.

Some cetacean stranding events have been associated with infections by resident or opportunistic bacteria occurring during or after periods of immune suppression (e.g., Buck et al., 1987, 1991; Parsons & Jefferson, 2000; Kreuder et al.,

2003; Pereira et al., 2008; McFee & Lipscomb, 2009). Antimicrobial susceptibility patterns have been described for populations and individuals of Atlantic bottlenose dolphins (*Tursiops truncatus*), Pacific bottlenose dolphins (*Tursiops aduncus*), Risso's dolphins (*Grampus griseus*), beluga whales (*Delphinapterus leucas*), sea otters (*Enhydra lutris*), and pinniped species (Wong et al., 2002; Greig et al., 2007; Stoddard et al., 2008; Rose et al., 2009; Schaefer et al., 2009; Brownstein et al., 2011; Wallace et al., 2013). Strains of bacteria resistant to multiple antibiotics that are used for human and animal treatments were isolated from these animals, and some of these bacteria were recognized by the American Biological Safety Association (ABSA) as human pathogens (Bogomolni et al., 2008). Zoonotic transmission of antibiotic resistant bacteria between marine mammals and humans can occur during stranding response, rehabilitation, captive management, ecotourism activities, and research work. A case in point is the colonization of dolphins and walrus in a marine park by methicillin-resistant *Staphylococcus aureus* (MRSA) found to be of a human strain implicated in hospital and community infections (Faires et al., 2009). In general, there is evidence of pathogenicity of the following human pathogens in marine mammals: *Aeromonas hydrophila*, *Candida albicans/glabrata*, *Edwardsiella tarda*, *Erysipelothrix rhusiopathiae*, *Klebsiella pneumoniae*, *Leptospira* spp., *Pasteurella multocida*, *Pseudomonas* spp., *Staphylococcus aureus* (especially coagulase positive strains), *Shewanella* spp., beta-hemolytic enterococci/streptococci, and several species of *Vibrio* (Buck et al., 2006; Cameron et al., 2008; Waltzek et al., 2012).

In this study, bacteria isolated from cetaceans stranded in the Philippines were tested for antibiotic susceptibility. Although the presence of these bacteria and their associated antibiotic resistances do not necessarily explain the causation of stranding cases, they indicate sources of biological pollution in relation to environmental factors to which the cetaceans are exposed.

Methods

Twenty-three bacterial isolates derived from nine cetaceans that stranded in different parts of the Philippines from January 2012 to March 2013 (Figure 1) and identified as *Achromobacter* spp., *Aeromonas* spp., *Burkholderia cepaci*a, *Enterococcus* sp., *Moraxella* sp., *Proteus mirabilis*, *Providencia stuartii*, *Rhizobium radiobacter*, *Serratia marcescens*, *Sphingomonas* sp., *Staphylococcus epidermidis*, and *Vibrio* spp. were tested for antibiotic susceptibility. The isolation,

culture, phenotypic, and molecular identifications of these bacteria were described in Obusan et al. (2015). The response, rehabilitation, and collection of biological materials during stranding events were carried out in collaboration with the Philippine Marine Mammal Stranding Network (PMMSN) and the Bureau of Fisheries and Aquatic Resources (BFAR), Department of Agriculture (DA). Swab samples were obtained based on (1) animal disposition (e.g., live, dead, or stressed), (2) physical preservation (i.e., based on the expanded version of the code system established by the Smithsonian Institution's Marine Mammal Events Program; Geraci & Lounsbury, 2005), and (3) sampling conditions (e.g., handling limitations that may result in sample contamination). The physical preservation codes used were Code 1 – live animal, Code 2 – fresh (carcass in good condition), Code 3 – fair (decomposed but organs basically intact), Code 4 – poor (advanced decomposition), and Code 5 – mummified or skeletal remains.

In the laboratory, bacterial suspensions of 18 to 24 h pure cultures were prepared using 0.1% peptone water standardized to 0.5 McFarland (approximately 1.5×10^8 CFU/mL). Inocula from the suspension were uniformly swabbed (three times with 60° rotations) onto MHA (Mueller-Hinton Agar) plates. MHA cultures were incubated at $35 \pm 2°C$ (Cavalieri et al., 2005). Antimicrobial controls included discs representing ten classes of antibiotics that were previously used to evaluate bacterial resistance in bottlenose dolphins (Greig et al., 2007) as well as other marine mammals (Bogomolni et al., 2008; Stoddard et al., 2008): penicillins (amoxicillin+clavulanic [AMC] 30 µg), aminoglycosides (amikacin [AMK] 30 µg), quinolones and fluoroquinolones (ciprofloxacin [CIP] 5 µg), macrolides (erythromycin [ERY] 15 µg), folate pathway inhibitors (sulphamethoxazole/trimethoprim [SXT] 25 µg), cephems (cefazoline [CFZ] 30 µg), carbapenems (meropeneme [MEM] 10 µg), tetracyclines (tetracycline [TE] 30 µg), phenicols (chloramphenicol [CHL] 30 µg), and nitrofurantoins (nitrofurantoin [F] 300 µg).

