



## Antimicrobial-resistance profiles of gram-negative bacteria isolated from green turtles (*Chelonia mydas*) in Taiwan

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### ABSTRACT

The green turtle (*Chelonia mydas*) is listed as a globally endangered species and is vulnerable to anthropogenic threats, including environmental pollution. This study investigated the antimicrobial resistance of Gram-negative bacteria isolated from wild green turtles admitted to a sea turtle rehabilitation center in Taiwan. For this investigation, cloacal and nasal swab samples were collected from 28 green turtles between 2018 and 2020, from which a total of 47 Gram-negative bacterial isolates were identified. Among these, *Vibrio* spp. were the most dominant isolate (31.91%), and 89.36% of the 47 isolates showed resistance to at least one of 18 antimicrobial agents tested. Isolates resistant to one (6.38%), two (8.51%), and multiple (74.47%) antimicrobials were observed. The antimicrobial agents to which isolates showed the greatest resistance were penicillin (74.47%), followed by spiramycin, amoxicillin, and cephalexin. The antimicrobial-resistance profiles identified in this study provide useful information for the clinical treatment of sea turtles in rehabilitation facilities. The results of our study also imply that wild green turtles may be exposed to polluting effluents containing antimicrobials when the turtles traverse migratory corridors or forage in feeding habitats. To benefit sea turtle conservation, future research should focus on (1) how to prevent pollution from antimicrobials in major green turtle activity areas and (2) identifying sources of antimicrobial-resistant bacterial strains in coastal waters of Taiwan.

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### 1. Introduction

Five of the seven globally recognized marine turtle species are found in the coastal regions of Taiwan, including the green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricata*), olive ridley turtle (*Lepidochelys olivacea*), leatherback turtle (*Dermochelys coriacea*), and loggerhead turtle (*Caretta caretta*) (Kuo et al., 2017; Cheng et al., 2019a). Of these five marine turtle species, the green turtle (*C. mydas*) is the most abundant in Taiwan (Kuo et al., 2017; Cheng et al., 2019a). In fact, the coastal waters of Taiwan are known to contain feeding habitats and migratory corridors for green turtles (Cheng 2000; Cheng et al., 2018, 2019b; Ng et al., 2018a; Kuo et al., 2017; Li et al., 2020). The green turtle is listed as a globally

endangered species (International Union for Conservation of Nature, IUCN, 2016) and it is also listed under the Schedule of Protected Marine Species (Ocean Affairs council, Executive Yuan) in Taiwan. Anthropogenic activities, including bycatch, illegal egg harvesting, artificial coastal construction, marine debris, global environmental change, and environmental pollution, pose major threats to green turtle populations (Aguirre and Lutz, 2004; Hamann et al., 2010; Jones et al., 2016; Perrault et al., 2017; Godoy and Stockin, 2018; Ng et al., 2018b). The current study focused on threats from environmental pollutants, which is a priority research area for marine turtle conservation (Hamann et al., 2010; Rees et al., 2016).

Antimicrobial agents are a significant type of aquatic environmental pollutant (Foti et al., 2009; Al-Bahry et al., 2011; Marinho et al., 2013; Prichula et al., 2016; Pace et al., 2019; Huijbers et al., 2020). Sources of antimicrobial agents in seawater include aquaculture waste and wastewater discharges from land (Al-Bahry et al.,

