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EVIDENCE OF ANTIBIOTIC RESISTANCE IN FREE-SWIMMING, TOP-LEVEL MARINE PREDATORY FISHES

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Abstract: Antibiotic resistance in bacteria is a growing problem in both human and veterinary medicine. Several studies documented the presence of resistant bacteria in humans, livestock, and domestic animals; however, limited research is available on the presence of antibiotic drug resistance in wildlife species. A cross-sectional study was conducted to estimate the prevalence of resistant bacteria collected from wild-caught, marine predatory fishes. Seven species of sharks and a single teleost species were opportunistically sampled from six different study sites in coastal Belize, coastal and nearshore waters of Louisiana, the Florida Keys, and Martha's Vineyard, Massachusetts. A total of 134 viable bacteria samples were isolated from the cloacal swabs of predatory fishes. Isolates were characterized by Gram-stain morphology and tested for resistance by using the Kirby-Bauer disc diffusion method. Thirteen drugs (penicillin G, piperacillin, ticarcillin, cefotaxime, ceftazidime, ceftiofur, amikacin, gentamicin, ciprofloxacin, enrofloxacin, doxycycline, chloramphenicol, and sulfamethoxazole) were selected for this study. Prevalence was calculated as the total number of isolates resistant to one or more drugs against the total number of samples in that study area or fish population. Sharks sampled in the Florida Keys exhibited the greatest resistance to a wide selection of drugs. Resistance to at least one drug was found in each of the six study sites and in all of the fish species sampled. Multidrug resistance was also documented in most of the study sites. Interspecific comparisons between redfish, *Sciaenops ocellata*, and sharks from Louisiana offshore waters (which represent species of the *Carcharhinus* genus) demonstrated a significantly higher prevalence in redfish, which may be because of the older age of the population. The findings of this study confirmed the presence of antibiotic-resistant bacteria in marine predatory fishes from multiple taxa and multiple geographic locations.

Key words: Antibiotic resistance, bull shark, elasmobranchs, nurse shark, smooth dogfish, redfish.

INTRODUCTION

Antibiotic resistance in bacteria is a growing problem in human and veterinary medicine. Since the introduction of antibiotics in the 1940s, bacteria have shown an increased evolutionary response, at the genetic level, to develop resistance. Several recent studies documented the presence of drug-resistant bacterial infections in humans[21,32] and in domestic and livestock animal cases[3,8,20,40]; however, limited research is available on the presence of drug-resistant bacterial flora in zoo animals[18] or wildlife species.[17,30] Several articles presented data on the presence of drug-resistant bacteria in marine mammal species from the Pacific coast of California,[22,42] Washington State,[25] Florida and South Carolina,[39] New England waters,[5,6] and the waters off England.[41]

To date, relatively few studies have attempted to estimate the prevalence of antibiotic resistance in marine top-level predatory fishes. There is a more substantive body of literature available on feral fishes and aquaculture operations where farmed fishes may interact with wild fishes.[26] There is also limited information available on the mechanisms for the acquisition, accumulation, and transmission of antibiotic resistance in marine fishes, especially those unrelated to fish farming operations. One study on wild-caught fishes presented data on two opportunistically sampled dogfish, *Mustelus mento*, from a small study focused on several commercially important fishes and found that antibiotic-resistant bacteria were present; however, no systematically collected data were presented in these or other sharks.[28]

The specific objectives of this study were to determine if antibiotic-resistant bacteria were present in marine predatory fishes that represent different populations and different species from different geographic locations. The hypotheses tested were that antibiotic-resistant bacteria

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would be detectable in the flora of wild predatory fish populations, that the prevalence of antibiotic-resistant bacteria would decrease as populations were collected further from shore, that sharks of the same species sampled in different geographic locations would exhibit different resistance patterns, and that patterns of antibiotic resistance would vary between species within the same study sites, possibly because of species-specific biology or age.

