Bad Waters: Antibiotic Resistance Bacteria in Sea Turtles

Andreia Garcês[1, 2*] and Isabel Pires[3]

ABSTRACT

Sea turtles play a vital role in maintaining the health of marine ecosystems and should be considered as environmental sentinels to determine pollution rates in marine environments. Several species of sea turtles are endangered or threatened due to a variety of threats, including habitat loss, hunting, accidental catch, and pollution. Antibiotic-resistant bacteria in sea turtles have become a new threat that could affect their health and survival. The spread of antibiotic resistance is a major concern in the 21st century, particularly in aquatic environments. Turtles can be exposed to antibiotics from various sources, such as through contaminated water, food or medical treatment. It is quite common to discover antibiotic-resistant genes in the environment and in animals that have never been exposed to antibiotic treatments. However, data on the worldwide incidence of antibiotic-resistant bacteria in wildlife is still very limited, specifically in marine animals such as sea turtles. Sea turtles can transmit bacteria and antimicrobial resistance (AMR) genes to humans and other animals. Humans can acquire antibiotic-resistant bacteria by handling contaminated animals or water. The present review aims to compile the most recent studies describing the presence of AMR bacteria in sea turtles and determine how they can impact the ecosystem and human health under the One Health concept.

Keywords: Antibiotic resistance, Bacteria, Conservation, Pollution, Sea turtle

INTRODUCTION

Sea turtles are air-breathing marine reptiles of the order Testudines (Al-Bahry *et al*., 2011; Alfaro-Núñez *et al*., 2014). Seven different species of sea turtles can be divided into two categories: hard-shelled (cheloniid) and leathery-shelled (dermochelyid). They can be found in all oceans except the polar regions. Most of their life is spent at sea, and only the females come ashore to lay their eggs on sandy beaches during the nesting season, after long migrations (Gable *et al*., 2021). These animals have a long lifespan of up to 50 years, taking between 20 and 30 years to fully mature to reproduce (Gable *et al*., 2021).

The world's sea turtle populations face numerous threats from anthropogenic activities, including illegal trade in animals and body parts, illicit egg harvesting, artificial coastal construction, destruction of nesting areas, boat traffic, marine debris like nets, environment pollution from light, noise and oil spills, domestic animals, climate change, and bushmeat hunting. These factors collectively represent the major threats to sea turtle populations (see Figure 1 and 2) (Tsai *et al*., 2021). Six of the seven existing species of sea turtles are now classified as threatened or endangered according to the IUCN Red List of Threatened Species. These species include the hawksbill turtle (*Eretmochelys imbricata*), Kemp's ridley turtle

---

[1]Exotic and Wild Animal Service, Veterinary Hospital of University of Trás-os Montes e Alto Douro (UTAD), Vila Real, Portugal
[2]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro, Vila Real, Portugal
[3]Veterinary and Animal Science Research Centre (CECAV), University of Trás-os Montes e Alto Douro, Vila Real, Portugal
[*]Corresponding author. E-mail address: andreiamvg@gmail.com
Received 30 April 2023 / Accepted 10 October 2023

(*Lepidochelys kempii*), green turtle (*Chelonia mydas*), loggerhead turtle (*Caretta caretta*), olive ridley turtle (*Lepidochelys olivacea*), and leatherback turtle (*Dermochelys coriacea*) (Mortimer and Donnelly, 2008).

Antimicrobial resistance (AMR) bacteria are increasing in the environment at a worrying proportion, becoming one of the most significant concerns in the 21st century (Al-Bahry *et al.*, 2011; Bonnedahl and Järhult, 2014). Currently, it is quite

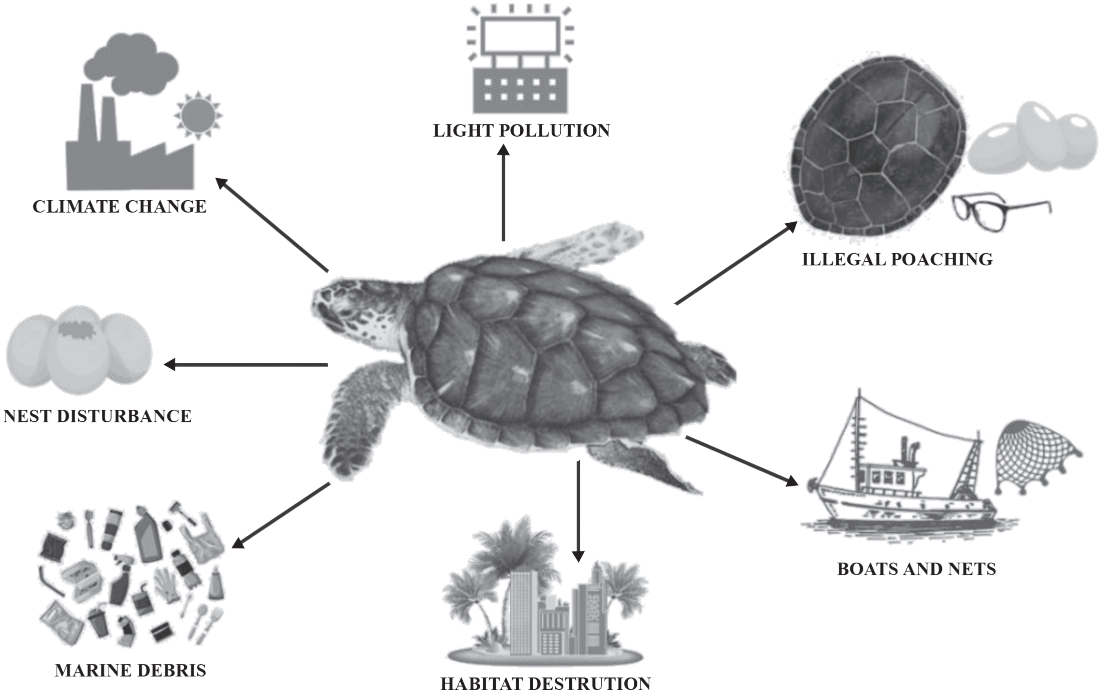

Figure 1. Examples of some major threats to sea turtle populations.