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2009a; Kummerer, 2009; Zhang et al., 2009; Gullberg et al., 2011; Suzuki et al., 2017; Huijbers et al., 2020). Because antimicrobial residues become highly diluted in seawater (Al-Bahry et al., 2012), antimicrobial-resistant bacteria may easily develop in marine creatures; thus, the distribution of bacteria can be used to detect antimicrobial pollution in marine environments (Al-Bahry et al., 2011, 2012; Marinho et al., 2013; Meena et al., 2015; Prichula et al., 2016; Pace et al., 2019). Several studies have also suggested using antimicrobial-resistance data as indices to quantify marine pollution (Foti et al., 2009; Marinho et al., 2013; Meena et al., 2015). As sea turtles play a key role in maintaining the health of marine ecosystems (Aguirre and Lutz, 2004), previous studies have employed these animals as bioindicators to determine pollution levels in local marine environments (Foti et al., 2009; Al-Bahry et al., 2011; Ahasan et al., 2017; Pace et al., 2019). Due to their high fidelity to coastal foraging grounds and nesting sites, long life-spans, and frequent use of nearshore habitats affected by anthropogenic activities, green turtles are often exposed to antimicrobial residues and antimicrobial-resistant bacteria, which are common in polluted areas (Al-Bahry et al., 2009b, 2011; Foti et al., 2009; Alduina et al., 2020; Blasi et al., 2020). Therefore, investigating the presence of antimicrobial-resistant bacteria in sea turtles may be an alternative to evaluating levels of antimicrobial pollution in marine environments (Al-Bahry et al., 2012; Galgani et al., 2014; Pace et al., 2019). Furthermore, as antimicrobial-resistant bacteria have been detected in both healthy wild sea turtles and injured or sick turtles undergoing rehabilitation (Foti et al., 2009; Innis et al., 2014; Prichula et al., 2016; Delli Paoli Carini et al., 2017; Pace et al., 2019; Tsai et al., 2019; Alduina et al., 2020; Ciccarelli et al., 2020), the effectiveness of clinical treatments for microbial infections in rescued sea turtles may be severely limited (Guthrie et al., 2010; Innis et al., 2014; Pace et al., 2018; Tsai et al., 2019; Ciccarelli et al., 2020).

Coastal areas in Taiwan contain major foraging and nesting sites for green turtles (Cheng 2000; Cheng et al., 2018, 2019b; Ng et al., 2018a; Kuo et al., 2017; Li et al., 2020; Ng and Matsuzawa, 2020). However, no previous studies have investigated antimicrobial resistance profiles among bacteria isolated from endangered green turtles in Taiwan (Ng and Matsuzawa, 2020). The current study sought to fill this research gap. Our findings should help inform the management, conservation, and protection of green turtles, which are important research areas for this species.

## 2. Materials and methods

### 2.1. Sample collection

In this study, samples from green turtles were collected under the following permits: the Institutional Animal Care and Use Committee (IACUC) of the National Museum of Marine Biology and Aquarium (NMMBA) (Permit no. 2017005, 2,018,007, and 2020001), the Forestry Bureau (Permit no. 1061702798), and the Ocean Conservation Administration (Permit no. 1070003700 and 1080007820). The study included a total of 28 green turtles that had been admitted to the sea turtle rehabilitation center at the NMMBA in Checheng Township, Pingtung County, Taiwan, between 2018 and 2020. Samples were collected after the turtles were admitted but before any antimicrobials had been administered. Of the 28 turtles, 27 (96%) were captured in southern Taiwan and 1 (4%) was captured in northern Taiwan. All turtles were identified through the official reporting system of the Marine Animal Rescue Network (established by the Ocean Conservation Administration) and brought to the rehabilitation center due to severe injuries or other issues. Reasons for admission included the following: being accidentally caught by fishermen/entanglement in fishing nets (13

cases), unknown cause of stranding (10 cases), buoyancy disorders (4 cases), and ingestion of hooks (1 case). During the rehabilitation period, all sea turtles were separately housed in individual rehabilitation tanks to eliminate aggressive behavior and reduce contamination. Conditions in all rehabilitation tanks were specific for rescued sea turtles and involved a continuous flow of sand-filtered natural seawater, the temperature of which varied from approximately 22 to 28 °C. No other rescued wild animals, including mammals, birds, or other reptiles, were kept at our rehabilitation facility during the study period. Thus, none of the sea turtles included in our study had contact with any other animals while being treated at our facility. Most green turtles were fed a mainly vegetarian diet (including seagrass and seaweed). However, more emaciated turtles were initially fed a diet primarily comprised of seafood (including squid, fish, and shrimp). No sea turtles that had been treated with antimicrobial drugs were included in this study.