Zones of inhibition in test and control replicates were measured in millimetres (mm) by electronic caliper. All stranded cetaceans sampled were not given any antibiotic at the time of swab collection, except in the case of the second sample collection from a rough-toothed dolphin (*Steno bredanensis*) that stranded in Candelaria, Zambales. The animal received antibiotic treatment during rehabilitation and additional bacteria (*Achromobacter xylosidans* and *Enterococcus faecalis*) were isolated from the lungs during necropsy. These additional isolates were identified through VITEK 2 system (Biomeriuex, Craponne, France), and their susceptibility (based on MIC values [µg/mL]) to the following antibiotics was
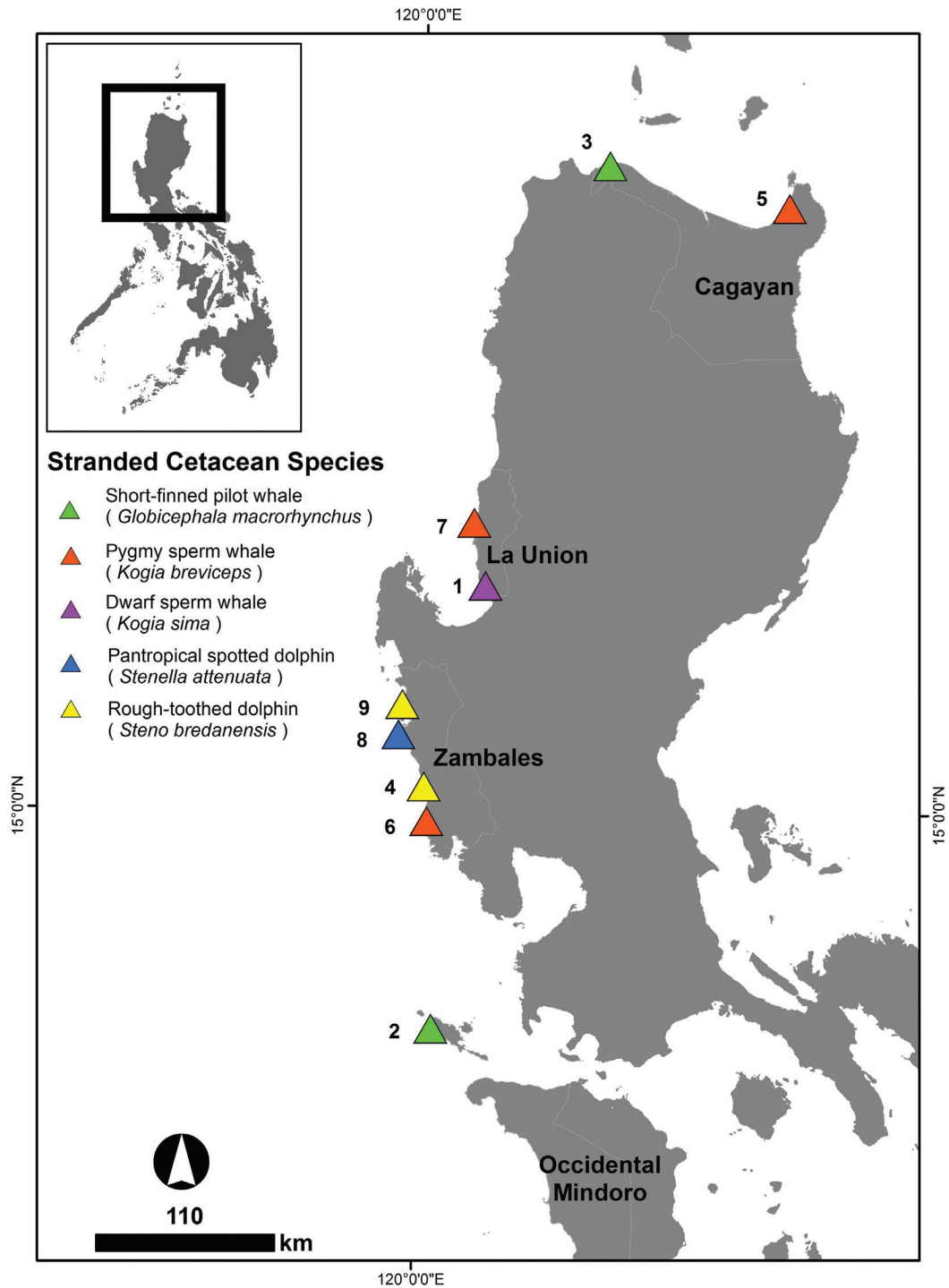
Figure 1. Stranding sites in the Philippines of cetaceans sampled in the study with numerical codes (Strander Codes) corresponding to dates of stranding events: (1) 7 February 2012, (2) 16 March 2012, (3) 9 April 2012, (4) 7 June 2012, (5) 12 September 2012, (6) 16 September 2012, (7) 17 September 2012, (8) 7 November 2012, and (9) 14 January 2013

tested: amikacin (AMK), cefepime (Cpe), ceftazidime (Caz), ceftriaxone (Cax), ciprofloxacin (CIP), clindamycin (Cd), colistin (CL), daptomycin (Dap), erythromycin (ERY), gentamicin (Gm), gentamicin (Gm) high level (synergy), imipenem (Imp), levofloxacin (Lvx), linezolid (Lzd), moxifloxacin (Mox), piperacillin/tazobactam (PT), streptomycin (S) high level (synergy), tetracycline (TE), sulfamethoxazole/trimethoprim (SXT), and vancomycin (Va). The reaction of each isolate (in terms of diameter of zone of inhibition) to antibiotics were interpreted as S (susceptible; growth of isolate inhibited *in vitro* with a high likelihood of therapeutic success), I (intermediate; growth of isolate inhibited *in vitro* but therapeutic success is uncertain), and R (resistant; growth of isolate not inhibited *in vitro* with a high likelihood of therapeutic failure) (Rodloff et al., 2008). Interpretations were based on criteria published by the Clinical Laboratory Standards Institute (CLSI) (2002) for bacteria isolated from animals (NCCLS M31-A2), and for bacteria with breakpoints reported in other CLSI documents, such as CLSI M100-S17 (CLSI, 2007) and CLSI M100-S24 (CLSI, 2014), and literature (Indian Council of Medical Research [IMCR], 2009).

Results

Twenty-three bacteria representing 13 consensus genera (*Achromobacter*, *Acinetobacter*, *Aeromonas*, *Burkholderia*, *Enterococcus*, *Moraxella*, *Proteus*, *Providencia*, *Rhizobium*, *Serratia*, *Sphingomonas*, *Staphylococcus*, and *Vibrio*) based on previous phenotypic and genotypic identifications were tested for susceptibility to selected antibiotics. These bacteria were isolated from nine cetacean individuals belonging to the following species: short-finned pilot whale (*Globicephala macrorhynchus*), pygmy sperm whale (*Kogia breviceps*), dwarf sperm whale (*Kogia sima*), rough-toothed dolphin, and spinner dolphin (*Stenella longirostris*). Of these bacteria, 21 were isolated at the time when there was no antibiotic given to the stranders. Among these 21 isolates, 14 (67%) exhibited single or multiple resistances to AMK, CFZ, CHL, ERY, MEM, F, TE, and SXT (Table 1). Three isolates were resistant to 50% of antibiotics: *P. mirabilis* (S8H) isolated from the heart of an adult male pygmy sperm whale that stranded in San Fernando, La Union, on 17 September 2012; and *Vibrio* spp. (S9BH1 and S9BH2) isolates from the blowhole of a subadult male pantropical spotted dolphin (*Stenella attenuata*) that stranded in Palauig, Zambales, on 7 November 2012.

