EcoHealth
https://doi.org/10.1007/s10393-023-01662-7

EcoHealth

Original Contribution

Potentially Pathogenic Bacteria in Nesting Olive Ridley Turtles in Northwestern Mexico

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Abstract: Olive ridleys (*Lepidochelys olivacea*) are the most common sea turtle found in the Gulf of California. Unfortunately, the bacterial flora of nesting olive ridley turtles is still unknown. We conducted a study to identify, characterize, serotype, and determine the antibiotic resistance of potentially pathogenic bacteria isolated from olive ridley turtles nesting in northwestern Mexico. Bacteria were isolated and identified from the oral cavity and cloaca of 47 postnesting turtles. *Escherichia coli* and *Vibrio parahaemolyticus* were characterized, and antibiotic resistance testing was performed. One hundred bacteria belonging to 21 species were isolated, 53 from the oral cavity and 47 from the cloaca, the most prevalent being *Pseudomonas aeruginosa*, followed by *Aeromonas hydrophila*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Klebsiella pneumoniae*, and *E. coli*, among others. Moreover, two to three different bacterial species were found co-colonizing both anatomical sites in some turtles. *E. coli* phylogroups B1, A, F, and unknown were identified as diarrheagenic *E. coli* (enteroaggregative and enteropathogenic *E. coli*). O1, O4, K8, K12, OUT, and KUT of *V. parahaemolyticus* serogroups were identified, also comprising pathogenic and nonpathogenic strains. Finally, 100% of the bacterial species tested were antibiotic resistant, and both MDR and XDR strains were found. In conclusion, olive ridley turtles are colonized by a diversity of bacterial species with a high rate of antibiotic resistance, some with pathogenic potential to turtles, representing a health risk factor for the species.

Keywords: Bacteria, *Escherichia coli*, *Vibrio parahaemolyticus*, Nesting, Olive ridley turtles

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Introduction

Sea turtles play a critical role in terrestrial and aquatic ecosystems, such as coral reefs and seagrass beds, bringing energy dispersing seeds to aid mineral cycling and carbon storage between oceanic and terrestrial habitats (Lovich et al. 2018; Turtle 2022) due to long migratory journeys between feeding and nesting grounds (Mérida-López 2011). However, due to anthropogenic impacts such as fishing and other anthropogenic activities in coastal areas, sea turtle populations have been in steep decline for several decades; in fact, six of the seven species of sea turtles are considered vulnerable, including olive ridley turtles (*Lepidochelys olivacea*) (IUCN 2015).

Olive ridleys are the most common turtle species in the Gulf of California, specifically at Ceuta beach, Sinaloa, in northwestern Mexico. Ceuta beach is considered a sea turtle sanctuary because it is a priority nesting beach for the species, with an average of 621 nests per year on a 35 km beach (CONANP 2009; Sosa-Cornejo I 2016). Unfortunately, there are several activities that affect the species in Sinaloa, including incidental capture during fishing, illegal meat and egg trafficking, and water contamination with plastics or agricultural runoff (Aguirre and Lutz 2004). Previously, our research team analyzed the bacterial microbiota of olive ridley sea turtles in a feeding ground in northwestern Mexico. That study demonstrated the presence of several potential pathogens in these sea turtles (Zavala-Norzagaray et al. 2015). However, there is no information on the bacterial microbiota of olive ridley turtles in the nesting areas of northwestern Mexico or the possible presence of pathogenic bacteria that could pose a potential health risk for turtle nesting and reproduction at this site.

The microorganisms that colonize turtles, including *Fusarium* spp., *Enterococcus* spp., and *Salmonella* spp., among others (Nowakiewicz et al. 2015), can also represent a health risk for potential infectious diseases in immunosuppressed turtles during rehabilitation activities. In addition, recent reports have isolated antibiotic-resistant bacteria from turtles (Trotta et al. 2021). Multidrug-resistant bacteria can complicate the recovery of turtles with an infectious disease and may represent a health risk for humans if turtle meat and eggs are consumed. Antibiotic resistance is a public health problem and continues to grow in marine animals (Laborda et al. 2022). The aim of this study was to identify, characterize, and determine the antibiotic resistance of potentially pathogenic bacteria isolated from oropharyngeal and cloacal swabs of olive ridley turtles nesting in northwestern Mexico.

Methods

Study Site

From August to November 2018, nasopharyngeal and cloacal swabs were taken from postnesting olive ridley turtles at the Ceuta Beach Sanctuary (CBS), Sinaloa, Mexico (23°52′00″N 106°56′00″W) (Fig. 1). CBS is considered a protected natural area for the conservation, repopulation, development, and control of different sea turtle species found in northwestern Mexico. Unfortunately, in the CBS, as in the rest of Sinaloa´s Beach, there are some activities that can affect the nesting process of turtles, including aquaculture, agriculture practices, and human settlement (Fig. 1).