### 2.2. Isolation and identification of bacterial species from green turtles

After peri-cloacal and peri-nasal areas were disinfected with a 70% alcohol solution, one cloaca swab and one nasal cavity swab were respectively collected from each green turtle using sterile swabs (Ahasan et al., 2017; Pace et al., 2019). Swabs were plated onto tryptic soy agar (TSA; Difco, USA) containing 1.5% NaCl (TSN), McConkey agar (Difco, USA), thiosulphate-citrate-bile salts-sucrose (TCBS; Difco, USA) agar, and TSA containing 5% sheep blood. The plates were incubated at 25 °C for 24–48 h. In this study, a total of 25 isolates from cloaca swabs and 22 isolates from nasal cavity swabs were recovered from cultured plates. All isolates were Gram-stained, tested for catalase and oxidase activity, and identified using API 20 E and API 20 NE systems (Biomérieux, France) (Al-Bahry et al., 2009b). Additionally, typical *Vibrio* colonies were further verified by polymerase chain reaction (PCR) using specific primers according to previously described methods (Haldar et al., 2010).

### 2.3. Determination of antimicrobial resistance

Antimicrobial resistance profiles were determined using the Kirby-Bauer method on Muller Hinton agar (MHA; Difco, USA), and *Vibrio* spp. isolates were cultured on MHA plates supplemented with 1% NaCl. Similar to previous research (Tsai et al., 2019), all isolates in this study were tested against 18 different antimicrobial discs that contained penicillin (10 µg), doxycycline (30 µg), enrofloxacin (5 µg), nitrofurantoin (300 µg), cephalexin (30 µg), oxytetracycline (30 µg), amoxicillin/clavulanic acid (30 µg), gentamicin (10 µg), amikacin (30 µg), piperacillin (100 µg), ciprofloxacin (5 µg), cefoperazone (30 µg), sulfamethoxazole/trimethoprim (25 µg), spiramycin (100 µg), chloramphenicol (30 µg), ceftiofur (30 µg), azithromycin (15 µg), or amoxicillin (25 µg) (Oxoid Ltd, Basingstoke, United Kingdom). The plates were incubated at 25 °C for 18 h. Results were subsequently evaluated in accordance with standards from the Clinical and Laboratory Standards Institute (CLSI Volume 36, 2016) to determine resistance against various antimicrobial agents (Foti et al., 2009; Innis et al., 2014; CLSI, 2016; Prichula et al., 2016; Alduina et al., 2020; Blasi et al., 2020). In this study, bacterial isolates resistant to at least three antimicrobial agents were defined as having multiple antimicrobial resistance (Prichula et al., 2016; Ahasan et al., 2017; Delli Paoli Carini et al., 2017).

### 3. Results

#### 3.1. Bacterial isolation

This study collected samples from 28 green turtles (Curved Carapace Length; CCL: mean 46.75 cm ± S.D. 8.99 cm; range: 37.00–80.00 cm) using nasal (n = 22) and cloacal (n = 25) swabs. All sea turtles in this study were identified as immature green turtles (including 27 juveniles and 1 sub-adult) on the basis of CCL: turtles with CCL < 67 cm were classified as juveniles (n = 27), whereas the animal with a CCL between 67 and 84 cm was classified as sub-adult (n = 1) (Ng et al., 2018b). A total of 47 Gram-negative bacterial isolates were identified from the samples. *Vibrio* spp. were the most dominant species, followed by *Aeromonas*

spp., species from the *Shewanella putrefaciens* group, unidentified Gram-negative bacteria, *Citrobacter freundii*, *Photobacterium damsela*, *Acinetobacter junii*, *Mannheimia haemolytica*, *Pseudomonas alcaligenes*, *Sphingomonas paucimobilis*, *Klebsiella oxytoca*, species from the *Proteus vulgaris* group, and *Shigella* spp. (Fig. 1). After performing further species identification tests for *Vibrio*, the isolates (n = 15) were found to be the following: *Vibrio alginolyticus*, *Vibrio harveyi*, *Vibrio vulnificus*, *Vibrio cholerae*, and *Vibrio metschnikovii*, according to their presence frequencies (Fig. 2).