On the other hand, the following isolates were not resistant to any antibiotic: (1) *Moraxella* sp. (SIG) from the genital area of an adult male dwarf sperm whale that stranded in Santo Tomas,

La Union, on 7 February 2012; (2) *Aeromonas* sp. (S2LU) from the lung of a subadult female short-finned pilot whale that stranded in Occidental Mindoro on 16 March 2012; (3) *R. radiobacter* (S4A) from the anal area of a subadult male rough-toothed dolphin that stranded in Cabangan, Zambales, on 7 June 2012; (4) *Vibrio* sp. (S7BH2) from the blowhole of the adult male pygmy sperm whale that was rescued from San Antonio, Zambales, on 16 September 2012; and three bacteria cultured from an adult male rough-toothed dolphin that stranded in Candelaria, Zambales, on 14 January 2013, namely (5) *Providencia* sp. (S10A1) isolate from the anal area, and (6) *Providencia* sp. (S10BH1) and (7) *Vibrio* sp. (S10BH2) isolates, both from the blowhole (Table 2). Based on the number of susceptible isolates, the order of antibiotic effectiveness was CIP and MEM > AMC > SXT > AMK > TE > F and CHL > CFZ > ERY. Observed resistances to antibiotics were not limited to bacteria isolated from body parts (e.g., anal and blowhole areas) exposed to the environment as these were also exhibited by bacteria that were isolated from internal tissues (e.g., lungs and liver), indicating that the bacteria were not contaminants from the environment.

In the case of a rough-toothed dolphin that stranded in Candelaria, Zambales, on 14 January 2013, additional bacteria were isolated after an antibiotic regimen. The animal was sustained for 24 h at the BFAR facility in Masinloc, Zambales, after which it was transferred to a rehabilitation site in Subic, Zambales, on 15 January 2013. The cetacean, based on preliminary physical assessment and dosage requirement (i.e., with body weight of approximately 90 kg), was given (through a feeding tube) the following antibiotics until 28 January 2013: amoxicillin (10 mg/kg po bid) and enrofloxacin (10 mg/kg po bid) starting 15 January 2013 onwards; nystatin (500,000 iu po bid) starting 18 January 2013 onwards; and clindamycin (7.7 mg/kg po bid) starting 20 January 2013 onwards. Amoxicillin was discontinued and replaced with cefuroxime (10 mg/kg po bid) on 28 January 2013 (M. B. Flores & L. J. Suarez, pers. comm., January 2013). Later during its rehabilitation, this animal was assessed to have a worsening infection as inferred from blood cell counts and transaminase production. The dolphin died after 2 wks of care by the facility's veterinarians and PMMSN volunteers. During necropsy, swab samples were obtained from the lungs, and additional isolates (identified as *A. xylosidans* and *E. faecalis*) were tested for susceptibility. Early isolates from this animal included two *P. stuartii* (S10A1 and S10BH1) from anal and blowhole areas, and *Vibrio* sp. (S10BH2) from the blowhole. Common susceptibility of all these isolates

Table 1. Resistance profiles of bacterial isolates from stranded cetaceans without (or prior to) antibiotic treatment; isolates with no profile entries are either susceptible or intermediate to antibiotics tested.

Strander Code	Cetacean species	Sex and age group	Physical condition code[a]	Source of swab sample	Isolated bacteria (Code)	Resistance profile[c]
1	*Kogia sima* (Dwarf sperm whale)	Male; adult	Fresh	Genital area	*Moraxella* sp. (SIG)	
2	*Globicephala macrorhynchus* (Short-finned pilot whale)	Female; subadult	Fresh	Lung	*Aeromonas* sp. (S2LU)	
				Skin lesion	*Vibrio* sp. (S2LE1)	SXT
					Burkholderia cepacia (S2LE2)	CFZ, ERY
3	*G. macrorhynchus*	Male; adult	Fresh	Blowhole	*Aeromonas* sp. (S3BH)	AMK
				Liver	*Enterococcus* sp. (S3LV)	AMK, CFZ, ERY, MEM
4	*Steno bredanensis* (Rough-toothed dolphin)	Male; subadult	Live	Anal	*Rhizobium radiobacter* (S4A)	
5	*Kogia breviceps* (Pygmy sperm whale)	Female; adult	Live	Blowhole	*Sphingomonas* sp. (S5BH1)	SXT
					Staphylococcus epidermidis (S5BH2)	ERY
					Serratia marcescens (S5BH3)	CFZ
					Vibrio sp. (S5BH4)	AMC, CFZ
6	*K. breviceps*	Male; adult	Live	Blowhole	*Vibrio* sp. (S7BH1)	CFZ
7	*K. breviceps*	Male; adult	Fresh	Heart	*Proteus mirabilis* (S8H)	CFZ, CHL, ERY, SXT, TE
				Liver	*Acinetobacter* sp. (S8LV)	AMK, CFZ, F
				Lung	*Acinetobacter* sp. (S8LU)	CFZ, CHL, ERY, F
8	*Stenella attenuata* (Spinner dolphin)	Male; subadult	Live	Blowhole	*Vibrio* sp. (S9BH1)	CFZ, CHL, ERY, F, TE
					Vibrio sp. (S9BH2)	CFZ, CHL, ERY, F, TE
9	*S. bredanensis*[b]	Male; adult	Live	Anal	*Providencia stuartii* (S10A1)	
				Blowhole	*P. stuartii* (S10BH1)	
					Vibrio sp. (S10BH2)	

[a]Condition codes at the time of sampling based on Smithsonian Institution's Marine Mammal Events Program (Geraci & Lounsbury, 2005)

[b]Additional bacteria isolates and sensitivity profiles presented in Table 3

[c]*Antibiotics*: AMK = amikacin, AMC = amoxicillin+clavulanic acid, CFZ = cefazoline, CHL = chloramphenicol, CIP = ciprofloxacin, ERY = erythromycin, MEM = meropenem, F = nitrofurantoin, TE = tetracycline, and SXT = sulfamethoxazole/trimethoprim