Figure 2. Two individuals of the specie *Caretta caretta* were found trapped in fishing nets and ended up drowning (2016, Spain).

frequent to discover antibiotic-resistance genes in the environment and in animals that have never been exposed to antibiotic treatment (Gambino *et al*., 2022). AMR is associated with the overuse and misuse of antibiotics in both human and veterinary medicine, and they are released into the ecosystems from non-treated waste (garbage, sewage, and carcasses) in many parts of the world. AMR in bacteria can occur due to genetic mutations, or horizontal transfer of resistance genes. Horizontal transfer is possible to occur between phylogenetically unrelated bacteria (Alduina *et al*., 2020). Both phenomena can occur naturally due to the normal evolution of the bacteria or be accelerated due to anthropogenic influence (e.g., contaminated wastes, or use of antibiotics not indicated for this treatment) (Alduina *et al*., 2020). The prevalence of AMR bacteria in the environment may be predisposed by the use of antibiotic (spread of resistant strains or genes from human or animal production), by the evolution or selection of new resistant strains, or by the expansion of pre-existing AMR in the ecosystem (Sherley *et al*., 2000). Wild animals can come into contact with antibiotics (even in sub-inhibitory manner) due to discharges of contaminated wastewater from the soil, leading to the selection of AMR microbiota (Alduina *et al*., 2020).

Although in aquatic environments bacterial concentrations are low compared to soil, marine animals are completely submerged in water throughout their lives and can be useful bioindicators, reservoirs and disseminators of AMR bacteria (Alduina *et al*., 2020). Antimicrobial waste pollution can be an index of marine pollution, that can be used to identify antimicrobial pollution in aquatic environments (freshwater and saltwater) (Foti *et al*., 2013; Tsai *et al*., 2021). Sea turtles play a vital role in maintaining the health of marine ecosystems (Aguirre and Lutz, 2004). These animals have been proposed as sentinel species to determine pollution levels in marine environments. They have already been used as sentinels in other contexts such as fibropapillomatosis (Aguirre and Lutz, 2004). Ecological and biological features such as long longevity, life cycles (juveniles on beaches and adults in aquatic systems), advanced age when reaching sexual maturity, migratory behavior and preference for feeding in habitats close to the coast, make them reliable bio-indicators (Foti *et al*., 2013). Due to these characteristics, sea turtles tend to bioaccumulate compounds such as heavy metals, chemical pollutants and pathogens (Trotta *et al*., 2021b; Gambino *et al*., 2022). The present review aims to present studies on antibiotic resistance and resistance genes in sea turtles and determine how they can impact the ecosystem and human health under the One Health concept.

SEA TURTLE'S DISPERSION AND CONTAMINATION SOURCE OF PATHOGENIC BACTERIA

Sea turtles can acquire antibiotic resistance bacteria from a variety of sources such as contaminated waters, contaminated food, direct contact with other animals/humans, vertical transmission from mother to hatchling and treatments in wildlife rehabilitation centers (Drane *et al*., 2021) (Figure 3).

These reptiles host a very diverse microflora. The different anatomical regions of the sea turtle including plastron, carapace, respiratory, reproductive and gastrointestinal tract are inhabited by a wide range of bacterial species. This microbiota can vary with age, physiological conditions, feeding and migration behavior (Drane *et al*., 2021). Some of these microorganisms from the turtle`s microbiota can become pathogenic when the animal is debilitated or when changes occur in its environment. This can also occur due to the dissemination of resistance genes to other marine bacteria (Trotta *et al*., 2021b). The dietary variations (omnivores when juveniles and herbivores when adults) of some turtle species and their migratory nature make them vulnerable to AMR bacteria. For example, Dermochelys coriacea which consumes almost exclusively jellyfish as a staple of its diet may accumulate antibiotics, resistance genes or AMR bacteria from multiple areas where the jellyfish has traveled. Chelonia mydas feeds mainly on seagrasses and algae and is non-migratory. This species can provide more accurate site-specific information about pollution in a given region due to its sedentary nature in near-coastal environments (Drane *et al*., 2021).