MATERIALS AND METHODS

A cross-sectional study was conducted to determine the prevalence of antibiotic-resistant bacteria from cloacal/distal anus samples of wild-caught, marine predatory fishes. Six study sites were used to sample marine top-level predators. Five sites were selected to sample sharks, and one location was opportunistically sampled for a teleost species. Nurse sharks, *Ginglymostoma cirratum*, were collected in northern Belize, within the boundaries of the Hol Chan Marine Reserve; and in the western most Florida Keys (USA), within the boundaries of the Dry Tortugas National Park. Bull sharks, *Carcharhinus leucas*, blacktip sharks, *Carcharhinus limbatus*, and a lemon shark, *Negaprion brevirostris*, were collected in the coastal waters of Timbalier Bay, Louisiana (USA). Spinner sharks, *Carcharhinus brevipinna*, were collected near an oil rig in the offshore waters of Louisiana (USA). Redfish, *Sciaenops ocellata*, a predatory teleost species, were sampled at a second offshore oil rig in Louisiana (USA). Dogfish, *Mustelus canis*, were sampled in Vineyard Haven Harbor, Massachusetts (USA). Each location represented a separate population of fish and a separate geographic and/or ecological setting. The locations of sampling sites, the number of individual fishes at each site, the collection method, and the total number of bacterial isolates by Gram stain are summarized in Table 1. Sampling-site description and capture methods used were similar as previously described by Blackburn.[4] Length measurements and species-specific body condition were collected from each individual fish to assess age (sharks,[23,34,37] redfish[36]). All animal lengths were recorded in centimeters.

Bacterial samples were collected from all of the fish by swabbing the cloaca or anus and distal colon with a sterile rayon-tip applicator (CULTURETTE, Becton Dickinson Microbiology Systems, Becton Dickinson and Company, Sparks, Maryland, USA). The swab was inserted into the cloaca-anus approximately 5–6 cm and rotated several times. The swab applicator was then placed into one ampule of 0.5-ml modified Stuart's transport media. The swabs were held on wet ice and then transported, on wet ice, within 1 to 7 days to the Louisiana State University School of Veterinary Medicine (LSU-SVM) for processing.

At the conclusion of each sampling trip, bacterial samples were inoculated on 5% blood agar plates (Remel, Lenexa, Kansas, USA) by using the swab applicator from the transport media and were incubated at 37°C for 24 hours under aerobic conditions at LSU-SVM. Presumptive organisms were described by using Gram stain and colony morphology. Gram-negative isolates were also cultured on MacConkey agar for 24 hours at 37°C under aerobic conditions to determine if they fermented lactose. Isolates were characterized and described by Gram stain and morphology.

Bacteria were tested for antibiotic resistance by using the Kirby-Bauer disc diffusion technique.[2] Thirteen drugs (penicillin G [P], piperacillin [PIP], ticarcillin [TIC], cefotaxime [CTX], ceftazidime [CAZ], ceftiofur [XNL], amikacin [AN], gentamicin [GM], ciprofloxacin [CIP], enrofloxacin [ENO], doxycycline [D], chloramphenicol [C], sulfamethoxazole [SXT]) were evaluated in this study (see Table 2 for dosages). The plates were then incubated at 37°C for 24–48 hr under aerobic conditions. After the samples had been incubated, the diameter of the zone of inhibition around each disc was measured against the National Committee for Clinical Laboratory Standards distances and recorded as resistant (R) or susceptible (S).[33]

Total resistance (TR) was calculated as the total number of isolates in each population with resistance to at least one drug divided by the total number of isolates in each population. Multidrug resistance (MDR) was calculated as the total number of isolates with resistance to more than one drug in each population divided by the total number of samples in that population. Because 13 drugs were tested for three major categories of Gram-stain–specific organisms, the prevalence of resistant bacteria was reported graphically for each geographic region.

Given the limited sample sizes, all graphed prevalence estimates included standard error bars.[19] The Pearson chi-square tests were calculated to quantify potential interpopulation differences between spatially explicit groups of animals. The Pearson chi-square test was also used to test for interspecific differences between Louisiana offshore sharks and offshore redfish.