Specimen Collection

Two nasopharyngeal and cloacal swabs were collected from each turtle for microbiological identification, and the samples were taken after the turtles completed the nesting process. Once this procedure was completed, the turtles were released unharmed to continue their return crawl to the sea. Swab samples were placed in alkaline peptone water at pH 8.5 (APW) for *Vibrio* spp. and in buffered peptone water pH 7.2 (BPW) for Enterobacteriaceae and then transported for bacterial strain isolation and identification. Ethical issues: The research was approved by the Mexican Environment and Natural Resources Ministry (SEMARNAT), and sampling, handling, and care of individuals were carried out under the proper research permits: SGPAC/DGVS/08562/17 and SGPA/DGVS/010518/18. Our study complied with all local, state, and national regulations. Meticulous efforts were made to ensure that animals were subjected to the least suffering possible, as well as to reduce external sources of stress, pain, and discomfort.

Isolation and Identification of Bacterial Strains

For *Vibrio* spp., all nasopharyngeal and cloacal swabs were placed in APW and streaked onto thiosulfate citrate bile salt sucrose agar (TCBS; Becton–Dickinson, USA) and CHROMagar Vibrio (CHROMagar Paris, France). The plates were incubated overnight at 37°C. From each plate, green and yellow colonies in TCBS or blue and violet co-

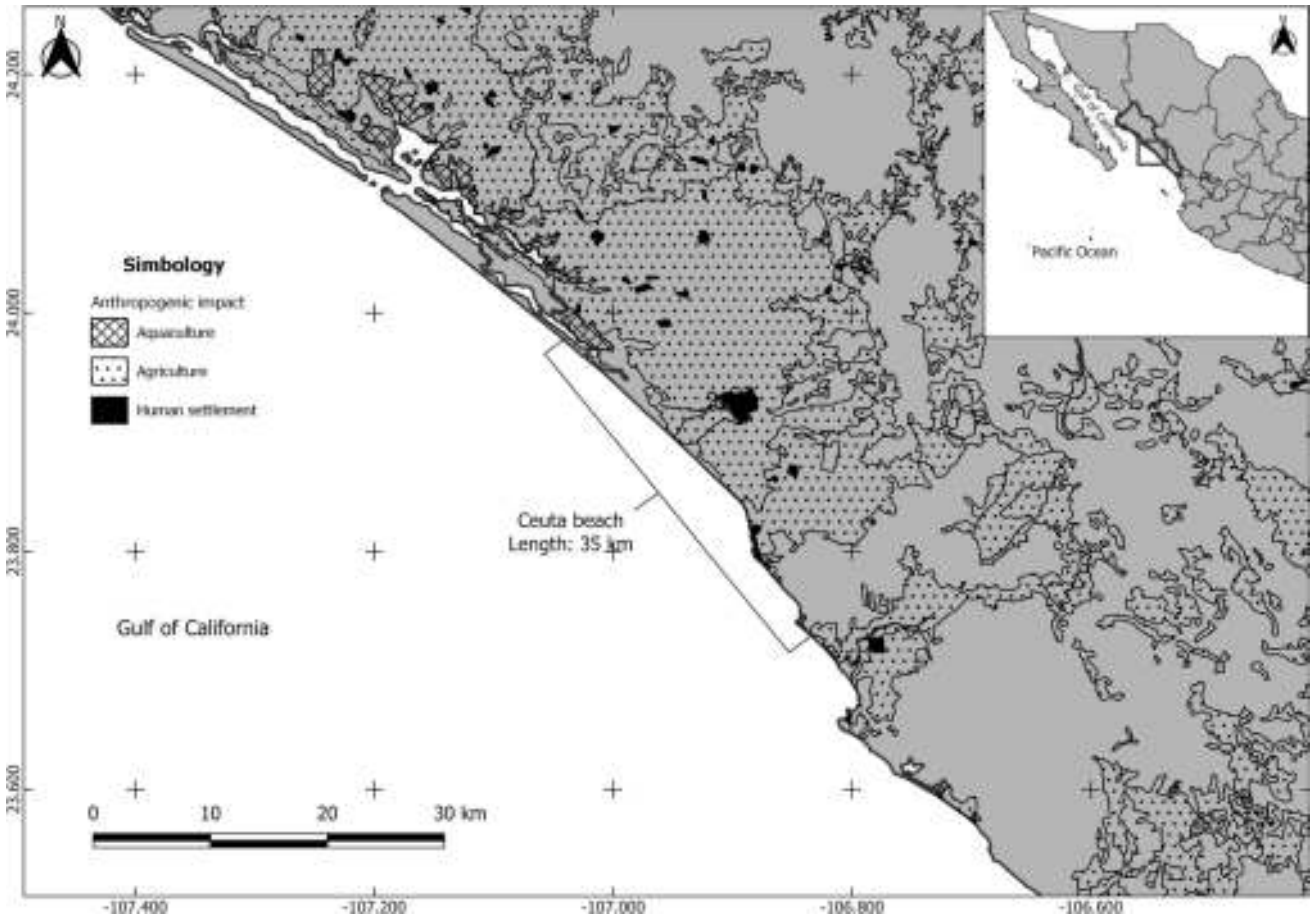

Figure 1. Map of nesting and sampling sites in the region of Ceuta, Sinaloa, northwestern Mexico. Map showing the location of anthropogenic factors near *Olive ridleys* (*Lepidochelys olivacea*) nesting beaches. These include aquaculture, agricultural practices, and human settlements.