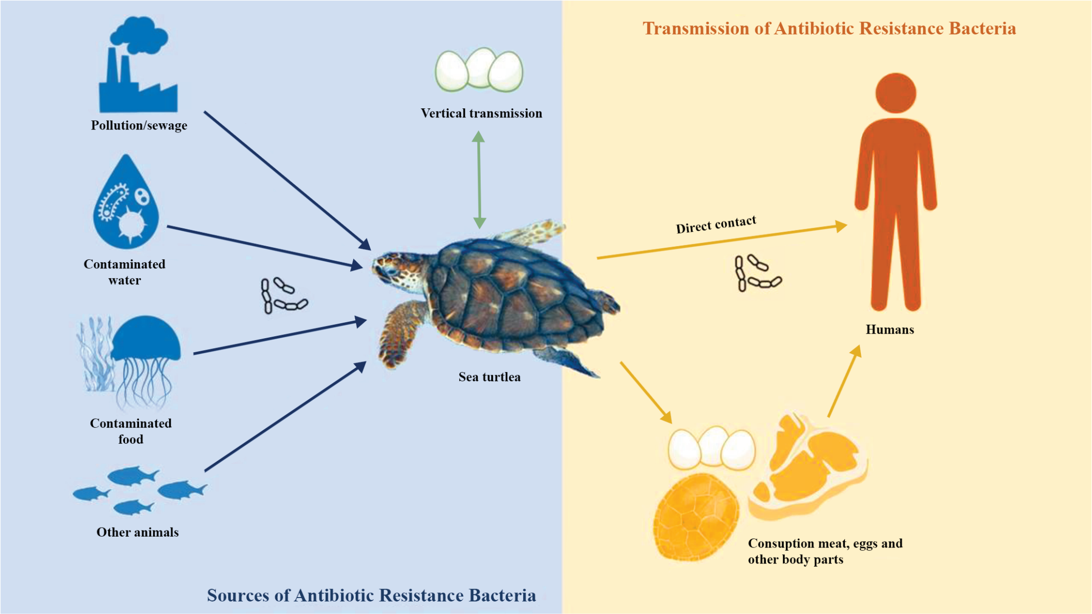

Figure 3. Transmission routes of antibiotic resistance bacteria in sea turtles.

Antimicrobial sources in seawater are mainly from aquaculture wastes, and land-based effluent discharges (agriculture, industry, and domestic), hospitals (veterinary and human), farms, agriculture, open-air dumps, aquacultures, and wastewater treatment plants are considered "hotspot environments" where bacteria can be exposed to high and repetitive doses of antibiotics in a very rich environment where they can multiply (Gambino *et al*., 2022). It is estimated that 90% of all sewage effluents are discharged (untreated) directly into lakes, streams, or oceans (Alduina *et al*., 2020). There is a strong indication that infection of marine animals by AMR bacteria is directly correlated with sewage effluent (Al-Bahry *et al*., 2011). Coastal regions serve as landfills for these wastewaters that are contaminated with antimicrobial residues (Gambino *et al*., 2022). The antimicrobial residue can resist the chlorination process and remain viable in treated effluent even after disinfection (Al-Bahry *et al*., 2011) persisting for long periods in the aquatic environment (Alduina *et al*., 2020). As a result, the aquatic environment has become a reservoir for AMR bacteria that can be isolated from the environment, animals and even humans (Al-Bahry *et al*., 2011). This constant flow of wastewater has greatly affected the coastal water, especially in shallow basins (Trotta *et al*., 2021b). When antimicrobial residues reach the sea waters, they become highly diluted and bacteria presented in the marine environment begin to develop resistance to antibiotic (Tsai *et al*., 2021).

AMR bacteria naturally exists in soil. Therefore, another source of contamination could be sand from nesting areas. Particularly beaches with a high tourist influx may harbor a greater diversity of AMR bacteria (Laborda *et al*., 2022).

Another major threat to turtles is ingesting plastic that they mistake for food. Plastic ingestion can lead to malfunctions and traumatic lesions of the gastrointestinal tract, while also serving as a potential pathway for the transfer of pollutants and microorganisms (Caron *et al*., 2018). The surfaces of this plastic debris (micro and macro) have a distinct microbial habitat composed of a diverse bacterial population. Gammaproteobacteria and *Vibrio* in particular are found in these fragments. After ingestion, a portion of this bacterial population may survive (*Vibrio* species, in particular, are especially resistant to digestion) and alter the normal microflora of the gastrointestinal tract or transmit resistant genes to the resident population (Pace *et al*., 2019).

Climate change also play a role in the dispersion of AMR bacteria. Increased temperatures can lead to a lower host immune response or affect the survival and evolution of the pathogen. An example is Vibrionaceae which prefers warm waters. It is very likely that increasing seawater temperatures will see an increase in the geographic and seasonal distribution of these pathogens. Therefore, the probability of turtles coming into contact with this agent is greater (Pace *et al*., 2019).

Sea turtles can also transmit AMR bacteria and resistance genes to humans and other animals (see Figure 3). Humans can acquire these AMR bacteria by interacting with animals, and in some cases, this is associated with consuming their meat or eggs in illegal bushing meat trade. In certain coastal communities where this turtle species is available, it is a common practice to use the entire animal to consumption. The meat can be prepared by grilling or stewing, internal organs (e.g., liver) can be used to make soup, oil can be extracted from the fat and used in alternative medicine to treat respiratory problems. Additionally, the eggs and blood are consumed raw and believed to have medical properties for treating conditions like anaemia, asthma or as an aphrodisiac (Zavala-Norzagaray *et al*., 2015). Illegal consumption of sea turtle meat or eggs can have negative impacts on humans. As these products are not inspected, wild animals are host to numerous agents (some zoonotic) and the hygiene of the places where they are processed is very low, potentially carrying pathogenic agents. There are already reports of humans becoming sick to the consumption of meat/eggs of sea turtles with *Vibrio mimicus*, *V. cholerae*, *Morganella* spp., *Pseudomonas* spp., and *Salmonella chester* (Zavala-Norzagaray *et al*., 2015).

As migratory animals (in some species) it is expected that, similar to what has been observed in birds, turtles can acquire AMR bacteria while feeding or in their breeding sites and can subsequently spread these bacteria through defecation when they move between different geographical areas. Additionally, during their migrations, these animals may enter highly polluted waters near the coast and carry these AMR bacteria to other regions (Laborda *et al*., 2022).

ANTIBIOTIC RESISTANCE BACTERIA IN SEA TURTLE

Of the 19 papers presented (Table 1), 47.4% (9/19) were performed in *Caretta caretta*, 21% (4/19) *Chelonia mydas*, 5.3% (1/19) *Lepidochelys olivacea*, 5.3% (1/18) *Dermochelys coriacea*. The remaining 4 studies were carried out on several species simultaneously.