Table 2. Susceptibility profiles of bacteria isolated from stranded cetaceans without (or prior to) antibiotic treatment

Bacterial isolates	Source cetacean species	Strander Code	Isolate Code	AMK	AMC	CFZ	CHL	CIP	ERY	MEM	F	SXT	TE
Acinetobacter sp.	*K. breviceps*	7	S8LU	I	S	R	R	S	R	S	R	I	I
Acinetobacter sp.	*K. breviceps*	7	S8LV	R	S	R	I	S	I	S	R	I	S
Aeromonas sp.	*G. macrorhynchus*	2	S2LU	S	S	S	S	S	I	S	S	S	S
Aeromonas sp.	*G. macrorhynchus*	3	S3BH	R	S	I	S	S	I	S	S	S	S
Burkholderia cepacia	*G. macrorhynchus*	2	S2LE(2)	S	S	R	S	S	R	S	S	S	I
Enterococcus sp.	*G. macrorhynchus*	3	S3LV	R	S	R	S	S	R	R	I	S	S
Moraxella sp.	*K. sima*	1	S1G	S	S	S	I	S	I	S	S	S	S
Proteus mirabilis	*K. breviceps*	7	S8H	S	S	R	R	S	R	S	I	R	R
Provencia stuartii	*S. bredanensis*	9	S10A1	S	S	S	S	S	I	S	S	S	S
P. stuartii	*S. bredanensis*	9	S10BH1	S	S	S	S	S	I	S	S	S	S
Rhizobium radiobacter	*S. bredanensis*	4	S4A	S	S	S	S	S	S	S	S	S	S
Serratia marcescens	*K. breviceps*	5	S5BH3	S	S	R	S	I	I	S	S	S	S
Sphingomonas sp.	*K. breviceps*	5	S5BH1	S	S	S	S	S	S	S	S	R	S
Staphylococcus epidermidis	*K. breviceps*	5	S5BH2	S	S	S	S	S	R	S	S	S	S
Vibrio sp.	*G. macrorhynchus*	2	S2LE(1)	S	S	S	S	S	S	S	S	R	S
Vibrio sp.	*K. breviceps*	5	S5BH4	S	R	R	S	S	I	S	S	S	S
Vibrio sp.	*K. breviceps*	6	S7BH1	S	S	R	S	S	I	S	S	S	S
Vibrio sp.	*K. breviceps*	6	S7BH2	S	S	S	S	S	I	S	S	S	S
Vibrio sp.	*S. attenuata*	8	S9BH1	S	I	R	R	S	R	S	R	S	R
Vibrio sp.	*S. attenuata*	8	S9BH2	S	I	R	R	S	R	S	R	S	R
Vibrio sp.	*S. bredanensis*	9	S10BH2	S	S	I	S	S	I	S	S	S	S

S = susceptible, I = intermediate, and R = resistant

a*Antibiotics:* AMK = amikacin, AMC = amoxicillin+clavulanic acid, CFZ = cefazoline, CHL = chloramphenicol, CIP = ciprofloxacin, ERY = erythromycin, MEM = meropenem, F = nitrofurantoin, TE = tetracycline, and SXT = sulfamethoxazole/trimethoprim

Table 3. Susceptibility test results for additional isolates from the rehabilitated rough-toothed dolphin (*Steno bredanensis*) (Strander Code 9)

Isolate identification	Source of swab sample	Sensitivity to antibiotics		
		Susceptible	Intermediate	Resistant
A. xylosidans	Lung	AMK Caz CL MEM Imp PT SXT	Cax CIP	Cpe Gm
E. faecalis	Lung	ERY Dap Gm high level (synergy) Lvx Lzd Mox S high level (synergy) TE Va	CIP	Cd

Antibiotics tested: AMK = amikacin, Cpe = cefepime, Caz = ceftazidime, Cax = ceftriaxone, CIP = ciprofloxacin, Cd = clindamycin, CL = colistin, Dap = daptomycin, ERY = erythromycin, Gm = gentamicin, Gm high level = gentamicin high level (synergy), Imp = imipenem, Lvx = levofloxacin, Lzd = linezolid, MEM = meropenem, Mox = moxifloxacin, PT = piperacillin/ tazobactam, S = streptomycin high level (synergy), TE = tetracycline, SXT = sulfamethoxazole/trimethoprim, and Va = vancomycin

to AMK, MEM, TE, and SXT was found. The early isolates were not resistant to any antibiotic, whereas those recovered during the necropsy were already resistant to some antibiotics (Table 3).

Discussion

More than half of the bacteria isolated from stranded cetaceans were resistant to the antibiotics tested. This is in contrast to the minimal evidence of antimicrobial resistance and absence of strains with unusual or clinically significant multiple-drug resistance patterns in sea otters from California and Alaska (Brownstein et al., 2011). Most of the resistances determined in this study were against cefazoline (27%) and erythromycin (19%), although the mechanism by which this resistance developed is unknown. In comparison, the most common resistance was against penicillin among isolates from the wild bottlenose dolphins of Florida and South Carolina, USA (Greig et al., 2007). Furthermore, no isolate was resistant to ciprofloxacin, a second-generation fluoroquinolone antibiotic. As the growth of isolates (95%) were inhibited by this antibiotic *in vitro*, there is high likelihood of therapeutic success if ciprofloxacin were used to treat bacterial infections in cetaceans during their stranding events or rehabilitation. This may also be supported by parallel findings such as those of Rose

et al. (2009) in which ciprofloxacin was found to be 99% effective against bacteria isolated from marine vertebrates (including marine mammals) along the northeastern coast of the United States. Likewise, the U.S. Navy Marine Mammal Program reported low incidence of ciprofloxacin-resistant bacteria (2.5% out of 688 bacterial isolates from 1988 to 2001) from free-ranging trained cetaceans (Wong et al., 2002). Conversely, significant increases in fluoroquinolone resistances were observed in some bacteria isolated from stranded pinnipeds in the Northwest Atlantic (Wallace et al., 2013). Based on these limited reports, it can be inferred that ciprofloxacin resistance is not yet prevalent among bacteria found in studied cetacean populations as compared to pinnipeds. This has implications for the treatment of cetaceans suffering from bacterial infections, especially since ciprofloxacin-resistant *E. coli* was associated with morbidity in two dolphins in the U.S. (Wong et al., 2002).