#### 3.2. Profiles of antimicrobial resistance among bacterial isolates

Antimicrobial resistance profiles revealed that 89.36% of the isolates identified in this study (42/47) were resistant to at least one

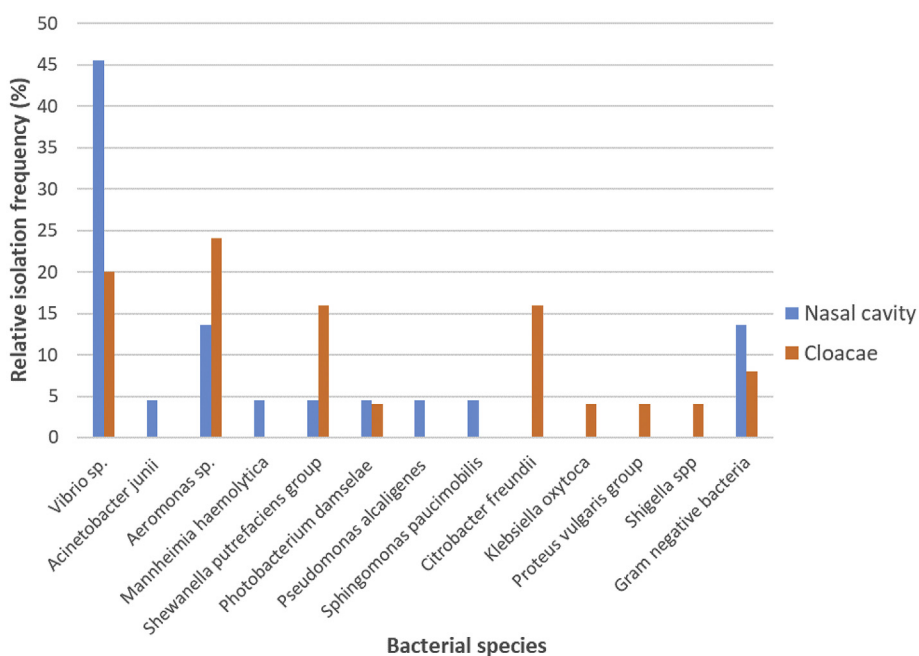


Fig. 1. Frequency distribution of isolates recovered from nasal cavities (n = 22) and cloacae (n = 25) of *Chelonia mydas*.

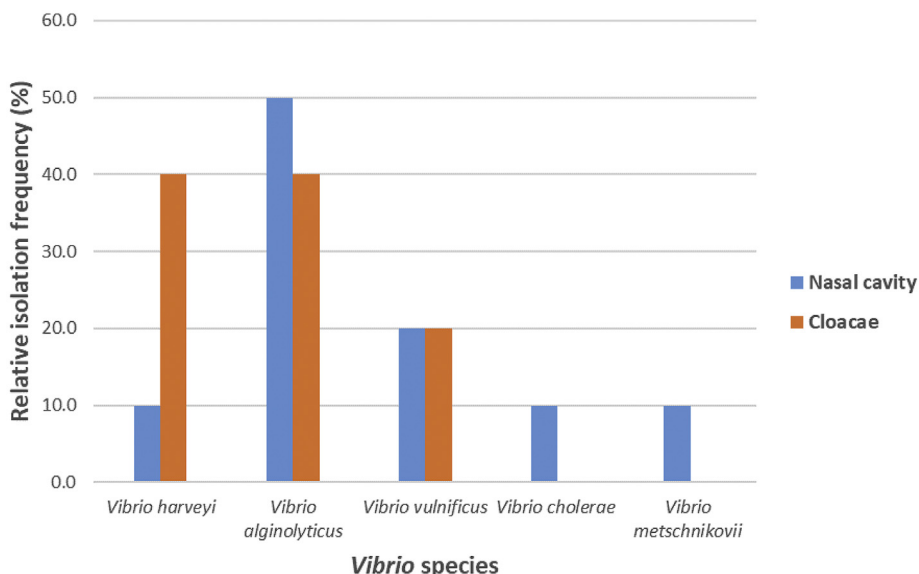
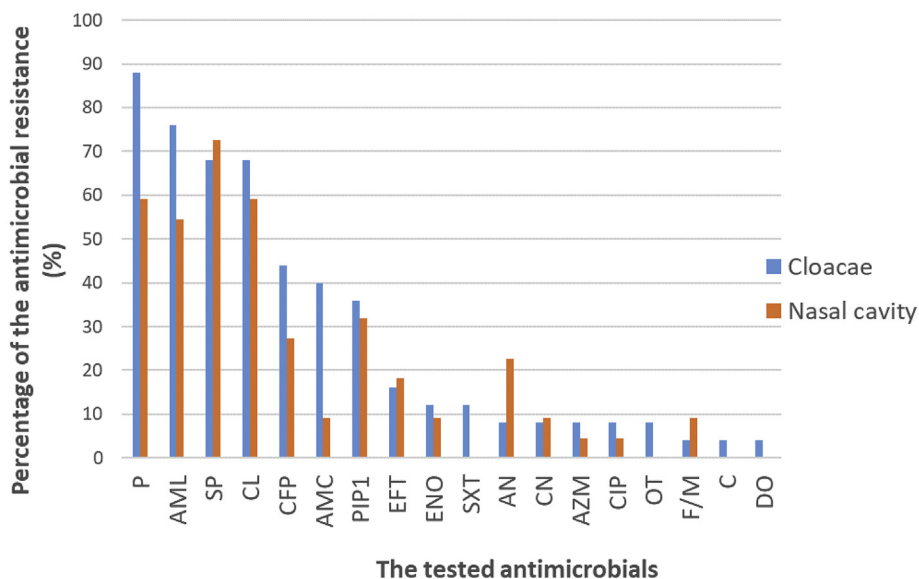