lonies in CHROMagar Vibrio exhibiting diverse morphology were transferred to TSA with 2% NaCl agar for purity. These plates were incubated overnight at 37°C, and identification was performed using a single isolated colony. Each colony was examined using the oxidase test, and all biochemical tests were performed as described in the Bacteriological Analytical Manual of the Food and Drug Administration for *Vibrio* spp. (Kaysner et al. 2004; Canizalez-Roman et al. 2011). At least three typical colonies of *Vibrio* spp. were isolated from each plate and subjected to identification by biochemical tests and PCR. After identification of *V. parahaemolyticus,* a single colony from each sample was used to continue the analysis (serotyping, virulence genes, or antibiotic susceptibility testing). For Enterobacteriaceae, specimens were placed in BPW and streaked onto Salmonella-Shigella, Hektöen, and McConkey agar (Becton–Dickinson, USA). The plates were incubated overnight at 37°C. The presumptive colonies were transferred to TSA agar for purity. These plates were incubated overnight at 37°C and proceeded with identification using a single isolated colony. Each colony was examined using the biochemical tests for *Vibrio spp., Aeromonas* spp., *Burkholderia* spp., *Chromobacterium* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Providencia* spp., *Pseudomonas* spp., *Rahnella* spp, *Raoultella* spp., and *Serratia* spp. (Andrews and Jacobson 2013; Andrews et al. 2018; Feng et al. 2020).

PCR Assays

PCR assays were performed to identify different bacterial species. To identify *Vibrio parahaemolyticus,* we used a protocol previously described (Velazquez-Roman et al. 2012; de Jesús Hernández-Díaz et al. 2015), using primers targeting the following genes: *tl*, *pR72H* plasmid and *tdh;* moreover, for pandemic strains, the genes *trh*, *toxRS/New,* and *orf8* were used. Regarding diarrheagenic *E. coli* (DEC) strains, we based our protocol on a previously published work (Canizalez-Roman et al. 2013). *E. coli* strains positive

for the *eae* and *bfp* genes were classified as enteropathogenic (EPEC), strains positive for the *aggR*, *Pcvd432*, *aap*, and/or *aafII* genes as enteroaggregative *E. coli* (EAEC), *E. coli* positive for the *lt* and/or *st* genes as enterotoxigenic *E. coli* (ETEC), diffusely adherent *E. coli* (DAEC) positive for the *daaE* gene, enteroinvasive *E. coli* (EIEC) strains positive for the *virF* and/or *ipaH* genes, Shiga toxin-producing *E. coli* (STEC) positive for the *stx1* and/or *stx2* genes, and enterohemorrhagic *E. coli* (EHEC) positive for *hylA* for the O157:H7, *rfbEO157*, and *fliCH7* genes. PCR assays were carried out in a 25 µL volume consisting of 1X GoTaq green master mix (Promega) and purified genomic DNA template (0.5 µg), with the remaining volume consisting of molecular biology grade water. PCR was routinely conducted in a Thermal Cycler C1000 (Bio-Rad Laboratories, Hercules, California). Ten microliter aliquots of each amplification product were separated by electrophoresis in a 2% agarose gel. Red Gel staining (0.5 mg/ml) allowed for the visualization of DNA fragments with a digital imaging system (Model E1 logia 100 imaging system; Kodak). The sizes of the PCR fragments were compared against a 50-bp DNA ladder (Promega DNA step ladder).

Serotyping

Serotyping of *V. parahaemolyticus* isolates was performed by using a commercially available *V. parahaemolyticus* antiserum test kit (Denka Seiken, Tokyo, Japan) with O1–O11 antisera and 71 K antisera according to the manufacturer's instructions. Briefly, strains were grown overnight at 37°C on LB agar containing 3% NaCl. A pool of colonies was suspended in 1 mL of saline and then split into two 500 µl aliquots. One aliquot was heated to 121°C for 1 h for O serotyping; if the serotype could not be obtained, the bacterial lysate was heated for an additional hour and then used for O serotyping. The second aliquot was used for serotyping based on the K antigen.