P. mirabilis (isolated from the pygmy sperm whale) and *Vibrio* isolates (from the spinner dolphin) were resistant to five antibiotics. *Proteus* was also reported by DebMandal et al. (2011) to demonstrate the highest percentage of resistance among bacteria isolated from environmental samples, with one of the strains, *P. mirabilis*, being resistant to all the antibiotics used except gentamicin, although an explanation for this resistance

was not discussed. As for *Vibrio* spp., multiple resistances were also observed in those isolated from marine organisms (Smaldone et al., 2014).

The administration of antibiotics in stranded cetaceans qualified for rehabilitation is usually done even at early periods to address both ongoing and secondary infections (e.g., commonly pneumonia) that may result from stress, malnutrition, and immunosuppression (Barnett, 2002; Flores et al., 2013). However, prolonged antibiotic use is said to increase the risk of precipitating antimicrobial resistance in the animal's normal microflora (Barnett, 2002). It can be noted that bacteria isolated prior to antibiotic treatment of the rehabilitated rough-toothed dolphin were not resistant to any antibiotic; however, bacteria that were isolated after antibiotic treatment were already resistant to some antibiotics. In particular, *E. faecalis* was already resistant to clindamycin, one of the antibiotics given. Also, the latter isolates had an intermediate reaction to ciprofloxacin, the antibiotic with the most inhibitory effect on the growth of all other isolates tested.

A. xylosidans is typically isolated from pulmonary sources and clinically implicated as a causative agent of pneumonia in immunosuppressed individuals (Claassen et al., 2011). Its isolation from the lung of the rough-toothed dolphin is not surprising as it is reportedly common in sea water (Yin et al., 2013). It is likely to be an opportunistic pathogen given the prospect of immunosuppression brought about by the stress arising from the stranding event and rehabilitation setting. On the other hand, *E. faecalis* has a wide range of niches and is known to cause urinary tract infections, hepatobiliary sepsis, endocarditis, neonatal sepsis, surgical wound infection, bacteremia, and other nosocomial infections (Fisher & Phillips, 2009). This species was implicated in pulmonary hypertension syndrome (PHS) among broiler chickens and, as such, was suggested to be used as a model for investigating the involvement of bacterial insult in PHS among humans (Tankson et al., 2001). PHS is known to cause ascites ("water belly") in chickens, a debilitating cardiopulmonary disease which is characterized by physical and physiological changes in the heart and lungs (Julian et al., 1986; Julian & Boulianne, 1988). The rough-toothed dolphin in which *E. faecalis* was isolated had high levels of aspartate aminotransferases (AST) (M. B. Flores, pers. comm., January 2013), a known early indicator of myocardial damage in both humans and chickens (Tankson et al., 2002). However, this was not further investigated in relation to the likelihood of *E. faecalis*-induced pulmonary hypertension in this animal, an aspect worthy of future investigation in relation to the medical management of

dolphins during long-term care or rehabilitation. Also, as this particular isolate was susceptible to vancomycin, it is not a concern for acquired community antibiotic resistance wherein the spread of vancomycin-resistant enterococci (VRE) due to antibiotic misuse is a major problem across the globe (Barbosa & Levy, 2000).

Bacteria isolated from pygmy sperm whales yielded the highest percentage resistance to antibiotics tested. This finding raises questions on the extent of antimicrobial resistance in marine habitats since this species has a deep-water distribution and limited ecological information (Bloodworth & Odell, 2008). The type of bacteria found in this species, as well as the associated antibiotic resistances, may be related to sources of coastal contamination and sinks of drug-resistant genes in the pelagic zone. Multiple resistances were also reported by Bogomolni et al. (2008) for bacteria (e.g., *Acinetobacter calcoaceticus-baumannii* resistant to ampicillin, ceftazime, cephalothin, and chloramphenicol) isolated from a Cuvier's beaked whale (*Ziphius cavirostris*), another deep-water species. However, in the present study, resistance to any antibiotic was not observed among the isolates from the dwarf sperm whale. While this is another deep-diving species, the absence of resistant isolates as compared to their presence in pygmy sperm whales, in particular, may be attributed to differences in exposure to potential sources and mechanisms of antimicrobial resistance development. It is important to note that information on the migration patterns of kogiids in the Philippines is lacking. In general, an extensive migration of dwarf sperm whales is not proposed, while a seasonal migration of pygmy sperm whales is hypothesized based on stranding data (i.e., more strandings occur in winter in U.S. and Europe since the dolphins migrate to the open sea during summer) (Culik, 2004).

How and why cetacean species acquire antimicrobial resistance in the marine environment remains poorly understood. Antimicrobial resistance has been attributed to the use of antibiotics for therapeutic, growth-promoting, and prophylactic purposes in different areas of animal production such as in aquaculture (Coker et al., 2011; Buschmann et al., 2012). For instance, vibrios resistant to oxytetracycline, furazolidone, oxolinic acid, and chloramphenicol were isolated from shrimp ponds where antimicrobials have been used (Tendencia & de la Peña, 2001). Significant increases in the number of bacteria and their antimicrobial-resistant fractions to oxytetracycline, oxolinic acid, and florfenicol were also detected in sediments of a salmon aquaculture site (Buschmann et al., 2012). These should pose alarm in areas where strict surveillance and guidance

on antibacterial use is lacking. As an example, Holmström et al. (2003) reported the inadvertent use of antibiotics in Thailand by shrimp farmers without proper information on efficient and safe application practices. Similar information is not yet available in the Philippines at the time of this study. Such information is vital to investigating the spread of antimicrobial resistance in aquatic environments given that local aquaculture is ranked eleventh globally for its 0.791 million metric tons of production (BFAR, 2012). Aside from aquaculture, land-based discharges to aquatic ecosystems are known sources of antibiotics and antimicrobial resistance. It has been proven that sewage waters are sources of resistances against ciprofloxacin, tetracycline, ampicillin, trimethoprim, erythromycin, and sulfamethoxazole/trimethoprim (Costanzo et al., 2005). These result in an emergence of antibiotic-resistant bacteria in water bodies, an increase of antibiotic resistance in aquatic species, the transfer of resistance determinants to pathogens of land animals and humans, and alterations of the bacterial flora composition in sediments and the water column (Cabello, 2006).