The studies were carried out in different locations around the globe including The Gulf of Oman and the Arabian Sea, the Great Barrier Reef, Taiwan, the Gulf of California, the Pacific Ocean, the Adriatic Sea, Italy, the Mediterranean, Brazil, the Gulf of Guinthea, St. Kitts, West Indies (Table 1). Different types of samples were collected in the different studies such as oviductal fluid, eggs, nasopharynx-geal, nasal, cloacal, oral, tissue (e.g., spleen) ocular and faeces from healthy individuals. The type of sample that predominated was the cloacal swabs (10/18), followed by oral swabs. Four studies were performed on oviductal fluid and egg content. Three studies collected samples from animals showing symptoms of infections (e.g. abscess, septicaemia) (Trotta *et al*., 2021a). As most of the studies were performed on cloacal and oral samples, the bacteria isolated were mostly Enterobacteriaceae. These bacteria constitute a large portion of the microflora of these anatomical regions. Other bacteria observed in other families are *Listeria monocytogenes*, *Bacillus* spp., *Staphylococcus* spp., and *Vibrio* spp.

It is important to refer that some zoonotic bacteria have been isolated from sea turtles that are resistant to medically important drugs. Some examples are *Escherichia coli* resistant to cephalexin, ceftiofur, enrofloxacin, and doxycycline (Ahasan *et al*., 2017), or *Pseudomonas* spp. and *E. faecalis* to gentamicine (Al-Musharafi *et al*., 2015; Tsai *et al*., 2019).

Table 1. Antibiotic resistance in sea turtles regarding turtle species, captured location, sampling year, type of sample, bacteria, antibiotic resistance and resistance genes.

Turtle species	**Captured location**	**N**	**Sampling year**	**Type of sample**	**Bacteria**	**Antibiotic resistance**	**Resistance gene**	**Ref.**
Chelonia mydas	Gulf of Oman and the Arabian Sea	20	2011	Oviductal fluids	*Citrobacter* spp., *Pseudomonas* spp., *Proteus* spp., *Morganellas* spp., *Enterobacter* spp., *Salmonella* spp., *Escherichia coli*, *Edwardsiella* spp., *Providencia* spp., *Shigella* spp., *Arcomobacter* spp.	Ampicillin, tetracycline, sulphamethoxazole	cephamycinase (CMY), 39.1% extended-spectrum beta-lactamases (EBSLs), 18.6% AmpC type beta-lactamases C (AmpC)	(Al-Bahry *et al*., 2011)
	Gulf of Oman and the Arabian Sea	40	2010	Oviductal fluids	*Citrobacter* spp. (51.4%), *Pasteurella* spp. (16.3%), *Pseudomonas* spp. (11.6%), *Salmonella* spp. (11.6%), *Proteus* spp. (4.7%), *Shigella* spp. (2.3%), *Aeromonas* spp. (2.1%),	Ampicillin, streptomycin, and sulphamethoxazole	-	(Al-Bahry *et al*., 2012)
	Great Barrier Reef	73	2015–2016	Cloacal	*Citrobacter* spp. (30.52%), *Escherichia coli* (12.34%), *Edwardsiella* spp. (21.43%)	Penicillin (76.6%), ampicillin (74.0%), amoxicillin-clavulanic acid (67.5%), cephalexin (68.9%), ceftiofur (55.8). nalidixic acid (46.1%), enrofloxacin (46.8%,), doxycycline (46.1%,)	-	(Ahasan *et al*., 2017)
	Taiwan	28	2018–2020	Cloacal and nasal	*Vibrio* spp.	Penicillin (74.47%), spiramycin, amoxicillin, and cephalexin	-	(Tsai *et al*., 2021)
	Gulf of Oman in the Arabian Sea	20	2015	Albumen and yolk	*Citrobacter* spp., *Aeromonas* spp., *Pseudomonas* spp.	Amikacin, trimethoprim-sulfamethoxazole, gentamicin, ampicillin	-	Al-Musharafi *et al*., 2015)
Chelonia mydas agassizii, *Lepidochely olivacea*	Gulf of California, Pacific Ocean	420	2012	Nasopharyngeal and cloacal	*Vibrio alginolyticus* (60%), *V. parahaemolyticus* (26%), *V. cholerae* (9%).	Ampicillin	29.4% tdh+gene, 11.7%tdh+and toxRS/new+, 100% non-O1/non-O139, 66% gene ace	(Zavala-Norzagaray *et al*., 2015)

Table 1. (Continued)