Fig. 2. Relative percentages of *Vibrio* species identified in nasal cavity (n = 10) and cloacal swabs (n = 5) from *Chelonia mydas*.



**Fig. 3.** Percentages of antimicrobial resistance in Gram-negative bacteria from *Chelonia mydas*. Abbreviations on the x-axis denote the tested antimicrobial agents, as follows: P. penicillin, AML. amoxicillin, SP. spiramycin, CL. cephalixin, CFP. cefoperazone, AMC. amoxicillin-clavulanic acid, PIP1. piperacillin, EFT. ceftiofur, ENO. enrofloxacin, SXT. sulfamethoxazole/trimethoprim, AN. amikacin, CN. gentamicin, AZM. azithromycin, CIP. ciprofloxacin, OT. oxytetracycline, F/M. nitrofurantoin, C. chloramphenicol, DO. doxycycline.

of the 18 tested antimicrobial agents. Specifically, isolates resistant to one (3/47 or 6.38%), two (4/47 or 8.51%), and multiple (35/47 or 74.47%) antimicrobials were observed. Among the various antimicrobial agents, the largest percentage of isolates (74.47%) were resistant to penicillin (88% and 59.09% in cloaca and nasal swabs, respectively), followed by spiramycin (68% and 72.72% in cloaca and nasal swabs, respectively), amoxicillin (76% and 54.55% in cloaca and nasal swabs, respectively), and cephalixin (68% and 59.09% in cloaca and nasal swabs, respectively) (Fig. 3). Furthermore, among the 47 Gram-negative bacterial isolates identified in this study, 40.42% (19/47), mainly *Vibrio* spp. (9/19) and *Aeromonas* spp. (4/19), were resistant to six or more antimicrobial agents (Table 1).

#### 4. Discussion

Current research on green turtles in Taiwan is primarily focused on captivity and husbandry, blood profiles, infectious diseases, and nesting ecology. Published articles pertaining to marine pollution and antimicrobial resistance in bacteria isolated from sea turtles are limited. Our previous research (Tsai et al., 2019) revealed that one olive ridley turtle (*Lepidochelys olivacea*) infected with multiple antimicrobial-resistant bacteria (*Enterococcus faecalis*) suffered from osteomyelitis. Because antimicrobial-resistant bacteria are a worldwide concern, this study aimed to advance our knowledge and understanding of antimicrobial resistance profiles among bacteria isolated from wild green turtles in Taiwan.

Marine pollution is considered to be a priority research area for global sea turtle conservation (Hamann et al., 2010; Rees et al., 2016). In wild marine turtles, antimicrobial resistant bacteria may be indicative of coastal contamination (Al-Bahry et al., 2009b; Delli Paoli Carini et al., 2017; Pace et al., 2019). In terms of pathogenicity, Gram-negative bacteria are generally more virulent than Gram-positive bacteria. To the best of our knowledge, no previous study has investigated antimicrobial resistance in Gram-negative bacteria isolated from green turtles in the East Asia Region. Using cloacal and nasal cavity swabs from wild green turtles in Taiwan, we isolated a total of 47 Gram-negative bacteria strains. Among these, the most common and second most common species identified were *Vibrio* spp. (31.91%) and *Aeromonas* spp. (19.15%), respectively.