More widespread antibiotic resistance in stranded as compared to free-ranging marine mammals were reported by Rose et al. (2009), and it remains unknown whether this has something to do with the tendency of immunosuppressed individuals to strand as compared to healthy ones. This cannot be confirmed by the present study as the samples used were comprised only of stranded individuals. Also, associations between increased prevalence of antibiotic resistant bacteria in marine mammals and proximity to human activities have been strongly suggested (Stoddard et al., 2008; Miller et al., 2009). Although such associations are not yet explored in the Philippines, the antibiotic susceptibility patterns of bacteria isolated from sampled cetaceans provide clues on possible contributing factors (e.g., antibiotic classes used in local aquaculture activities) for the development and extent of antimicrobial resistance in cetacean populations and their habitats. As this is the first time that stranded cetaceans in the country were investigated for antibiotic susceptibility, the baseline information generated by the study finds utility in the work of providing animal care during stranding events and rehabilitation as well as in monitoring the health of these ocean sentinels.

Conclusion
Antibiotic resistance in marine mammals is an urgent concern, not only because these sentinels can serve as vectors of antibiotic resistant bacteria and the diseases they cause, but more so because they can indicate sources and mechanisms of antimicrobial resistance development in the marine environment. This study provided the first report on antibiotic susceptibility patterns of bacteria isolated from nine stranded individuals representing five cetacean species that stranded in the Philippines. The findings have implications for providing medical intervention during cetacean stranding response and rehabilitation, explaining circumstances surrounding stranding events, and preventing zoonotic transmission of potentially pathogenic or antibiotic resistant bacteria from cetaceans to humans and other species.

Literature Cited

Acevedo-Whitehouse, K., de la Cueva, H., Gulland, F. M. D., Aurioles-Gamboa, D., Arellano-Carbajal, F., & Suarez-Guemes, F. (2003). Evidence of *Leptospira interrogans* infection in California sea lion pups from the Gulf of California. *Journal of Wildlife Diseases*, *39*(1), 145-151. https://doi.org/10.7589/0090-3558-39.1.1455

Aragones, L. V. (2013). An overview of Philippine marine mammals. In L. V. Aragones, G. E. Laule, & B. G. Espinos (Eds.), *Marine mammal stranding response manual: A guide for the rescue, rehabilitation, and release of stranded cetaceans and dugong in the Philippines* (pp. 6-9). Subic Bay Freeport, Philippines: Wildlife In Need (WIN) and Ocean Adventure.

Aragones, L .V., Laggui, H. L. M., & Amor, A. K. S. (2017). *The Philippine marine mammal strandings from 2005 to 2016*. Quezon City: Philippine Marine Mammal Stranding Network (PMMSN).

Aragones, L. V., Roque, M. A., Flores, M. B., Encomienda, R. P., Laule, G. E., Espinos, B. G., . . . Braun, R. C. (2010). The Philippine marine mammal strandings from 1998 to 2009: Animals in the Philippines in peril? *Aquatic Mammals*, *36*(3), 219-233. https://doi.org/10.1578/AM.36.3.2010.219

Barbosa, T. M., & Levy, S. B. (2000). The impact of antibiotic use on resistance development and persistence. *Drug Resistance Updates*, *3*, 303-311. https://doi.org/10.1054/drup.2000.0167

Barnett, J. (2002). *Evaluation of rehabilitation as an option for stranded dolphins, porpoises, and whales*. Retrieved from www.bdmlr.org.uk/uploads/documents/reports/rehabilitation-report.pdf

Bloodworth, B. E., & Odell, D. K. (2008). *Kogia breviceps* (Cetacea: Kogiidae). *Mammalian Species*, *819*, 1-12. https://doi.org/10.1644/819.1

Bogomolni, A. L., Gast, R. J., Ellis, J. C., Dennett, M., Pugliares, K. R., Lentell, B. J., & Moore, M. J. (2008). Victims or vectors: A survey of marine vertebrate zoonoses from coastal waters of the Northwest Atlantic. *Diseases of Aquatic Organisms*, *81*, 13-38. https://doi.org/10.3354/dao01936

Bossart, G. D. (2011). Marine mammals as sentinel species for oceans and human health. *Veterinary Pathology*, *48*(3), 676-690. https://doi.org/10.1177/0300985810388525

Brownstein, D., Miller, M. A., Oates, S. C., Byrne, B. A., Jang, S., Murray, M. J., . . . Jessup, D. A. (2011). Antimicrobial susceptibility of bacterial isolates from sea otters (*Enhydra lutris*). *Journal of Wildlife Diseases*, *47*(2), 278-292. https://doi.org/10.7589/0090-3558-47.2.278

Buck, J. D., Shepard, L. L., & Spotte, S. (1987). *Clostridium perfringens* as the cause of death of a captive Atlantic bottlenose dolphin (*Tursiops truncatus*). *Journal of Wildlife Diseases*, *23*(3), 488-491. https://doi.org/10.7589/0090-3558-23.3.488

Buck, J. D., Wells, R. S., Rhinehart, H. L., & Hansen, L. J. (2006). Aerobic microorganisms associated with free-ranging bottlenose dolphins in coastal Gulf of Mexico and Atlantic Ocean waters. *Journal of Wildlife Diseases*, *42*(3), 536-544. https://doi.org/10.7589/0090-3558-42.3.536

Buck, J. D., Overstrom, N. A., Patton, G. W., Anderson, H. F., & Gorzelany, J. F. (1991). Bacteria associated with stranded cetaceans from the northeast USA and southwest Florida Gulf coasts. *Diseases of Aquatic Organisms*, *10*, 147-152. https://doi.org/10.3354/dao010147

Bureau of Fisheries and Aquatic Resources (BFAR). (2012). *Philippine fisheries profile 2012*. Quezon City, Philippines: Fisheries Policy and Economics Division, BFAR, Department of Agriculture.