Turtle species	Captured location	N	Sampling year	Type of sample	Bacteria	Antibiotic resistance	Resistance gene	Ref.
Lepidochelys olivacea	Taiwan	1	2019	Abscess	*E. faecalis*	Doxycycline, enrofloxacin, erythromycin, neomycin, oxytetracycline, gentamicin, amikacin, ciprofloxacin, spiramycin, chloramphenicol, ceftiofur, azithromycin	tet(M)	(Tsai *et al*., 2019)
Caretta caretta	Adriatic Sea	200	2018–2021	Oral, cloacal samples, skin wounds, biopsy	*E. coli*, *raxella* spp., *Kluyvera* spp., *Salmonella* spp., *Acinetobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Morganella* spp.	Ampicillin, amoxicillin-clavulanic acid, ceftazidime, cefuroxime, gentamicin, doxycycline, ciprofloxacin, enrofloxacin	-	(Trotta *et al*., 2021b)
	Italy	52	2016–2019	Clinical samples	*Aeromonas hydrophila*, *Vibrio vulnificus*, *V. alginolyticus*, *V. metschnikovii*, *V. fluvialis*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *P. putida*, *P. putrefaciens*, *Acinetobacter calcoaceticus*, *Morganella morganii*, *Proteus vulgaris*, *Enterobacter cloacae*, *Alcaligenes faecalis*, *Klebsiella oxythoca*	Ampicillin (97.5%), amoxicillin-clavulanic acid (90%), cephalexin (90%), pradofloxacin (82.5%), cefotaxime (77.5%), ceftazidime (70%), danofloxacin (77.5%), cefuroxime (72.5%), moxifloxacin (67.5%), ciprofloxacin (67.5%), aztreonam (60%), enrofloxacin (60%), tetracycline (58%), trimethoprim-sulphamethoxazole (50%), doxycycline, (47.5%)	$bla_{ACT\text{-}24}$, $bla_{ACT\text{-}2}$, $bla_{ACT\text{-}17}$, $bla_{DHA\text{-}4}$, $bla_{CMY37,\ CTX+AmpC}$, $bla_{ACT\text{-}2+TEM\text{-}236+SHV\text{-}12}$, $bla_{CTX\text{-}M\text{-}3+ACT\text{-}24+TEM\text{-}236}$	(Trotta *et al*., 2021a)
	Mediterranean	35	2015–2016	Oral and cloacal	*Morganella morganii,* *Citrobacter* spp., *Proteus* spp., *Pseudomonas aeruginosa*	Penicillins, tetracyclines, phenicols, quinolones, cephalosporins	-	(Pace *et al*., 2019)
	Italy	107	2016–2020	Spleen	*Salmonella enteritidis*	-	bla_{TEM} tet(D), tet(E) sulI	(Gambino *et al*., 2022)

Table 1. (Continued)

Turtle species	Captured location	N	Sampling year	Type of sample	Bacteria	Antibiotic resistance	Resistance gene	Ref.
Caretta caretta	South Tyrrhenian Sea, Ionian Sea	14	2006–2007	Oral and cloacal	*Citrobacter* spp., *Proteus* spp., *Enterobacter* spp., *Escherichia* spp., *Providencia* spp., *Morganella* spp., *Pantoea* spp., *Pseudomonas* spp., *Shewanella* spp.	Carbenicillin (100%), cephalothin (92.6%), oxytetracycline (81.3%), amoxicillin (77.8%), colistin (72.0%), tetracycline (64.9), ampicillin (63.6%) ticarcillin-clavulanic acid (52.9%), lomefloxacine (51.9%).	-	(Foti *et al*., 2009)
	Island of Maio	33	2021	cloacal (oviductal fluid), oral, and egg content	*Shewanella putrefaciens, Morganella morganii, Vibrio alginolyticus, Enterobacter cloacae, Aeromonas hydrophila/caviae, Brevundimonas vesicularis, Burkholderia cepacia,* and *Citrobacter* spp.	Imipenem, enrofloxacin, tetracycline, piperacillin	-	(Fernandes *et al*., 2021)
	Italy	1	2021	Organs	*Listeria monocytogenes*	Fosfomycin, quinolones, sulfamethoxazole, oxacillin, and cephalosporins	fosX, lin, mprF, norB, sul	(Di Renzo *et al*., 2022)
	Mediterranean	33	2014	Cloacal	*Enterobacteriaceae* (59%), *Shewanellaceae* (31%), *Vibrionaceae families* (5%)	Ampicillin (70%), sulfamethoxazole/ trimethoprim (30%), tetracycline, ciprofloxacin, chloramphenicol, kanamycin, streptomycin, nalidixic acid	-	(Blasi *et al*., 2020)
Eretmochelys imbricata, Chelonia mydas	Brazil	17	2012–2014	Faecal samples	*Enterococcus* spp.	Tetracycline, rifampin, enrofloxacin, norfloxacin	-	(Prichula *et al*., 2016)
Eretmochemys imbricata, Chelonia mydas,	Gulf of Guinea	12	2010	Oral and cloacal	*Alcaligenes faecalis, Pseudomonas aeruginosa, P. stutzeri, P. mendocina*	Ticarcillin, ticarcillin-clavulanic acid, aztreonam	-	(Oliveira *et al*., 2017)
Dermochelys coriacea	St. Kitts, West Indies	21	2011	cloacal	*Salmonella enterica*	-	-	(Dutton *et al*., 2013)

Table 1. (Continued)

Turtle species	Captured location	N	Sampling year	Type of sample	Bacteria	Antibiotic resistance	Resistance gene	Ref.
Chelonia mydas, Caretta caretta, Eretmochelys imbricata, Lepidochelys olivacea	Brazil	36	2018	Ocular	*Bacillus* spp., *Staphylococcus* spp.	Oxacillin, polymyxin B	-	(Cardoso-Brito *et al.*, 2019)

Regarding antibiotic resistance, the majority was observed in the classes of penicillins, tetracyclines, phenicols, quinolones, and cephalosporins. Penicillin resistance (ampicillin, penicillin, and amoxicillin) was the most observed in a total of 11 studies. In only six studies resistance genes were studied. Some of the genes that were detected were: bla_{TEM}, tet(D), tet(E) sulI, tet(M), bla_{ACT-24}, bla_{ACT-2}, bla_{ACT-17}, bla_{DHA-4}, and bla_{CMY37}, among others.

The production of extended-spectrum beta-lactamases (ESBLs) and carbapenem resistance by Enterobacteriaceae, methicillin-resistant *Staphylococcus pseudointermedius* (MRSP), methicillin-resistant *Staphylococcus aureus* (MRSA), and vancomycin-resistant *Enterococci* (VRE), are some of the core public health concerns in the 21st century regarding AMR (Garcês *et al*., 2019). ESBL has only been recorded to date in the specie *Chelonia mydas* from individuals recovered from the Arabian Sea (Al-Bahry *et al*., 2011).