Furthermore, 60.00% of *Vibrio* spp. isolates (9/15) and 44.44% of *Aeromonas* spp. isolates (4/9) in our study were found to be resistant to six or more different antimicrobial agents. Therefore, the effectiveness of many antimicrobial agents could be limited in treating infected green turtles. *Vibrio* species have often been found in sea turtles around the world (Work et al., 2003; Santoro et al., 2006; Fichi et al., 2016; Pace et al., 2019; Alduina et al., 2020; Blasi et al., 2020) and have been reported in previous green turtle studies (Work et al., 2003; Delli Paoli Carini et al., 2017). The most prevalent *Vibrio* species identified in our study was *V. alginolyticus* (46.66%; 7 isolates), followed by *V. harveyi* (20.00%; 3 isolates), *V. vulnificus* (20.00%; 3 isolates), *V. cholerae* (6.66%; 1 isolate), and *V. metschnikovii* (6.66%; 1 isolate). *V. alginolyticus* plays various roles in sea turtles. For example, it is part of the common flora in the oral cavity and cloaca in healthy sea turtles captured from foraging grounds (Zavala-Norzagaray et al., 2015), is an opportunistic pathogen (Orós et al., 2004, 2005), in gastrointestinal lesions of captive juvenile sea turtles (Chuen-Im et al., 2010), and can be responsible for skin disease in an aquarium-housed loggerhead turtle (Wiles and Rand, 1987). Work et al. (2003) reported that *V. harveyi* was the most prevalent bacteria isolated from wild green turtles suffering from bacteraemia. Taken together, findings from both the current and previous studies indicate that *V. alginolyticus* and *V. harveyi* should be considered important pathogens in sick and injured sea turtles in rehabilitation centers.

*Aeromonas* spp. (19.15%) were the second most frequently isolated species in our study. *Aeromonas* species are widely distributed in aquatic environments worldwide (Igbinsosa et al., 2012). *Aeromonas* spp. cultured from diseased sea turtles have also been identified in previous studies, including from digestive lesions in stranded and captive sea turtles (Orós et al., 2004; Chuen-Im et al., 2010) as well as from traumatic ulcerative skin lesions in stranded loggerhead turtles (Orós et al., 2005). In addition, *A. hydrophila* with multiple antimicrobial resistance was isolated from a loggerhead sea turtle with a bone infection (Pace et al., 2018). Veterinarians treating debilitated sea turtles with *Aeromonas* infections should be aware that *Aeromonas* spp. are pathogenic to sea turtles and can carry multiple-antimicrobial resistance.

Antimicrobial resistance profiles in bacteria isolated from wild

**Table 1**  
Distribution of antimicrobial resistance patterns among 19 Gram-negative bacterial isolates from green turtles that showed resistance to six or more different antimicrobial agents.

Bacteria	Antimicrobial group/agents <sup>a</sup>																		No. of antimicrobial resistance
	Penicillin			Beta lactam - beta lactamase inhibitor	Cephem			Macrolides		Fluoroquinolones		Aminoglycosides		Tetracyclines				Folate Pathway Inhibitors	
	PIP	-	+		Phenicol			AZM	SP	CIP	ENO	AN	CN	OT	DO	Nitrofurans		AML	
				AMC	EFT	CL	CFP									SXT	C	F/M	
<i>Proteus vulgaris</i> group	-	+	+	+	-	+	-	-	+	+	+	+	+	+	-	-	-	+	11
<i>Citrobacter freundii</i>	+	+	+	+	-	+	-	+	+	+	+	-	-	-	-	+	-	-	10
<i>Aeromonas hydrophila/caviae</i>	+	+	-	-	-	+	+	-	+	-	-	-	-	+	+	+	+	-	9
<i>Vibrio harveyi</i>	+	+	+	-	+	+	+	-	+	-	+	-	-	-	-	-	-	-	8
<i>Acinetobacter junii</i>	-	+	-	-	+	+	+	-	+	+	+	-	-	-	-	-	-	+	8
<i>V. harveyi</i>	+	+	+	-	+	+	+	-	+	-	-	-	+	-	-	-	-	-	8
<i>V. vulnificus</i>	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	8
<i>A. hydrophila/caviae</i>	+	+	+	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	7
<i>C. freundii</i>	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	7
<i>V. harveyi</i>	+	+	+	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	7
<i>V. alginolyticus</i>	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	7
<i>V. vulnificus</i>	+	+	+	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	7
<i>V. alginolyticus</i>	+	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	-	-	7
<i>V. alginolyticus</i>	+	+	+	-	-	+	+	-	+	-	-	-	+	-	-	-	-	-	7
<i>Shewanella putrefaciens</i> group	+	+	-	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	6
<i>A. sobria</i>	+	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	6
<i>A. salmonicida</i>	+	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	6
<i>A. salmonicida</i> ssp <i>salmonicida</i>																			
<i>Pseudomonas alcaligenes</i>	-	-	+	-	-	+	+	-	+	-	+	+	-	-	-	-	-	-	6
<i>V. cholerae</i>	+	-	-	-	+	+	+	-	+	-	-	+	-	-	-	-	-	-	6

