Aquatic Mammals 2018, *44*(5), 568-579, DOI 10.1578/AM.44.5.2018.568

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

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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 cepacia*, *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 1.5×108 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 ± 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

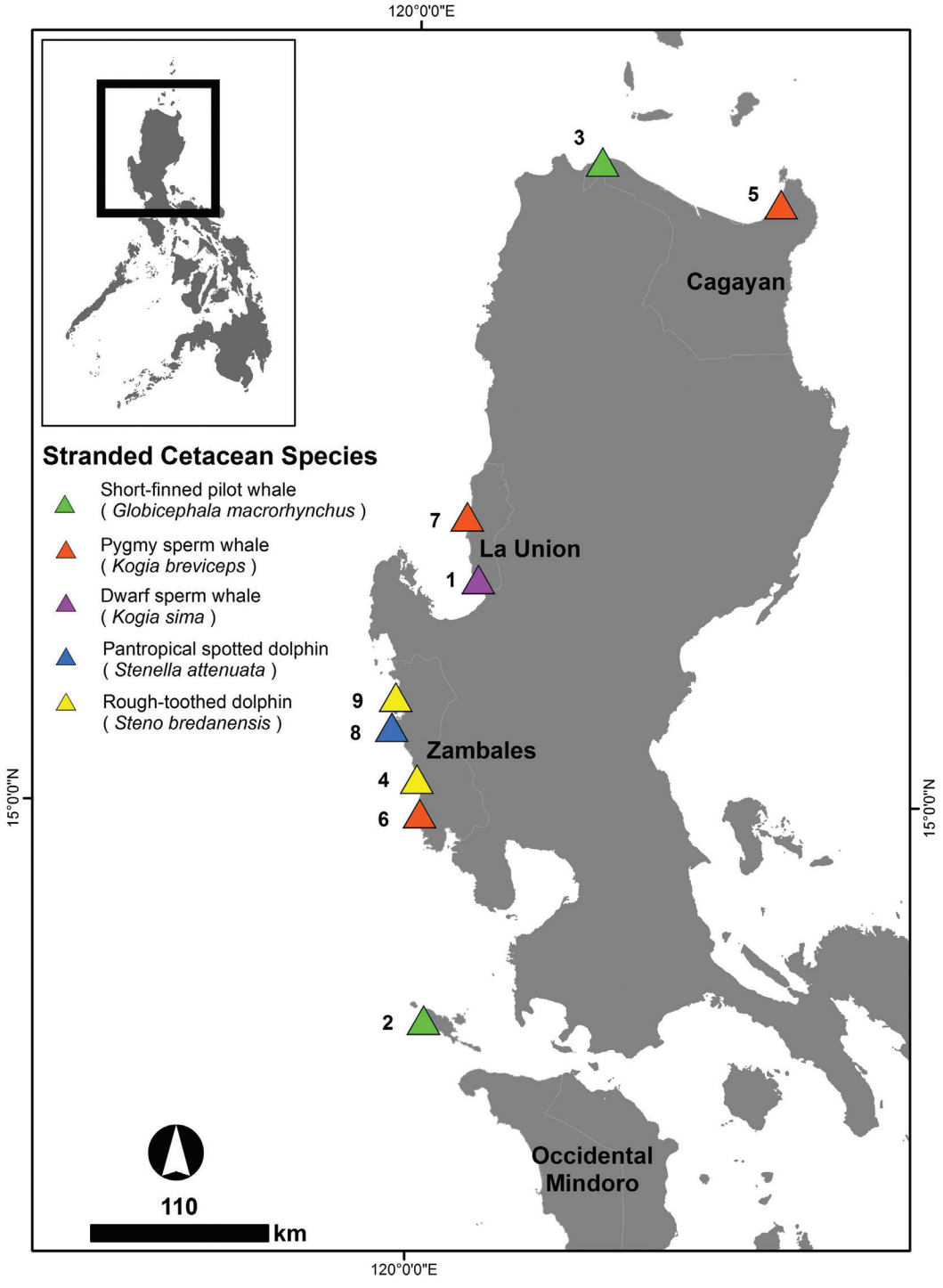

Figure 1. Stranding sites in the Philippines of cetaceans sampled in the study with numerical codes (Strander Codes) corresponding to dates of stranding events: (1) 7 February 2012, (2) 16 March 2012, (3) 9 April 2012, (4) 7 June 2012, (5) 12 September 2012, (6) 16 September 2012, (7) 17 September 2012, (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

				Antibiotics[a]									
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)

		Sensitivity to antibiotics		
Isolate identification	Source of swab sample	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.

Acknowledgments

The authors thank Leo Jonathan Suarez DVM, the staff and veterinarians of the Bureau of Fisheries and Aquatic Resources (BFAR) of the Department of Agriculture (DA), volunteers of Philippine Marine Mammal Stranding Network (PMMSN), the staff of Ocean Adventure, and Honey Leen M. Laggui. Special thanks to Mariel Buccat Flores DVM for the assistance provided. Funding was provided by the Philippine Council for Health Research and Development–Department of Science and Technology (PCHRD-DOST) through the Accelerated Science and Technology Human Resource Development Program (ASTHRDP) scholarship grant for MCMO.

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