The emergence of antibiotic resistance bacteria in sea turtles is a growing concern because it can affect their health and survival. With several species under threat, the consequences can be very serious, not only for the biodiversity of this group of animals but also for the health of other animals and the environment. Living mainly in the sea, they can provide us with valuable information about the state of the marine ecosystem. Unfortunately, as many of these animals are on verge of extinction it is sometimes very difficult to access samples to perform studies because they are protected animals and because there are so few exemplars. This condition increases the risk of infections and reduces the effectiveness of medical treatments, reducing therapeutic options, which can lead to chronic or recurrent infections and even death. It can also compromise the health of sea turtles, impairing their intestinal microbiota and disrupting their metabolic and immune functions. Turtles can be exposed to antibiotics in a number of ways, such as contaminated water, food, or medical treatment. Although it is not yet a widely studied subject, some studies have considered the prevalence and diversity of antibiotic-resistant bacteria in sea turtles, as well as the factors that contribute to the appearance and spread of AMR bacteria in these animals.

Several studies have investigated antibiotic resistance in sea turtles. For instance, Pace *et al*. (2019) found that gut bacteria from green turtles exhibited high levels of resistance to several antibiotics, including ampicillin, erythromycin, and tetracycline. Another study by Blasi *et al*. (2020) showed that AMR bacteria was present in the skin and oral cavities of loggerhead turtles. In this review, it was possible to observe that in most studies, animals show resistance, especially to the penicillin class. This can be associated with the fact that this category of antibiotic was the first to be used for many years worldwide, so high resistance is expected (Dube *et al*., 2021; Fernandes *et al*., 2021).

Based on the data, sea turtles can acquire and carry AMR bacteria in various parts of their anatomy. The origin of these bacteria is almost impossible to determine, but three studies showed that they can be present from birth through vertical transmission (Al-Bahry *et al*., 2011; 2012; Fernandes *et al*., 2021). Some authors have suggested that the fluid produced during oviposition may contain antimicrobial properties that protect against potential pathogens in the sand and mucosa (Praja *et al*., 2021). There is the possibility of this natural antibiotic carrying out some selection of genes when associated with other agents that may be present in the water or soil.

Enterobacteriaceae are often blamed for the spreading of resistant genes. Sea turtle microbiome change throught their lives. Several authors refer that the prevalence of Enterobacteriaceae is higher in younger animals. This has been described in other animal species. In older turtles, Enterobacteriaceae are outcompeted by other species of microorganisms (Al-Bahry *et al*., 2011; 2012; Fernandes *et al*., 2021). Furthermore, changes in the blood parameters, influence the occurrence of Enterobacteriaceae, as implied by the lower values of packed cell volume and lymphocytes found in younger turtles. This variation may make turtles more susceptible to acquiring RAM bacteria at certain times (Trotta *et al*., 2021a).

The proliferation of AMR bacteria in sea turtles can harm their growth, maturation, and overall health. Antimicrobial resistance and resistance genes have been detected in both healthy sea turtles and sick sea turtles during rehabilitation. According to Drane *et al*. (2021) 58% and 49% of AMR bacteria isolates from sea turtles in all published studies are from animals that have been in a rehabilitation center. In this review, seven studies examined animals held in captivity at a rehabilitation center recovering from injuries or other diseases. This result is not surprising because they use broad-spectrum antibiotic, as complementary exams are expensive and sometimes unavailable (Drane *et al*., 2021). What could lead to the development of AMR or the selection of pathogenic strains. The antibiotics most used in human and veterinary medicine are quinolones (enrofloxacin and nalidixic acid), beta-lactams (amoxicillin-clavulanic acid and ampicillin), and tetracyclines (tetracycline and doxycycline). In several of the studies presented, it is possible to observe that these groups of antibiotics are those with the highest rates of resistance. Therefore, it is possible to hypothesize that this high frequency of resistance could potentially originated from wastewater treatment plants (sewage, agriculture waste) and turtle rehabilitation centers (Drane *et al*., 2021). Both acquired and intrinsic antibiotic resistance can reduce therapeutic options and complicate treatment during rehabilitation. Data regarding resistance patterns can be helpful in rehabilitation facilities for efficient treatment and to reduce the risk of releasing animals back into the wild that could disseminate AMR bacteria into their natural environment (Pace *et al*., 2019).

RECOMMENDATION

Because sea turtles can be hosts for AMR bacteria, they are undoubtedly an important biological indicator of environmental health. However, there are still gaps in information about the dynamics and mechanisms of action of these pathogenic agents. Wild animals such as sea turtles, are often not involved in epidemiological surveillance for disease monitoring. Due to its status as an endangered species, accessing samples of it is even more complicated. Sometimes, it is only possible in a rehabilitation center when they are already in contact with other contaminated animals or exposed to the antibiotic.

It is therefore important to control the prevalence and diversity of antibiotic-resistant bacteria among sea turtles, as well as the sources and mechanisms of resistance. To mitigate the risks of antibiotic resistance in sea turtles, it is essential to implement strategies that promote the responsible use of antibiotics in animal and human medicine, as well as in aquaculture and other sectors. The use of antibiotics in aquaculture is a worrying situation because the regulation is rare. Such strategies may include reducing the use of antibiotic, strengthening hygiene and biosecurity practices, and promoting the development of alternative therapies and treatments.

Without a doubt, antibiotic resistance is a global problem that impacts even the most isolated wild animals. It is important to advise the coastal populations to be careful with these animals when manipulating them or consuming their meat or eggs. Although bacteria producing ESBL or MRSA have not yet been reported in these animals, it is possible that they carry these agents, but sufficient investigation has not yet been carried out. In the future, additional studies will be needed within the One Health system to establish the role of sea turtles in the spread and acquisition of AMR bacteria in the marine ecosystem.