Table 1. Sampling locations, ecological settings, fish species collected, sampling gear used, and the number of bacterial isolates from each study site.

Sampling site	Geographic location, longitude; latitude	Ecological setting	Species collected (n[a])	Sampling gear	No. bacterial isolates
Amber Gris Caye, Belize	87°59′28″W; 17°50′ 23″N	Shallow reef with sharks conditioned to human swimmers	Nurse shark, *Ginglymostoma cirratum* (8)	Free caught during swimmer/ shark interactions	Gram+ 3 Gram− 9
Dry Tortugas, Florida Keys, USA	82°50′45″W; 24°36′24″N	Shallow reef near boat basin and tourist facility with damaged leach field septic system	Nurse shark, *G. cirratum* (7)	Collected in hand nets from underneath reef crops	Gram+ 10 Gram− 19
Timbalier Bay, coastal Louisiana, USA	90°16′32″W; 29°8′19″N	Shallow nearshore waters with proximity to petroleum support industry	Bull sharks, *Carcharhinus lecuas* (28); blacktip sharks, *Carcharhinus limbatus* (3); lemon shark, *Negaprion brevirostris* (1)	Experimental gillnet	Gram+ 19 Gram− 27
Offshore costal waters of Louisiana	89°32′58″W; 29°7′13″N (site 1); 89°31′24″N; 29°3′17″W (site 2)	Open bay waters with proximity to oil production platforms	Site 1: spinner sharks, *Carcharhinus brevipinna* (7); blacktip shark, *C. limbatus* (1); site 2: Redfish, *Sciaenops ocellata* (7)	Rod and reel with bait and tackle	Site 1: Gram+ 6 Gram− 17 Site 2: Gram+ 7 Gram− 5
Vineyard Haven Harbor, Martha's Vineyard, Massachusetts, USA	70°35′32″N; 41°28′42″W	Shallow water bay with proximity to development and heavy boat usage	Smooth dogfish, *Mustelus canis* (3)	Experimental longline	Gram+ 1 Gram− 7

[a] Sample sizes reflect the total number of sharks with viable bacterial samples recovered and tested for antibiotic resistance.

Table 2. Drug names, dosages, and abbreviations used in this study.

Drug	Dosage (μg)	Abbreviation
Penicillin G	10	P
Piperacillin	100	PIP
Ticarcillin	75	TIC
Cefotaxime	30	CTX
Ceftazidime	30	CAZ
Ceftiofur	30	XNL
Amikacin	30	AN
Gentamicin	10	GM
Ciprofloxacin	5	CIP
Enrofloxacin	5	ENO
Doxycycline	30	D
Chloramphenicol	30	C
Sulfamethoxazole	25	SXT

The Fisher exact test was used to test for intrapopulation TR differences between sexes in bull sharks and to test for interspecific differences between nurse shark populations, because some cell counts were fewer than five. Power analysis was performed for any comparison that was found to not be significant. A probability value ($P < 0.5$) was used to determine statistical significance. Statistical analyses were conducted by using S-Plus 6.0 Professional software (Insightful Corporation, Seattle, Washington, USA).

RESULTS

A total of 130 bacterial isolates from the six populations were characterized by Gram stain and morphology. Seventy-seven of those were