<sup>+</sup> Indicates that the isolate was resistant to the antimicrobial agent.

<sup>-</sup> Indicates that the isolate was susceptible to the antimicrobial agent.

sea turtles may be useful as indices of marine pollution (Foti et al., 2009; Al-Bahry et al., 2011; Marinho et al., 2013). In the current study, a majority (89.36%) of isolates showed resistance to at least one of the 18 tested antimicrobial agents. Importantly, none of the sea turtles captured from the wild had been treated with any antimicrobial agents prior to sample collection in our study. With regard to antimicrobial resistance patterns, we found that a high proportion (74.47%) of Gram-negative bacteria isolated from green turtles possessed multiple antimicrobial resistance (i.e., resistance to at least three antimicrobial agents). These results are similar to findings reported in previous research. For example, one previous study found that more than 60% of bacteria strains isolated from green turtles in Oman were resistant to multiple antimicrobial agents (Al-Bahry et al., 2011). Pace et al. (2019) also recently reported that more than 60% of bacteria isolated from loggerhead sea turtles in the western Mediterranean exhibited resistance to at least three antimicrobial agents. In addition, Ciccarelli et al. (2020) recently isolated several multidrug-resistant Gram-negative bacteria (*Citrobacter freundii* and *Pseudomonas putrefaciens*) from loggerhead sea turtles with pulmonary diseases. Moreover, Ahasan et al. (2017) reported that bacterial isolates from rehabilitated green turtles (which had not received any antimicrobial treatment prior to sample collection) were more likely to possess multiple antimicrobial resistance compared to isolates from free-ranging sea turtles.

Because wild sea turtles should not have a history of exposure to antimicrobial therapy under natural conditions, resistance in bacteria isolated from wild sea turtles has become an ever-greater concern (Al-Bahry et al., 2009, 2011, 2012; Foti et al., 2009; Frazzon, 2016; Ahasan et al., 2017; Pace et al., 2019). In this study, 18 of the 28 turtles (64%) were administered antimicrobials by a certified veterinarian to prevent bacterial infections during the rehabilitation period. However, no sea turtle received antimicrobial drugs prior to sample collection. Why most bacterial isolates from sea turtles in our study were resistant to multiple antimicrobial agents remains unclear. It is possible that the multiple antimicrobial-resistant strains are related to coastal effluent pollution (Foti et al., 2009; Kummerer, 2009; Al-Bahry et al., 2009, 2012; Frazzon, 2016). In other words, selection pressure may cause bacteria in marine habitats to develop antimicrobial resistance following exposure to environmental effluents that contain antimicrobial agents (e.g., effluents from waste produced by agriculture, aquaculture, human medical, and veterinary medical industries) (Rice et al., 1995; Chee-Sanford et al., 2001; Cabello, 2006; Al-Bahry et al., 2009a, 2011, 2012; Foti et al., 2009; Meena et al., 2015; Frazzon, 2016; Ahasan et al., 2017; Pace et al., 2019). However, antimicrobial resistance in bacteria can also be influenced by other pollutants, such as heavy metals. For example, results of previous studies indicated that heavy metals were correlated with the selection of antimicrobial resistance genes (Yamaguchi et al., 1990; Palm et al., 2008; Gorovtsov et al., 2018; Chen et al., 2019), which suggests that heavy metals may induce antibiotic resistance in metal-contaminated environments. In the present study, all 28 green turtles were designated as immature (27 juveniles and 1 sub-adult) in accordance with CCL classifications (Ng et al., 2018b). Immature green turtles are considered to be resident animals that typically frequent inshore foraging habitats with a high degree of site fidelity (Limpus et al., 1994; Musick and Limpus, 1997; Jones et al., 2016). As documented in previous studies (Ng et al., 2018a; Kuo et al., 2017; Cheng et al., 2019b; Li et al., 2020), the inshore environments of Taiwan contain foraging areas for green turtles. These biological factors combined with the antimicrobial resistance profiles that were identified in the current research suggest that the green turtles in our study may have been exposed to coastal effluent pollution containing antimicrobial agents or heavy metals