ACKNOWLEDGEMENTS

The participation of Pires I was supported by the projects UIDB/CVT/00772/2020 and LA/P/0059/2020, funded by the Portuguese Foundation for Science and Technology (FCT). (Project UIDB/CVT/0772/2020). The participation of Garcês A. was supported by National Funds from FCT Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020.

LITERATURE CITED

Aguirre, A.A. and P.L. Lutz. 2004. Marine turtles as sentinels of ecosystem health: Is Fibropapillomatosis an Indicator. **EcoHealth** 1: 275–283. DOI: 10.1007/s10393-004-0097-3.

Ahasan, M.S., J. Picard, L. Elliott, R. Kinobe, L. Owens and E. Ariel. 2017. Evidence of antibiotic resistance in Enterobacteriales isolated from green sea turtles, *Chelonia mydas* on the Great Barrier Reef. **Marine Pollution Bulletin** 120: 18–27. DOI: 10.1016/j.marpolbul.2017.04.046.

Al-Bahry, S.N., I.Y. Mahmoud, M. Al-Zadjali, A. Elshafie, A. Al-Harthy and W. Al-Alawi. 2011. Antibiotic resistant bacteria as bio-indicator of polluted effluent in the green turtles, *Chelonia mydas* in Oman. **Marine Environmental Research** 71: 139–144. DOI: 10.1016/j.marenvres.2010.12.005.

Al-Bahry, S.N., M. Al-Zadjali, I.Y. Mahmoud and A. Elshafie. 2012. Biomonitoring marine habitats in reference to antibiotic resistant bacteria and ampicillin resistance determinants from oviductal fluid of the nesting green sea turtle, *Chelonia mydas*. **Chemosphere** 87: 1308–1315.

Al-Musharafi, S., I. Mahmoud and S. Al-Bahry. 2015. Heavy metals and antibiotic resistant bacteria in green turtles are indicators of environmental pollution. **International Journal of Biological, Food, Veterinary and Agricultural Engineering** 9: 356–359.

Alduina, R., D. Gambino, A. Presentato, A. Gentile, A. Sucato and D. Savoca. 2020 Is *Caretta Caretta* a carrier of antibiotic resistance in the Mediterranean Sea. **Antibiotics** 9: 116. DOI: 10.3390/antibiotics9030116.

Alfaro-Núñez, A., M.F. Bertelsen, A.M. Bojesen, I. Rasmussen, L. Zepeda-Mendoza and M.T. Olsen. 2014. Data from: Global distribution of Chelonid fibropapilloma-associated herpesvirus among clinically healthy sea turtles. **BMC Ecology and Evolution** 1: 82241. DOI: 10.5061/DRYAD.8R082.

Blasi, M.F., L. Migliore, D. Mattei, A. Rotini, M.C. Thaller and R. Alduina. 2020. Antibiotic resistance of gram-negative bacteria from wild captured loggerhead sea turtles. **Antibiotics** 9: 162. DOI: 10.3390/antibiotics9040162.

Bonnedahl, J. and J.D. Järhult. 2014. Antibiotic resistance in wild birds. Upsala. **Journal of Medical Sciences** 119: 113–116. DOI: 10.3109/03009734.2014.905663.

Cardoso-Brito, V., A.C.S. Raposo, T.T. Pires, M.H. Pinna and P.A. Oriá. 2019. Conjunctival bacterial flora and antimicrobial susceptibility of captive and free-living sea turtles in Brazil. **Veterinary Ophthalmology** 22: 246–55. DOI: 10.1111/vop.12584.

Caron, A.G.M., C.R. Thomas, K.L.E. Berry, C.A. Motti, E. Ariel and J.E. Brodie. 2018. Ingestion of microplastic debris by green sea turtles (*Chelonia mydas*) in the Great Barrier Reef: Validation of a sequential extraction protocol. **Marine Pollution Bulletin** 127: 743–751. DOI: 10.1016/j.marpolbul.2017.12.062.

Di Renzo, L., M.E. De Angelis, M. Torresi, V. Di Lollo, G. Di Teodoro and D. Averaimo. 2022. First report of Septicaemic Listeriosis in a loggerhead sea turtle (*Caretta caretta*) Stranded along the Adriatic Coast: Strain detection and sequencing. **Animals** 12: 2364. DOI: 10.3390/ani12182364.

Drane, K., R. Huerlimann, M. Power, A. Whelan, E. Ariel and M. Sheehan. 2021. Prevalence, risk factors and antimicrobial resistance of Salmonella infection in turtles in Khon Kaen province. **Antibiotics** 10: 775. DOI: 10.3390/antibiotics10070775.

Dube, F., R. Söderlund, M. Lampinen Salomonsson, K. Troell and S. Börjesson. 2021. Benzylpenicillin-producing *Trichophyton erinacei* and methicillin resistant *Staphylococcus aureus* carrying the mecC gene on European hedgehogs – A pilot-study. **BMC Microbiol** 21: 212. DOI: 10. 1186/s12866-021-02260-9.

Dutton, C.S., F. Revan, C. Wang, C. Xu, T.M. Norton and K.M. Stewart. 2013 *Salmonella enterica* prevalence in leatherback sea turtles (*Dermochelys coriacea*) in St. Kitts, West Indies. **Journal of Zoo and Wildlife Medicine** 44: 765–768. DOI: 10.1638/ 2012-0216R1.1.

Fernandes, M., M.L. Grilo, C. Carneiro, E. Cunha, L. Tavares and J. Patino-Martinez. 2021. Antibiotic resistance and virulence profiles of gram-negative bacteria isolated from loggerhead sea turtles (*Caretta caretta*) of the Island of Maio, Cape Verde. **Antibiotics** 10: 771. DOI: 10.3390/ antibiotics10070771.