Antibiotic Susceptibility Testing

All isolates of *Pseudomonas aeruginosa* (*P. aeruginosa*), *Vibrio alginolyticus* (*V. alginolyticus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *V. parahaemolyticus*, *E. coli*, *Proteus mirabilis* (*P. mirabilis*), *Vibrio mimicus* (*V. mimicus*), and *Vibrio furnissii* (*V. furnissii*) *were* tested for antimicrobial susceptibility. Antibiotic susceptibility testing of pathogenic isolates was performed by the Kirby-Bauer disk diffusion method (Bauer et al. 1966) following the guidelines developed by the Clinical Laboratory Standard Institute (CLSI) (Cockerill 2011). Suspensions of bacteria isolated from olive ridley nesting turtles were prepared in LB at a turbidity of 0.5 using the McFarland standard. Then, Mueller–Hinton agar plates were swabbed with these cultures, and antibiotic disks (BD BBL, Franklin Lakes, NJ) were placed aseptically on the inoculated agar. The antibiotics tested were ampicillin (10 µg), tetracycline (30 µg), trimethoprim–sulfamethoxazole (1.25 µg/23.75 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), gentamicin (10 µg), and cefotaxime (30 µg). The plates were incubated at 37°C for 18 to 20 h. The diameters (in mm) of clear zones of growth inhibition around the antimicrobial agent disks were measured using a precision digital caliper (Absolute, Mitutoyo, Japan) (Angulo-Zamudio et al. 2021). *E. coli* ATCC 25922 and *E. coli* ATCC 35218 obtained from the American Type Culture Collection (ATCC) were used as controls. Recommendations by CLSI were utilized to define breakpoints of antibiotics and thus categorize the isolates as resistant, intermediate, or sensitive (White et al. 2006). Isolates that showed resistance to ≥ 3 different categories of antibiotics were classified as multidrug resistant (MDR), and extremely drug-resistant (XDR) isolates were those resistant to ≥ 6 different categories of antibiotics (Magiorakos et al. 2012). Antibiotics were selected based on their use to treat human infections caused by gram-negative bacteria and represent different classes of antimicrobial agents that are available to treat these infections in Mexico.

Results

Isolated Bacterial Species

Oral and cloacal swabs were taken from 52 turtles at the Ceuta Beach Sanctuary (Fig. 1) for microbiology, of which bacteria were isolated from 47 turtles. A total of 100 bacteria were isolated, 53 from the oral cavity and 47 from the cloaca, from 21 different species. The most prevalent was *P. aeruginosa,* followed by *Aeromonas hydrophila* (*A. hydrophila*), *V. alginolyticus*, *V. parahaemolyticus*, and *K. pneumoniae* (Table 1).

E. coli Pathotypes and Phylogroups

A total of five *E. coli* cultures were isolated, four from oropharyngeal samples and one from the cloaca. The most

Table 1. Bacterial Species Isolated from Buccal Cavities and Cloacae of Nesting Olive Ridley Turtles (*Lepidochelys olivacea*) from a Rookery in Northwestern Mexico.

Bacterial species	Total bacteria $n = 100$ (%)	Anatomic isolation site	
		Oral $n = 53$ (%)	Cloacal $n = 47$ (%)
Pseudomonas aeruginosa	22 (22.0)	9 (16.6)	13 (27.6)
Aeromonas hydrophila	20 (20.0)	13 (24.0)	7 (14.9)
Vibrio alginolyticus	8 (8.0)	6 (11.1)	2 (4.2)
Vibrio parahaemolyticus	6 (6.0)	2 (3.7)	4 (8.5)
Klebsiella pneumoniae	6 (6.0)	2 (3.7)	4 (8.5)
Escherichia coli	5 (5.0)	4 (7.4)	1 (2.1)
Enterobacter amnigenus	4 (4.0)	0 (0.0)	4 (8.5)
Enterobacter sakazakii	4 (4.0)	3 (5.5)	1 (2.1)
Raoultella ornithinolytica	4 (4.0)	1 (1.8)	3 (6.3)
Proteus mirabilis	3 (3.0)	1 (2.2)	2 (4.2)
Pseudomonas fluorescens	3 (3.0)	2 (3.7)	1 (2.1)
Vibrio mimicus	3 (3.0)	2 (3.7)	1 (2.1)
Burkholderia cepacia	2 (2.0)	2 (3.7)	0 (0.0)
Enterobacter cloacae	2 (2.0)	2 (3.7)	0 (0.0)
Vibrio furnissii	2 (2.0)	0 (0.0)	2 (4.2)
Chromobacterium violaceum	1 (1.0)	0 (0.0)	1 (2.2)
Providencia alcalifaciens	1 (1.0)	0 (0.0)	1 (2.2)
Rahnella aquatilis	1 (1.0)	1 (1.8)	0 (0.0)
Serratia ficaria	1 (1.0)	1 (1.8)	0 (0.0)
Serratia fonticola	1 (1.0)	1 (1.8)	0 (0.0)
Serratia liquefaciens	1 (1.0)	1 (1.8)	0 (0.0)

All bacteria were isolated from a total of 47 olive ridley turtles.

prevalent phylogroup was B1 with 40% (2/5) isolated in both anatomical sites, then in the same proportion phylogroups A, F, and unknown 20% (1/5), all found in oral samples (Table 2). Phylogroups B2 of *E. coli* C, D, E, clade I, and clade II were not identified. On the other hand, *E. coli* pathotypes were also determined; EAEC 50% (2/4), EPEC 25% (1/4), and non-DEC 25% (1/4) were found in oral samples, while the only *E. coli* isolated from the cloacal sample was EAEC (Table 2).