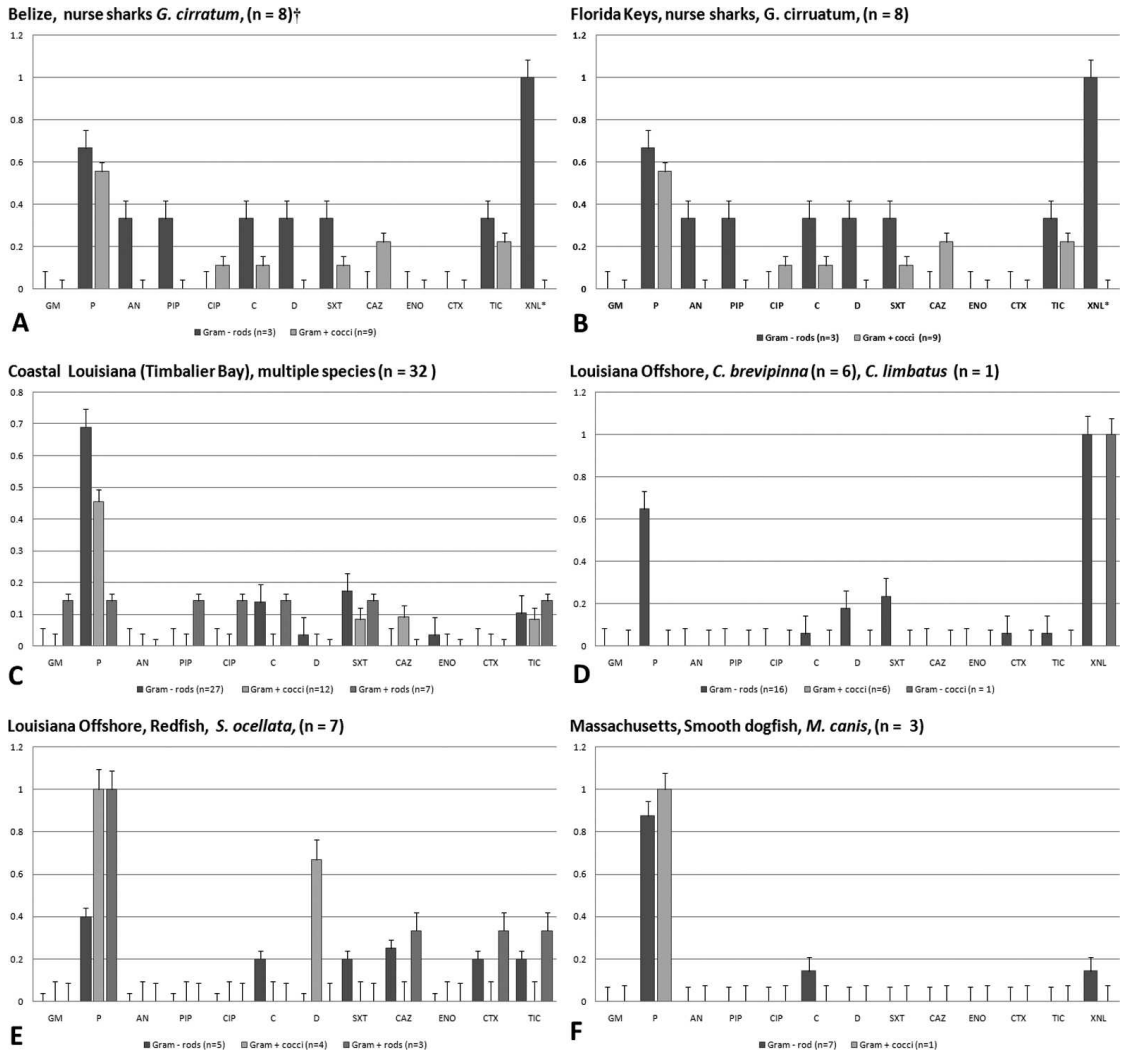

Figure 1. Antibiotic-resistance prevalence values by individual drug type for isolate groups categorized by Gram stain and morphology, and separated by study site. **A.** Belize, nurse sharks. **B.** Florida Keys, nurse sharks. **C.** Coastal Louisiana, bull sharks, blacktip sharks, and lemon shark. **D.** Offshore Louisiana, spinner sharks and blacktip shark. **E.** Offshore Louisiana, redfish. **F.** Massachusetts, dogfish. Error bars represent the 95% standard error. †Sample sizes in titles represent the number of fish per sample population.

Gram-negative rods, 38 were Gram-positive cocci, 14 were Gram-positive rods, and a single isolate was classified as Gram-negative cocci. With the exception of those bacteria collected from nurse sharks in Belize, Gram-negative rods were the most commonly identified isolates (Fig. 1). In the Belizean samples, Gram-positive cocci were the most common isolates.