after recruiting to nearshore foraging grounds. Given that sea turtles are sensitive bioindicators of pollution in marine ecosystems (Foti et al., 2009; Al-Bahry et al., 2011; Ahasan et al., 2017; Pace et al., 2019), the presence of antimicrobial-resistant bacteria in wild green turtles in Taiwan highlights the need to appropriately manage polluting effluents in marine environments.

In our study, isolated Gram-negative bacteria were frequently resistant to penicillin (74.47%), spiramycin (70.20%), amoxicillin (65.94%), and cephalexin (63.83%). A high prevalence of penicillin resistance has also been reported in previous studies (Pinera-Pasquino, 2006; Ahasan et al., 2017). For example, Pinera-Pasquino (2006) found that Gram-negative bacteria isolated from loggerhead sea turtles in North Carolina, USA, were most frequently resistant to penicillin (95.2%). A high prevalence (76.6%) of penicillin-resistant bacteria was also found in a study on green turtles in the Great Barrier Reef, Australia (Ahasan et al., 2017). In our study, the resistance rate (65.94%) for amoxicillin (which belongs to the penicillin family of antimicrobials) was similar to the resistance rate reported in bacteria from loggerhead sea turtles in the central Mediterranean Sea (Foti et al., 2009). However, penicillin and amoxicillin are commonly used as antimicrobial agents in sea turtles with *Enterococcus* spp. infections during rehabilitation (Innis et al., 2014; Tsai et al., 2019). Our study also found a high resistance rate for cephalexin (63.83%), which is very similar to results reported in a previous study (68.9%) by Ahasan et al. (2017). A recent study by Ciccarelli et al. (2020) also found that 92% of isolates from sea turtles (*C. caretta*) with pulmonary disease were resistant to cephalexin. Antimicrobial therapy plays an important role in treating diseased sea turtles in rehabilitation centers. Bacteria with antimicrobial resistance can limit therapeutic options and further complicate treatment efficacy in sea turtles with bacterial infections (Guthrie et al., 2010; Innis et al., 2014; Pace et al., 2018; Tsai et al., 2019; Ciccarelli et al., 2020).

In our study, different antimicrobial-resistant bacteria were isolated from rescued green turtles captured from the wild in Taiwan. The presence of antimicrobial-resistant bacteria is considered to be a bioindicator of pollution in coastal marine areas (Foti et al., 2009; Al-Bahry et al., 2011). However, how these sea turtles acquired antimicrobial-resistant bacteria as well as the mechanisms that underlie the development of antimicrobial resistance in these bacteria require further elucidation.

## 5. Conclusions

To the best of our knowledge, this is the first study to report on antimicrobial resistance profiles in Gram-negative bacteria isolated from wild green turtles in the East Asia region. The identification of Gram-negative bacteria with multiple antimicrobial resistance isolated from wild green turtles raises serious concerns about the extent of antimicrobial residue pollution in coastal waters around Taiwan. Our findings provide important clinical references to benefit the treatment of rescued sea turtles that may have been infected with antimicrobial-resistant bacteria.

## Author contributions

Tsung-Hsien Li: conceived of the study, analyzed the data, carried out the statistical analysis, performed the veterinary care, and drafted the paper. Chao-Chin Chang: participated in the design of the study and helped draft the paper. Ming-An Tsai: performed the sample collection, isolation and identification of bacterial species, antimicrobial resistance testing, participated in the design of the study, and helped draft the paper.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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