V. parahaemolyticus Serovars and Virulence Genes

The virulence factors and serovars of *V. parahaemolyticus* were examined. Six strains were isolated, including two O (O1 and O4) and two K type (K8 and K12) (Table 3) serogroups. Two strains were not recognized by O antisera (OUT), four by K antisera (KUT), and one strain was negative for both O:K antisera (OUT:KUT). The serovars identified were OUT:KUT (1), O4:KUT (2), O1:KUT (1), OUT:K8 (1), and O4:K12 (1). Based on the virulence genes (*tdh, trh, toxRS/new,* and *orf8*) of *V. parahaemolyticus*, we identified one pathogenic strain (*tdh* +, *trh*−, *toxRS/new*−, *and orf8*−) belonging to serotype OUT:KUT and five nonpathogenic strains (*tdh*−, *trh*−, *toxRS/new*−-, *and orf8*−) (Table 3).

Cocolonization of Bacterial Species

In the anatomical sampling sites of the turtles, we found cocolonization of two to three different bacterial species. In the oral samples we found 11 combinations of bacteria cocolonizing with two different species: *E. coli/A. hydrophila*; *Burkholderia cepacian/V. alginolyticus*; *P. mirabilis/A. hydrophila; Rahnella aquatilis/Serratia ficaria; P. aeruginosa/A. hydrophila; Enterobacter sakazakii/A. hydrophila*; *K. pneumoniae/V. mimicus*; *Enterobacter sakazakii/A. hydrophila*; *Enterobacter cloacae/A. hydrophila*; *E. coli/A. hydrophila,* and *E. coli/V. alginolyticus*; while seven combinations

Table 2. Distribution of *Escherichia coli* Phylogroups and Pathotypes Isolated from Nesting Olive Ridley Turtles (*Lepidochelys olivacea*) from a Rookery in Northwestern Mexico.

E. coli phylogroups	Total *E. coli* $n = 5$ (%)	Anatomical isolation site			
		Oral $n = 4$ (%)		Cloacal $n = 1$ (%)	
		EAEC $n = 2$ (50)	EPEC $n = 1$ (25)	Non-DEC $n = 1$ (25)	EAEC $n = 1$ (100)
A	1 (20.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
B1	2 (40.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (100)
UNKNOWN	1 (20.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
F	1 (20.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)

The phylogroups B2. C, D, E, CLADE I, and CLADE II were not found.

Table 3. Virulence Genes and Serovars of *Vibrio parahaemolyticus* Isolated from Nesting Olive Ridley Turtles (*Lepidochelys olivacea*) from a Rookery in Northwestern Mexico.

Vibrio parahaemolyticus					
Serovar	Total no. of isolates n = 6 (%)	Presence of virulence gene			
		tdh	*trh*	*orf8*	*toxRS/New*
OUT:KUT	1	+	–	–	–
O4:KUT	2	–	–	–	–
		–	–	–	–
O1:KUT	1	–	–	–	–
OUT:K8	1	–	–	–	–
O4:K12	1	–	–	–	–

were found in cloacal samples: *P. aeruginosa*/*V. parahaemolyticus*; *Enterobacter amnigenus*/*V. parahaemolyticus*; *P. mirabilis*/*Raoultella. ornithinolytica; Providencia alcalifaciens*/*A. hydrophila*; *K. pneumoniae*/*A. hydrophila*; *V. parahaemolyticus*/*A. hydrophila*, and *E. coli*/*V. alginolyticus* (Table 4). In addition, colonization of three different bacterial species was found in both *Serratia fonticola*/*A. hydrophila*/*V. parahaemolyticus* in the oral sample, and *Raoultella ornithinolytica* / *P. mirabilis* / *A. hydrophila* in the cloacal sample (Table 4).

Antimicrobial Resistance

The results of antimicrobial resistance of bacterial species are shown in Table 5. Most of the bacterial species were resistant to ampicillin 85.4% (47/55), followed by cefotaxime 62.7% (37/55), nalidixic acid 54.5% (30/55), tetracycline, and sulfamethoxazole-trimethoprim 49% (27/55), and 45.4% (25/55), respectively, and were less resistant to chloramphenicol (34.5%; 19/55), gentamicin (16.3%; 9/55), and ceftazidime (5.4%; 3/55). All bacterial species were susceptible to ciprofloxacin (Table 5). *P. aeruginosa* was the most resistant bacterium to antimicrobials (> 50%, by drug class and category), except for gentamicin (27.2%) and ceftazidime (4.5%). All bacterial species were resistant to at least one antibiotic; 53% (29/55) of bacteria were resistant to 1–3 antibiotics, while 47% (26/55) were resistant to 4 or more antibiotics. In addition, 30.9% (17/55) of bacteria were MDR, and 29% (16/55) were XDR. *K. pneumoniae, P. mirabilis, P. aeruginosa*, and *V. mimicus* were MDR in 66.6%, 66.6%, 45.6%, and 33.3% of isolates, respectively. On the other hand, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, *V. mimicus,* and *E. coli* were classified as XDR in 50%, 33.3%, 33%, 33%, and 20%, respectively.