Twelve viable bacteria were isolated from eight nurse sharks from the Belize population, five females (63% [5/8]) and three males (37% [3/8]), and were tested for resistance from November 2001 ($n = 5$ sharks, $n = 6$ isolates) and May 2002 ($n = 3$ sharks, $n = 6$ isolates). Female sharks ranged from 140 to 170 cm in curved total length (CTL) ($W = 0.898$; $df = 5$, $P = 0.387$), with a mean length of 152-cm CTL. Male sharks ranged from 108 to 140 cm CTL (Kolomogorov-Smirnov $= 0.385$; $df = 3$, $P = 0.075$), with a mean length of 129 cm. All eight sharks were characterized as juveniles based on size comparisons with published data on nurse shark reproduction and biology.[10,12] Age estimates in years were unavailable, because no current age and growth study was available for the species. The resistance patterns for each Gram-stain group and each

drug are illustrated in Figure 1A. Gram-negative rods showed resistance to eight of the drugs tested, including C, D, and SXT. Belize was one of three geographic regions that showed resistance to XNL. Belize was the only sampling site to show CIP resistance in Gram-positive cocci.

Twenty-nine viable bacteria were isolated from seven nurse sharks from the Florida Keys in May 2002 ($n = 4$ sharks, $n = 22$ isolates) and January 2003 ($n = 3$ sharks, $n = 7$ isolates). One shark was female, and the remaining six were male. Again, all sharks were classified as juveniles based on size.[12] Several isolates from Florida demonstrated resistance, including resistance to CIP, C, and D (Fig. 1B). Florida isolates showed resistance to 11 of the drugs tested, the most diverse pattern of resistance from the six study sites.

Forty-six viable bacteria were isolated from 32 sharks in Louisiana coastal waters from April to September of 2002. Ten female bull sharks were sampled with fork length (FL) that ranged from 65 cm to 139 cm ($W = 0.918$; $df = 10$, $P = 0.384$), with a mean of 112 cm. Sixteen male bull sharks were sampled with FL that ranged from 79 to 139 cm ($W = 0.877$; $df = 16$, $P = 0.038$), with a median of 97 cm FL. Two female blacktip sharks were sampled, with FLs of 64 cm and 70 cm, and a single male blacktip was sampled with a 68 cm FL. A single female lemon shark was sampled with a 111 cm FL. Bull sharks were classified as juveniles younger than six years of age based on size comparisons with published age and growth estimates.[35] All other sharks collected in coastal Louisiana were also classified as juveniles after published working definitions for inshore waters.[34] Current blacktip and lemon sharks age and growth estimates were not available for the northern Gulf of Mexico. Resistance patterns for isolates from Louisiana inland waters are summarized in Figure 1C. In general, TR was low (<0.2) for all drugs but P. This was the only collection of isolates that showed resistance to ENO (Gram-negative rods).

Twenty-three bacteria were isolated from two female spinner sharks, four male spinner sharks, and one adult female blacktip shark from the offshore waters of Louisiana from July 2002 to November 2002. Five additional isolates were collected from two female spinner sharks and one female blacktip shark from approximately 35 km southwest of the Mississippi River. The female spinner sharks were 82 and 167 cm FL, respectively. The smallest female was estimated to be a yearling animal, probably born in the early spring of 2002 based on the presence of a healed umbilical scar.[34] The large female spinner was probably a mature animal based on the reported size of maturity.[13] The female blacktip was an adult animal based on published age and growth estimates.[13] Eighteen isolates were collected from four male spinner sharks at an oil rig approximately 10 km from shore. All male spinner sharks were approximately 60 cm in FL and classified as young of the year based on the presence of a healing umbilical scar.[34] Samples from these offshore sharks showed relatively little drug resistance (Fig. 1D), with only Gram-negative rods and cocci showing any resistance to the drugs tested. Of note, samples were resistant to C and D.