Buschmann, A. H., Tomova, A., Lopez, A., Maldonado, M. A., Henriquez, L. A., Ivanova, L., . . . Cabello, F. C. (2012). Salmon aquaculture and antimicrobial resistance in the marine environment. *PLOS ONE*, *7*(8), e42724. https://doi.org/10.1371/journal.pone.0042724

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*, *8*(7), 1137-1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Cameron, C. E., Zuerner, R. L., Raverty, S., Colegrove, K. M., Norman, S. A., Lambourn, D. M., . . . Gulland, F. M. D. (2008). Detection of pathogenic *Leptospira* bacteria in pinniped populations via PCR and identification of a source of transmission for zoonotic leptospirosis in the marine environment. *Journal of Clinical Microbiology*, *46*(5), 1728-1733. https://doi.org/10.1128/JCM.02022-07

Cavalieri, S. J., Harbeck, R. J., McCarter, Y. S., Ortez, J. H., Rankin, I. D., Sautter, R. L., . . . Spiegel, C. A. (2005). *Manual of antimicrobial susceptibility testing*. Seattle, WA: American Society for Microbiology.

Claassen, S. L., Reese, J. M., Mysliwiec, V., & Mahlen, S. D. (2011). *Achromobacter xylosidans* infection presenting as a pulmonary module mimicking cancer. *Journal of Clinical Microbiology*, *49*(7), 2751-2754. https://doi.org/10.1128/JCM.02571-10

Clinical and Laboratory Standards Institute (CLSI). (2007). *Performance standards for antimicrobial susceptibility testing; Seventeenth informational supplement* (CLSI document M100-S17). Wayne, PA: CLSI.

CLSI. (2014). *Performance standards for antimicrobial susceptibility testing; Twenty-fourth informational supplement* (CLSI document M100-S24). Wayne, PA: CLSI.

Coker, R., Rushton, J., Mounier-Jack, S., Karimuribo, E., Lutumba, P., Kambarage, D., . . . Rweyemamu, M. (2011). Towards a conceptual framework to support one-health research for policy on emerging zoonoses. *The Lancet Infectious Diseases*, *11*(4), 326-331. https://doi.org/10.1016/S1473-3099(10)70312-1

Costanzo, S. D., Murby, J., & Bates, J. (2005). Ecosystem response to antibiotics entering the aquatic environment. *Marine Pollution Bulletin*, *51*(1-4), 218-223. https://doi.org/10.1016/j.marpolbul.2004.10.038

Culik, B. M. (2004). *Review of small cetaceans: Distribution, behaviour, migration and threats* (Marine Mammal Action Plan/Regional Seas Reports and Studies No. 177). Bonn, Germany: United Nations Environment Programme (UNEP) and the Secretariat of the Convention on the Conservation of Migratory Species of Wild Animals (CMS).

DebMandal, M., Mandal, S., & Pal, N. K. (2011). Antibiotic resistance prevalence and pattern in environmental bacterial isolates. *The Open Antimicrobial Agents Journal*, *3*, 45-52. https://doi.org/10.2174/1876518101103010045

Faires, M. C., Gehring, E., Mergl, J., & Weese, J. S. (2009). Methicillin-resistant *Staphylococcus aureus* in marine mammals. *Emerging Infectious Diseases*, *15*(12), 2071-2072. https://doi.org/10.3201/eid1512.090220

Fisher, K., & Phillips, C. (2009). The ecology, epidemiology, and virulence of *Enterococcus*. *Microbiology*, *155*, 1749-1757. https://doi.org/10.1099/mic.0.026385-0

Flores, M. B., Suarez, L., Torno, C., Lopez, H., Luz, S., & Braun, R. (2013). Medical management of stranded marine mammals. In L. V. Aragones, G. E. Laule, & B. G. Espinos (Eds.), *Marine mammal stranding response manual: A guide for the rescue, rehabilitation, and release of stranded cetaceans and dugong in the Philippines* (pp. 59-77). Subic Bay Freeport, Philippines: Wildlife In Need (WIN) and Ocean Adventure.

Geraci, J. R., & Lounsbury, V. J. (Eds.). (2005). *Marine mammals ashore: A field guide for strandings* (2nd ed.). College Station: Texas A&M University Sea Grant College Program.

Gibbs, E. P. J. (2014). The evolution of one health: A decade of progress and challenges for the future. *Veterinary Record*, *174*, 85-91. https://doi.org/10.1136/vr.g143

Greig, T. W., Bemiss, J. A., Lyon, B. R., Bossart, G. D., & Fair, P. A. (2007). Prevalence and diversity of antibiotic resistant *Escherichia coli* in bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida, and Charleston Harbor Area, South Carolina. *Aquatic Mammals*, *33*(2), 185-194. https://doi.org/10.1578/AM.33.2.2007.185

Gulland, F. M. D., & Hall, A. J. (2007). Is marine mammal health deteriorating? Trends in the global reporting of marine mammal disease. *EcoHealth*, *4*, 135-150. https://doi.org/10.1007/s10393-007-0097-1

Holmström, K., Gräslund, S., Wahlström, A. Poungshompoo, S., Bengtsson, B-E., & Kautsky, N. (2003). Antibiotic use in shrimp farming and implications for environmental impacts and human health. *International Journal of Food Science & Technology*, *38*(3), 255-266. https://doi.org/10.1046/j.1365-2621.2003.00671.x

Indian Council of Medical Research (IMCR). (2009). *Detection of antimicrobial resistance in common gram negative and gram positive bacteria encountered in infectious diseases: An update* (Vol. 39, No. 103). Ansari Nagar, New Delhi: IMCR.

Julian, R. J., & Boulianne, M. (1988). Natural and experimental lung pathology causing pulmonary hypertension, right ventricular hypertrophy, right ventricular failure and ascites in broiler chickens. *American Journal of Veterinary Research*, *192*(209), 1781.