Discussion

Northwest Mexico provides important foraging and nesting areas for sea turtles during all stages of their life history with migratory movements between the coastal waters of the eastern Pacific and the Gulf of California (Sandoval-Lugo et al. 2020; Kot et al. 2022). Within the Gulf of California, the state of Sinaloa in its northern zone has an important foraging area, while in the south, it has several nesting areas considered olive ridley turtle sanctuaries (Hart et al. 2014, 2018; Zavala-Norzagaray et al. 2017). This interaction between the coastal zone and nesting beaches

Table 4. Cocolonization of Bacterial Species Isolated from Buccal Cavities and Cloacae of Nesting Olive Ridley Turtles (*Lepidochelys olivacea*) from a Rookery in Northwestern Mexico.

Number of bacteria species cocolonized	Bacterial species	
	Oral $n = 12$	Cloacal $n = 7$
Two	*Escherichia coli/Aeromonas hydrophila*	*Pseudomonas aeruginosa/Vibrio parahaemolyticus*
	Burkholderia cepacian/Vibrio alginolyticus	*Enterobacter amnigenus/Vibrio parahaemolyticus*
	Proteus mirabilis/Aeromonas hydrophila	*Proteus mirabilis/Raoultella ornithinolytica*
	Rahnella aquatilis/Serratia ficaria	*Providencia alcalifaciens/Aeromonas hydrophila*
	Pseudomonas aeruginosa/Aeromonas hydrophila	*Klebsiella pneumoniae/Aeromonas hydrophila*
	Enterobacter sakazakii /Aeromonas hydrophila	*Vibrio parahaemolyticus/Aeromonas hydrophila*
	Klebsiella pneumoniae /Vibrio mimicus	*Escherichia coli/Vibrio alginolyticus*
	Enterobacter sakazakii /Aeromonas hydrophila	–
	Enterobacter cloacae/Aeromonas hydrophila	–
	Escherichia coli/Aeromonas hydrophila	–
	Escherichia coli/Vibrio alginolyticus	–
	Escherichia coli/Vibrio alginolyticus	–

	Oral $n = 1$	Cloacal $n = 1$
Three	*Serratia fonticola/Aeromonas hydrophila/Vibrio parahaemolyticus*	*Raoultella ornithinolytica/Proteus mirabilis/Aeromonas hydrophila*

All bacteria cocolonizing bacteria were isolated from a total of 47 olive ridley turtles.

exposes sea turtles to opportunistic pathogens related to coastal pollution due to anthropogenic activities such as tourism, fisheries, mining, industry, agriculture, and aquaculture, which could influence the prevalence of some diseases in sea turtle populations (Pace et al. 2019). During this study, we found that nesting olive ridley turtles were colonized by a diversity of bacteria in the oropharynx and cloaca, some of which are potentially pathogenic, including *P. aeruginosa,* EPEC and EAEC *E. coli*, *K. pneumoniae,* and *V. parahaemolyticus.* Moreover, a combination of two or three different bacterial species were found cocolonizing both anatomical sites. Finally, a high level of antibiotic resistance was found in bacteria isolated from nesting turtles, as approximately 30% of bacterial species were MDR or XDR.

Previous studies have also found high bacterial diversity with differences between cloacal and oral or nasal flora in turtles. Zavala-Norzagaray et al. (2015) isolated 13 different species of bacteria from oral and cloacal samples of black turtles (*Chelonia myda sagassizii*) and olive ridley turtles (*Lepidochelys olivacea*) taken from the Pacific Ocean (Baja California Sur state) and Gulf of California (Sinaloa state), with the most commonly isolated strains being *V. parahaemolyticus* (pandemic and pathogenic strains), *V. cholereae*, and *V. alginolyticus* (Zavala-Norzagaray et al. 2015). Pace et al. (2019) examined oral and cloacal samples of 35 loggerhead turtles from the western Mediterranean and found the presence of opportunistic bacteria belonging to several families, including Aeromonadaceae (*Aeromonas hydrophila*); Enterobacteriaceae (*Citrobacter* spp., *Enterobacter* spp. *E. coli*, among others); Pseudomonadaceae (*Pseudomonas aeruginosa*); Shewanellaceae (*Shewanella putrefaciens*); and Vibrionaceae (*Vibrio parahaemolyticus*, *Vibrio vulnificus* and others *Vibrio*) (Pace et al. 2019). These studies are in line with our results because they also found a high diversity of bacterial species and differences among the flora of turtle cloacal and oral or nasal cavities. The difference in bacterial colonization of the oral and cloacal

Table 5. Antimicrobial Resistance of Bacterial Strains Isolated from Nesting Olive Ridley Turtles (*Lepidochelys olivacea*) from a Rookery in Northwestern Mexico.