Twelve viable bacteria were isolated from seven adult redfish collected at an oil rig in the offshore waters of Louisiana on 29 November 2002. Redfish specimens ranged from 80 to 105 cm standard length (SL) ($W = 0.563$; $df = 7$, $P = 0.01$), with a median length of 105 cm SL. Resistance patterns from redfish bacterial flora are summarized in Figure 1E. Redfish isolates showed high TR to P in Gram-positive rods and cocci, with only nurse sharks in Florida showing similarly high Gram-positive resistance to P. Also, redfish isolates of Gram-positive cocci showed relatively high prevalence to D (>0.6), with Floridian isolates being the only other group to show resistance to D in colonies of similar morphology, though those sampled had a lower TR (<0.3).

Eight bacterial isolates were collected from three smooth dogfish in Vineyard Haven Harbor on 25 September 2002. Five bacterial isolates were collected from two mature female dogfish. The female dogfish were considered adults based on published age estimates.[14] Three bacterial isolates were also collected from a single adult male smooth dogfish with a CFL of 71 cm. Dogfish resistance patterns are summarized in Figure 1F. These isolates showed the least resistance to all drugs, with resistance only documented in P, C, and XNL. Of note, the single Gram-positive cocci sample showed resistance to P. All other resistance was documented in Gram-negative rods. These results should be considered with caution, because this was the smallest sample size of viable organisms from this study.

Redfish had significantly higher TR than spinner sharks ($P = 0.0016$, $df = 1$). There was no significant difference in TR between Louisiana inshore sharks and offshore sharks ($\chi^2 = 2.0519$; $df = 1$, $P = 0.152$, power = 0.29). There was no

significant difference in TR between male and female bull sharks in Louisiana coastal waters ($\chi^2 = 0.0068$; df = 1, $P = 0.9343$, power = 0.05) or between nurse sharks from Belize and Florida ($P = 0.3407$, power = 0.1).

DISCUSSION

Antibiotic-resistant bacteria were present in all of the populations of top-level marine predators examined in this study. Likewise, MDR was documented in at least a single sample from each population and each location surveyed. The presence of drug-resistant bacteria in both elasmobranchs and teleosts was consistent with the findings of previous studies on marine fishes from Concepción Bay, Chile, where resistance was documented in *M. mento*, a shark species, and several teleost species.[28] Those results suggested that resistant bacteria are present in both demersal and pelagic food webs in South America. A previous study found antibiotic residues present in the tissues of marine fishes from South America tested within 400 m of an experimental aquaculture pen where antibiotics were being administered through fish feed.[26] Though the ultimate fate of those residues is unknown, they may provide selection pressures, which allows resistant bacteria to evolve in the environment. Likewise, that same study[26] also reported the presence of antibiotic residues in crabs and smaller teleosts that may be predated upon by elasmobranchs, which might serve as an intermediate step between antibiotic introduction into the environment and uptake by apex predators.

Although there is no known information regarding bacterial shedding in elasmobranchs, results of research performed on reptiles suggest that repeated samplings of the same individual are required to establish the actual presence of specific bacteria or infection.[9] One study did recover bacterial samples from the same free-ranging dolphins over multiple time periods and found variability, with most microorganisms being sporadic and only a few being confirmed in the same individual upon resampling (e.g., *Candida* yeast).[7] It should be noted, that study did not report on antibiotic resistance. In this current study, there was no way to determine the status of antibiotic resistance in the bacterial flora of fish sampled before or after the sampling period. Incidentally, these findings are likely underestimates of the true prevalence of drug resistance, because they are based on single samples[29] and represent relatively small sample populations. In addition, no analyses were undertaken to quantify the loss of bacterial diversity from transporting samples on wet ice, although the limited number of isolates in this study suggests a loss. The primary objective of this study was to determine if bacteria from the cloaca and/or distal anus of free-swimming apex predators from coastal waters surrounding the United States and Central America exhibit drug resistance. These findings confirmed their presence.