Julian, R. J., Friars, G. W., French, H., & Quinton, M. (1986). The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broiler and roaster chickens. *Avian Diseases*, *31*, 130-135. https://doi.org/10.2307/1590785

Kreuder, C., Miller, M. A., Jessup, D. A., Lowenstine, L. J., Harris, M. D., Ames, J. A., . . . Mazet, J. A. (2003). Patterns of mortality in southern sea otters (*Enhydra lutris nereis*) from 1998-2001. *Journal of Wildlife Diseases*, *39*(3), 495-509. https://doi.org/10.7589/0090-3558-39.3.495

McFee, W. E., & Lipscomb, T. P. (2009). Major pathologic findings and probable causes of mortality in bottlenose dolphins stranded in South Carolina from 1993 to 2006. *Journal of Wildlife Diseases*, *45*(3), 575-593. https://doi.org/10.7589/0090-3558-45.3.575

Miller, M. A., Byrne, B. A., Jang, S. S., Dodd, E. M., Dorfmeier, E., Harris, M. D., . . . Miller, W. A. (2009). Enteric bacterial pathogen detection in southern sea otters (*Enhydra lutris nereis*) is associated with coastal urbanization and freshwater runoff. *Veterinary Research*, *41*(1). https://doi.org/10.1051/vetres/2009049

Moore, S. E. (2008). Marine mammals as ecosystem sentinels. *Journal of Mammalogy*, *89*(3), 534-540. https://doi.org/10.1644/07-MAMM-S-312R1.1

National Committee for Clinical Laboratory Standards (NCCLS). (2002). *Performance standards for anti-microbial disk and dilution susceptibility tests for bacteria isolated from animals: Approved standard* (2nd ed., CLSI document M31-A2). Wayne, PA: CLSI.

Obusan, M. C. M., Rivera, W. L. R., Siringan, M. A. T., & Aragones, L. V. A. (2016). Stranding events in the Philippines provide evidence for impacts of human interactions on cetaceans. *Ocean & Coastal Management*, *134*, 41-51. https://doi.org/10.1016/j.ocecoaman.2016.09.021

Obusan, M. C. M., Aragones, L. V., Salibay, C. C., Siringan, M. A. T., & Rivera, W. L. (2015). Occurrence of human pathogenic bacteria and *Toxoplasma gondii* in cetaceans stranded in the Philippines: Providing clues on ocean health status. *Aquatic Mammals*, *41*(2), 149-166. https://doi.org/10.1578/AM.41.2.2015.149

Parsons, E. C. M., & Jefferson, T. A. (2000). Post-mortem investigations on stranded dolphins and porpoises from Hong Kong waters. *Journal of Wildlife Diseases*, *36*(2), 342-356. https://doi.org/10.7589/0090-3558-36.2.342

Pereira, C. S., Siciliano, S., Moreno, I. B., Ott, P. H., & Rodrigues dos Prazeres, D. (2008, May). *Occurrence of enteropathogens isolated from marine mammals from coastal regions in Brazil* (Paper SC/60/DW16-Workshop Proceedings). Cambridge, UK: IWC Scientific Committee.

Rodloff, A., Bauer, T., Ewig, S., Kujath, P., & Müller, E. (2008). Susceptible, intermediate, and resistant – The intensity of antibiotic action. *Deutsches Ärzteblatt International*, *105*(39), 657-662. https://doi.org/10.3238/arztebl.2008.0657

Rose, J. M., Gast, R. J., Bogomolni, A., Ellis, J. C., Lentell, B. J., Touhey, K., & Moore, M. (2009). Occurrence and patterns of antibiotic resistance in vertebrates off the northeastern United States coast. *FEMS Microbiology Ecology*, *67*(3), 421-431. https://doi.org/10.1111/j.1574-6941.2009.00648.x

Schaefer, A. M., Goldstein, J. D., Reif, J. S., Fair, P. A., & Bossart, G. D. (2009). Antibiotic-resistant organisms cultured from Atlantic bottlenose dolphins (*Tursiops truncatus*) inhabiting estuarine waters of Charleston, SC and Indian River Lagoon, FL. *Ecohealth*, *6*, 33-41. https://doi.org/10.1007/s10393-009-0221-5

Smaldone, G., Marrone, R., Cappiello, S., Martin, G. A., Oliva, G., Cortesi, M. L., & Anastasion, A. (2014). Occurrence of antibiotic resistance in bacteria isolated from seawater organisms caught in Campania Region: Preliminary study. *BMC Veterinary Research*, *10*, 161. https://doi.org/10.1186/1746-6148-10-161

Stoddard, R. A., Atwill, E. R., Gulland, F. M. D., Miller, M. A., Dabritz, H. A., Paradies, D. M., . . . Conrad, P. A. (2008). Risk factors for infection with pathogenic and antimicrobial-resistant fecal bacteria in northern elephant seals in California. *Public Health Reports*, *123*, 360-370. https://doi.org/10.1177/003335490812300316

Tankson, J. D., Thaxton, J. P., & Vizzier-Thaxton, Y. (2001). Pulmonary hypertension syndrome in broilers caused by *Enterococcus faecalis*. *Infection and Immunity*, *69*(10), 6318-6322. https://doi.org/10.1128/IAI.69.10.6318-6322.2001

Tankson, J. D., Thaxton, J. P., & Vizzier-Thaxton, Y. (2002). Biochemical and immunological changes in chickens experiencing pulmonary hypertension syndrome caused by *Enterococcus faecalis*. *Poultry Science*, *81*(12), 1826-1831. https://doi.org/10.1093/ps/81.12.1826

Tendencia, E. A., & de la Peña, L. D. (2001). Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture*, *195*(3-4), 193-204. https://doi.org/10.1016/S0044-8486(00)00570-6

Wallace, C. C., Yund, P. O., Ford, T. E., Matassa, K. A., & Bass, A. L. (2013). Increase in antibacterial resistance in bacteria isolated from stranded marine mammals of the Northwest Atlantic. *Ecohealth*, *10*(2), 201-210. https://doi.org/10.1007/s10393-013-0842-6

Waltzek, T. B., Cortés-Hinojosa, G., Wellehan, J. F. X., & Gray, G. (2012). Marine mammal zoonoses: A review of disease manifestations. *Zoonoses and Public Health*, *59*(8), 521-535. https://doi.org/10.1111/j.1863-2378.2012.01492.x

Wong, S., Jensen, E., & Van Bonn, W. (2002). *Ciprofloxacin resistance associated with marine mammals*. 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy (C2-118), San Diego, CA.

Yin, Q., Fu, B., Li, B., Shi, X., Inagaki, F., & Zhang, X. H. (2013). Spatial variations in microbial community composition in surface seawater from the ultra-oligotrophic center to rim of the South Pacific gyre. *PLOS ONE*, *8*(2), e55148. https://doi.org/10.1371/journal.pone.0055148