Class and antimicrobial	Total bacteria species	*P. aeruginosa*	*V. alginolyticus*	*K. pneumoniae*	*V. parahaemolyticus*	*E. coli*	*P. mirabilis*	*V. mimicus*	*V. furnisii*
	n = 55 (%)	*n* = 22 (%)	*n* = 8 (%)	*n* = 6 (%)	*n* = 6 (%)	*n* = 5 (%)	*n* = 3 (%)	*n* = 3 (%)	*n* = 2 (%)
Aminoglycoside									
Gentamicin	9 (16.3)	6 (27.2)	0 (0.0)	0 (0.0)	1 (16.6)	0 (0.0)	2 (66.6)	0 (0.0)	0 (0.0)
Quinolones and Fluoroquinolones									
Ciprofloxacin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nalidixic acid	30 (54.5)	21 (95.4)	0 (0.0)	3 (50.0)	0 (0.0)	2			
(40.0)	3 (100)	1 (33.3)	0 (0.0)						
Sulfonamides and potentiated sulfonamides									
Sulfamethoxazole-trimethoprim	25 (45.4)	17 (77.2)	0 (0.0)	4 (66.6)	0 (0.0)	1			
(20.0)	2 (66.6)	1 (33.3)	0 (0.0)						
Tetracyclines									
Tetracycline	27 (49.0)	17 (77.2)	0 (0.0)	3 (50.0)	0 (0.0)	2			
(40.0)	2 (66.6)	3 (100)	0 (0.0)						
Beta lactams									
Ampicillin	47 (85.4)	18 (81.8)	8 (100)	5 (83.3)	4 (66.6)	4			
(80.0)	3 (100)	3 (100)	2 (100)						
Cephalosporins									
Ceftazidime	3 (5.4)	1 (4.5)	1 (12.5)	0 (0.0)	1 (16.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cefotaxime	37 (67.2)	21 (95.4)	1 (12.5)	6 (100)	2 (33.3)	3			
(60.0)	3 (100)	1 (33.3)	0 (0.0)						
Phenicols									
Chloranphenicol	19 (34.5)	12 (54.5)	0 (0.0)	3 (50.0)	0 (0.0)	1			
(20.0)	1 (33.3)	2 (66.6)	0 (0.0)						
Category									
Susceptible	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Resistant to any antibiotic	55 (100)	22 (100)	8 (100)	6 (100)	6 (100)	5 (100)	3 (100)	3 (100)	2 (100)
MDR	17 (30.9)	10 (45.6)	0 (0.0)	4 (66.6)	0 (0.0)	0 (0.0)	2 (66.6)	1 (33.3)	0 (0.0)
XDR	16 (29.0)	11 (50.0)	0 (0.0)	2 (33.3)	0 (0.0)	1			
(20.0)	1 (33.3)	1 (33.3)	0 (0.0)						
Number of antimicrobials resistance to									
0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1	13 (23.6)	0 (0.0)	6 (75.0)	0 (0.0)	4 (66.6)	1			
(20.0)	0 (0.0)	0 (0.0)	2 (100)						
2	9 (16.3)	1 (4.2)	2 (25.0)	0 (0.0)	2 (33.3)	3			
(60.0)	0 (0.0)	1 (33.3)	0 (0.0)						
3	7 (12.7)	2 (9.0)	0 (0.0)	4 (66.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)
4	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
5	9 (16.3)	8 (36.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
6	14 (25.4)	9 (40.1)	0 (0.0)	2 (33.3)	0 (0.0)	1			
(20.0)	1 (33.3)	1 (33.3)	0 (0.0)						
7	2 (3.6)	2 (9.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

MDR; Multidrug-resistant: resistant to ≥ 3 different categories of antibiotics; XDR; extensively drug-resistant: resistant to ≥ 6 different categories of antibiotics.

cavities in turtles could be due to the hydromorphological characteristics of water, biotic and abiotic environmental factors, and anthropogenic pressures, all of which may influence bacterial communities (Araujo et al. 1996; do Gonçalves et al. 2004; Al-Bahry et al. 2011).

Another important finding in this work was the diversity of potentially pathogenic bacteria in turtles, for example, *V. parahaemolyticus*. In this study, we found *V. parahemolyticus* pathogenic strains but not pandemic strains, in comparison with the Zavala-Norzagaray study, in which they identified that 11.7% of *V. parahaemolyticus*

strains isolated from turtles belonged to pandemic clones (Zavala-Norzagaray et al. 2015). Other studies have also reported the presence of *V. parahaemolyticus* in turtles but have not investigated the presence or absence of virulence factors (genes) or serotypes (Santoro et al. 2006; Keene et al. 2014a, b; McNally et al. 2021). Additionally, *V. alginolyticus* is one of the most common bacteria in marine organisms (Jacobs Slifka et al. 2017; Matamp and Bhat 2019). It is considered an opportunistic pathogen of fish, and its consumption is associated with different pathologies, such as bronchopneumonia and kidney disease (Di Renzo et al. 2017). Therefore, due to its predominance and pathogenic potential, it should be considered a risk factor for the health of sea turtles.