Antibiotic resistance is generally thought to occur either through mutation or gene transfer. However, it is important to evaluate intrinsic resistance. The high overall prevalence of drug resistance found in this study may have been because of intrinsic resistance. In most cases, one can distinguish the effectiveness of a drug against bacteria based on Gram-stain characteristics. Because P is a drug that targets Gram-positive bacteria, resistance to P might not be unexpected in Gram-negative bacteria. When total resistance was calculated by excluding P, the prevalence of antibiotic resistance (ABR) changed dramatically. For example, total resistance in the Massachusetts population decreased by 75% when P was excluded from the analyses. The total resistance of 12.5% probably more truly represents the resistance in the Massachusetts samples, because seven of the eight bacterial isolates were Gram negative. Further characterization of the bacteria for the presence of resistance genes would have been beneficial but were beyond the resources and scope of this study. A similar situation was noted in a study of harbor porpoises, *Phoecena phoecena*, in English waters, where all *Salmonella* isolates were resistant to clindamycin.[41] The investigators noted that the high proportion of resistance may have been because of the nature of the drug and its inhibitory properties relative to the organism.

Twelve other drugs with more broad-spectrum antibacterial properties were also tested in this study, and resistance was identified for all of the drugs in at least some of the isolates. However, resistance to all drugs did not occur in all geographic locations, which suggests that there may be spatial differences in the ABR patterns of marine predatory fishes. The sample sizes evaluated in this study were small, and the 95% confidence intervals were large, which made it nearly impossible to draw conclusions regarding spatial differences. A larger sample size would be required to evaluate any spatial differences.

Of the relatively few articles published on antibiotic resistance in the marine environ-

ment,[22,25,27] most did not evaluate spatial patterns of resistance. One such study determined significant differences in drug-resistance patterns in wild-caught bottlenose dolphins, *Tursiops truncatus*, captured in the Indian River Lagoon, Florida, and the estuarine waters of Charleston, South Carolina.[39] Whereas, overall results suggested resistance to a number of drugs across both study sites, *Escherichia coli* showed significantly greater resistance to PIP, tetracycline, and trimethoprim-sulfamethoxazole in the Indian River Lagoon. Although limited to a single species and two coastal sites, this study confirmed that bacteria associated with marine apex predators can exhibit spatially explicit patterns of drug resistance.

Though direct comparisons cannot be made to other marine studies, all of those studies documented resistance to many of the antibiotics tested in this study. Bacteria isolated from pinnipeds,[22] dolphins,[39,42] and the shark populations and redfish from this current study all showed at least some resistance to C, with the exception of *M. mento* in Chile.[28] Bacteria isolated from pinnipeds,[22] dolphins,[42] and the sharks (Belize, Florida Keys, and coastal Louisiana) from this current study also showed resistance to CIP. In addition, bacteria isolated from pinnipeds[22] and sharks from this study showed resistance to GM (Florida Keys), AN (Belize, Florida Keys), ENO (Coastal Louisiana), and SXT (all sites minus Massachusetts). The studies on dolphins[39,42] and pinnipeds[22] all documented drug-resistant pathogenic bacteria that may impact humans. Whereas, the results of this current study were primary limited to Gram stain and morphology, resistant colonies of *Pseudomonas* were identified. These previous works and this current study support the hypothesis that marine predators can serve as reservoirs for drug-resistant bacteria and should be considered for further investigations as marine environmental sentinels. There were drugs tested in both the pinniped study and this current study that were not tested in both studies. Future work should be done to address possible similarities and differences to other drugs in both groups of animals.