Foti, M., C. Giacopello, T. Bottari, V. Fisichella, D. Rinaldo and C. Mammina. 2009. Antibiotic resistance of gram negatives isolates from loggerhead sea turtles (*Caretta caretta*) in the central Mediterranean Sea. **Marine Pollution Bulletin** 58: 1363–1366. DOI: 1016/j.marpolbul.2009.04.020.

Foti, M., C. Giacopello, V. Fisichella and G. Latella. 2013. Multidrug-Resistant *Pseudomonas aeruginosa* Isolates from captive reptiles. **Journal of Exotic Pet Medicine** 22: 270–274. DOI: 10.1053/j.jepm.2013.08. 007.

Gable, S.M., M.I. Byars, R. Literman and M. Tollis. 2021. A genomic perspective on the evolutionary diversification of turtles. **Systematic Biology** 10(14): 464421. DOI: 10.1101/2021.10.14.464421.

Gambino, D., D. Savoca, A. Sucato, V. Gargano, A. Gentile and L. Pantano. 2022. Occurrence of antibiotic resistance in the Mediterranean Sea. **Antibiotics** 11: 332. DOI: 10.3390/ antibiotics11030332.

Garcês, A., S. Correia, F. Amorim, J.E. Pereira, G. Igrejas and P. Poeta. 2019. First report on extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from European free-tailed bats (*Tadarida teniotis*) in Portugal: A one-health approach of a hidden contamination problem. **Journal of Hazardous Materials** 370: 219–224. DOI: 10.1016/j.jhazmat.2017.12.053.

Laborda, P., F. Sanz-García, L.E. Ochoa-Sánchez, T. Gil-Gil, S. Hernando-Amado and J.L. Martínez. 2022. Wildlife and antibiotic resistance. **Frontiers in Cellular and Infection Microbiology** 12: 873989. DOI: 10.3389/fcimb.2022.873989.

Mortimer, J.A. and M. Donnelly. 2008. ***Eretmochelys imbricata***. The IUCN Red List of Threatened Species 2016. https://dx.doi. org/10.2305/IUCN.UK.2008.RLTS.T8005 A12881238.en. Cited 14 Nov 2022.

Oliveira, M., I. Serrano, J.P. Santos, F. Bilocq, N. Pereira and N. de Santos Loureiro. 2017. Pseudomonads from wild free-living sea turtles in Príncipe Island, Gulf of Guinea. **Ecological Indicators** 81: 260–264. DOI: 10.1016/j.ecolind.2017.06.005.

Pace, A., L. Dipineto, A. Fioretti and S. Hochscheid. 2019. Loggerhead sea turtles as sentinels in the western Mediterranean: Antibiotic resistance and environment-related modifications of gram-negative bacteria. **Marine Pollution Bulletin** 149: 110575. DOI: /10.1016/j.marpolbul.2019.110575.

Praja, R.N., A. Yudhana, W. Haditanojo and V. Oktaviana. 2021. Short Communication: Antimicrobial properties in cloacal fluid of olive ridley sea turtle (*Lepidochelys olivacea*). **Biodiversitas Journal of Biological Diversity** 22(9): 3671-3676. DOI: 10.13057/biodiv/d220909.

Prichula, J., R.I. Pereira, G.R. Wachholz, L.A. Cardoso, N.C.C. Tolfo and N.A. Santestevan. 2016. Resistance to antimicrobial agents among enterococci isolated from fecal samples of wild marine species in the southern coast of Brazil. **Marine Pollution Bulletin** 105: 51–57. DOI: 10.1016/j.marpolbul.2016.02.071.

Sherley, M., D.M. Gordon and P.J. Collignon. 2000. Variations in antibiotic resistance profile in Enterobacteriaceae isolated from wild Australian mammals. **Environmental Microbiology** 2: 620–631. DOI: 10.1046/j.1462-2920.2000.00145.x.

Trotta, A., M. Cirilli, M. Marinaro, S. Bosak, G. Diakoudi and S. Ciccarelli. 2021a. Detection of multi-drug resistance and AmpC β-lactamase/extended-spectrum β-lactamase genes in bacterial isolates of loggerhead sea turtles (*Caretta caretta*) from the Mediterranean Sea. **Marine Pollution Bulletin** 164: 112015. DOI: 10.1016/j.marpolbul.2021.112015.

Trotta, A., M. Marinaro, A. Sposato, M. Galgano, S. Ciccarelli and S. Paci. 2021b Antimicrobial resistance in loggerhead sea turtles (*Caretta caretta*): A comparison between clinical and commensal bacterial isolates. **Animals** 11: 2435. DOI: 10.3390/ani11082435.

Tsai, M.A., C.C. Chang and T.H. Li. 2019 Multiple-antibiotic resistance of *Enterococcus faecalis* in an endangered olive ridley sea turtle (*Lepidochelys olivacea*): A case report. **Indian Journal of Animal Research** 1: 1064. DOI: 10.18805/ijar.B-1064.

Tsai, M.A., C.C. Chang and T.H. Li. 2021. Antimicrobial-resistance profiles of gram-negative bacteria isolated from green turtles (*Chelonia mydas*) in Taiwan. **Environmental Pollution** 277: 116870. DOI: 10.1016/j.envpol.2021.116870.

Zavala-Norzagaray, A.A., A.A. Aguirre, J. Velazquez-Roman, H. Flores-Villaseñor, N. León-Sicairos and C.P. Ley-Quiñonez. 2015 Isolation, characterization, and antibiotic resistance of *Vibrio* spp. in sea turtles from Northwestern Mexico. **Frontiers in Microbiology** 6: 635. DOI: 10.3389/fmicb.2015.00635.