DECs are a risk factor for the development of infectious diseases in humans through the consumption of these animals. We identified diarrheagenic *E. coli* (EPEC and EAEC); however, there are no studies identifying *E. coli* pathotypes in bacterial isolates from turtles. The presence of EPEC and EAEC could represent a risk factor for unknown impacts on sea turtle health; therefore, further research is necessary to determine the presence of DECs in healthy and immunocompromised turtles and their secondary effects.

The presence of *V. parahaemolyticus* and *E. coli* in nesting sea turtles is associated with lower sea turtle hatching success, particularly during early incubation when embryos are most vulnerable to bacterial infection (Keene et al. 2014a, b). Although bacteria such as *E. coli* could be present in the sand at the nesting site, it has been previously documented that some bacteria are already inside the eggs at the time of oviposition, so it is possible that they are contaminated by the female as they pass through the oviduct (Al-Bahry et al. 2009). The presence of these bacteria in nesting turtles could be related to interaction with domestic animals such as cats and dogs during nesting or exposure to human or domestic animal feces around turtle nests (Praja et al. 2021).

P. aeruginosa, *K. pneumoniae*, *A. hydrophila*, *Serratia* spp., and *Enterobacter* spp. were reported herein individually or with other bacteria in cocolonization. Cocolonization of bacteria has also been found in other marine animals, such as sea lions (Zavala-Norzagaray et al. 2022). Some of these bacteria could be opportunistic pathogens, normally present in the environment or as part of the bacterial flora of turtles, but may become pathogenic when the turtle's immune system is compromised by various environmental factors such as poor water quality, global warming, trauma, chronic weakness, and starvation, among others (Ahasan et al. 2017a, b; Vega-Manriquez et al. 2018; Pace et al. 2019). Although all species of sea turtles are currently protected by international regulations due to their decline, targeted illegal consumption of meat and eggs in coastal areas in Mexico continues, particularly for *L. olivacea* (Senko et al. 2009; Mancini et al. 2011). This can be a potential risk factor to human health, as turtles are colonized by potential pathogenic bacteria that can cause diseases of global public health importance (Aguirre et al. 2006; Fussy et al. 2007; Schmitt and De Haro 2013). Examples of these pathogens are *E. coli, Vibrio alginolyticus, V. parahaemolyticus* (pandemic clone), *V. cholerae, Salmonella enterica,* and *P. aeruginos*a (Zavala-Norzagaray et al. 2015; Wendt and Heo 2016; Edwards et al. 2021). In fact, an outbreak of *Vibrio cholerae* from the consumption of soft-shelled turtles was reported in China in 2009, and other cases of diarrhea due to *V. mimicus* or *S. enterica* from the consumption of turtles have also been reported (Campos et al. 1996; Tang et al. 2010; Braun et al. 2015).

All bacteria (100%) isolated in this study demonstrated resistance to at least one antibiotic, and approximately 30% were MDR or XDR. Previous data reported high antibiotic resistance in bacterial species isolated from loggerhead turtles (Pace et al. 2019). Zavala-Norzagaray et al. (2015) found similar resistance to antibiotics in *Vibrio* isolated from black and olive ridley turtles (Zavala-Norzagaray et al. 2015). However, Fernandes et al. (2021) reported low antibiotic resistance of bacterial species isolated from loggerhead turtles (Fernandes et al. 2021). Sea turtles living in ecosystems affected by human activities are at a higher risk of being exposed to antimicrobial environmental pressure, e.g., sewage effluent pumped into rivers, spreading of sewage sludge as fertilizer, or in the faces of treated livestock and pets (Arnold et al. 2016). Additionally, the interaction of rehabilitated sea turtles that were given antibiotics or exposed to antibiotic-resistant bacteria while undergoing treatment may play a vital role in the spread of these bacteria and their antibiotic resistance genes in their natural environment (Ahasan et al. 2017a, b).

To our knowledge, this is the first time that bacterial colonization and cocolonization and the presence of EPEC and EAEC of postnesting olive ridley turtles in Mexico have been reported.

Conclusion

The presence of bacteria with pathogenic potential in sea turtles indicates their importance as sentinel species of the environment and should be considered in sea turtle conservation efforts since the bacteria isolated from turtles reflect the feeding and nesting areas where they develop and may be associated with anthropogenic pollution sources. Many of these bacteria are associated with the development of diseases in sea turtles, which represents a health risk to their populations, including bacterial transmission from nesting turtles to eggs during oviposition. In addition, the zoonotic potential that sea turtles may represent, due to illegal consumption in the coastal areas of northwestern Mexico, should be considered as part of sea turtle protection and conservation programs, since many of the isolated bacteria such as *V. alginolyticus*, *V. parahaemolyticus*, and diarrheagenic *E. coli* are considered pathogenic to human health.

Acknowledgements

We thank the staff of the Sea Turtle Program (at the Ceuta beach sanctuary) of the Biology Faculty of the Universidad Autónoma de Sinaloa for their logistical support in the sampling.

Funding

None.

Availability of Data and Materials

Most data collected in this study are presented in the current manuscript. Raw data are also available upon request.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Consent to Participate Not applicable.

Consent to Publish Not Applicable.

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