Nurse sharks from both Belize and Florida showed a high prevalence of antibiotic-resistant bacteria, with the Florida Keys' isolates that exhibited the greatest amount of resistance. Direct comparisons were made between the populations, because the same species was sampled in both locations. In addition, the ages of the sampled sharks were similar between groups. Although it was not possible to quantify differences in the environments of both locations, it is of some interest to qualify them. Florida sharks were in close proximity to a failing septic facility that operated through leaching. The sharks from Belize spent a high proportion of their time within 8 km of the sewage system of Ambergris Caye, which could not keep up with the demands of a booming tourist city.[27] This polluted water may be transported from the island south through longshore transport. Studies provided evidence that raw sewage and sludge from southern Austria can harbor *E. coli* bacteria resistant to at least three drug types similar to those tested in this study.[38] In addition, sewage-treatment processes do not necessarily eradicate drug-resistant bacteria and, therefore, may disseminate these species into the environment.[38] Belize sharks may also be directly exposed to antibiotic residue from contact with human swimmers; however, this could not be determined. The findings of this current study may also support the findings of recent seroprevalence studies on selected pathogens in marine-foraging river otters[16] and Hawaiian monk seals,[24] which provided evidence that proximity to human population and associated anthropogenic shifts (e.g., domestic pet populations, runoff water patterns) directly increase the likelihood of exposure to pathogens, including bacterial agents. Work should address the potential for similar transmission patterns for drug resistance.

Although the samples from Massachusetts represent the smallest number of isolates and a small group of coastal sharks, other investigators corroborated this work by confirming antibiotic resistance in pelagic mako sharks, *Isurus paucus*, and thresher sharks, *Aliopias vulpinus*, collected during pelagic sampling off the coast of Martha's Vineyard.[6] Like this current study, the investigators noted that limited information on target species' natural history and movement patterns, coupled with limited knowledge on native bacterial flora, makes interpreting or identifying the role of the environment, proximity to coastal waters, or human activity difficult. It is worth noting that other large pelagic sharks, such as shortfin makos, *Isurus oxyrinchus*, are found in the offshore waters of Louisiana[11] and may represent an additional pelagic group for sampling.

Redfish samples were collected within 15 km of spinner shark samples, yet the redfish showed significantly higher TR than spinner sharks. Because both species feed on fish in similar

trophic levels, it is most likely that the redfish represented older animals and, therefore, potentially greater periods of exposure. Redfish age estimates ranged from 3 to >4 yr.[31] Redfish age estimates for equal length redfish greater than 100 cm SL can range from 4 to 30 yr of age. All of the spinner sharks sampled in this study were no older than 1 yr.

The Mississippi River Delta dominates the oceanographic conditions of Louisiana's coastal and nearshore environments. The river is the largest in the United States and drains the majority of the eastern United States. The Environmental Protection Agency reported that agricultural runoff from the Mississippi River is one of the primary sources of pollution to water quality in the northern Gulf of Mexico.[15] The Mississippi River may be a point source for antibiotic-resistant bacteria or genetic material,[1] or at least promotes the input of genetic material for resistance into the marine environment of Louisiana and the northern Gulf of Mexico. All sampling sites used in the analyses for this study were within the influence of the Mississippi River during at least some portion of the year. If freshwater runoff and agricultural pollution do promote the prevalence of antibiotic resistance in the marine environment, sharks and redfish in Louisiana may serve as important indicators for antibiotic resistance from free-ranging fishes.

CONCLUSIONS

Despite a limited sample size and a lack of speciation to the generic or species level, the findings of this study confirm the presence of antibiotic resistance in the bacterial flora of marine predatory fishes from multiple taxa and multiple geographic locations. The marine environment may be considered a reservoir for resistance to such drugs, and future surveillance of predatory fishes should continue. The marine predatory fishes sampled in this study may serve as valuable sentinels, because they are long lived and slow growing and, therefore, have a potentially long exposure to resistant bacteria in the ocean. In addition, these data support the hypothesis of previous work that resistance is present in marine species from multiple food webs and habitats.[28] Because fisheries remain an important component of the human diet, this information may be used to determine zoonotic health risks. Future data collection on bacteria from sharks should focus on a greater number of animals from each population and more specific efforts to compare data spatially. Collecting sharks, however, will likely remain a difficult procedure because of the vastness of the marine environment. Future efforts should concentrate on quantifying drug resistance and integrate methodologies for evaluating the genetics of resistance.

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