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Review of Human Pathogenic Bacteria in Marine Animals with Emphasis on Sharks[1]

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ABSTRACT

Bacteria belonging to the genus *Vibrio* were demonstrated as etiologic agents of disease in captive sharks, following their isolation from a dead sandbar shark, *Carcharhinus plumbeus*, and from experimentally infected lemon sharks, *Negaprion brevirostris*. Studies were expanded to healthy, free-ranging sharks captured and sampled for bacteria at Bimini, Bahamas. The bacterial flora of 28 neritic sharks, comprising five species, were examined. All 28 sharks were colonized with bacteria, primarily the genus *Vibrio*. All tissues and organs sampled contained *Vibrio* spp., including liver, spleen, kidney, eye, mouth, skin, pancreas, intestine, stomach, gall bladder, gill slits, and fetuses (from a pregnant sharpnose). The conclusion, based on over 300 bacterial isolates from over 50 healthy sharks, is that sharks contain an autochthonous flora in most tissues and organs. The bacteria typically number between 10^2 and 10^5 bacteria per gram of tissue except blood, which is free from both aerobic and anaerobic bacteria. Human pathogens among the isolates included *V. alginolyticus*, *V. parahaemolyticus*, *Listonella damsela*, and *Clostridium* spp. While their ecological niche remains an enigma, it is clear that bacteria in healthy sharks can derive nutrients from elasmobranchs and, under conditions of stress to the host, cause death. Equally clear is the fact that when used as food, shark meat must be thoroughly cooked to destroy potential pathogens. If not properly cooked, pathogens such as *V. parahaemolyticus* could initiate gastroenteritis.

Introduction

In 1982, the National Aquarium in Baltimore experienced unusually high morbidity and mortality among their display sharks and provided the author samples from a dead sandbar shark, *Carcharhinus plumbeus*. Two vibrios were isolated (Fig. 1), *Vibrio carchariae* from kidney and *Vibrio damsela* from liver samples (Grimes et al. 1984a). It was subsequently demonstrated that each isolate was capable of infecting and causing disease in healthy lemon sharks, *Negaprion brevirostris* (Grimes et al. 1985a). In subsequent studies, *V. carchariae* was found to have a unique 5S ribosomal RNA sequence (MacDonell and Colwell 1985a), confirming the original description (Grimes et al. 1984a) of it as a new species. *V. damsela* was subsequently placed into a new genus, *Listonella* (MacDonell and Colwell 1985b).

Examination of apparently healthy, control lemon sharks that had been inoculated intraperitoneally with sterile saline during reinfectivity studies (Grimes et al. 1985a) revealed the presence of potentially pathogenic *Vibrio* species. Consequently, additional healthy, free-ranging sharks were examined, and *Vibrio* species, as well as other pathogenic bacteria, were found to reside in shark tissue (Grimes et al. 1985b). This unusual finding suggests that sharks are a potential health risk to humans, and this review will discuss the risk in context with other marine animals known to harbor human pathogens. In addition, the ecology of elasmobranch-borne pathogens will be discussed.

Human Pathogens in Marine Animals

Shellfish frequently become colonized with human pathogens (Table 1). In past years, typhoid fever, caused by *Salmonella typhi*, was the major disease associated with consumption of contaminated bivalve molluscs. In recent years, however, *S. typhi* has been virtually eliminated as a shellfish contamination problem, in part due to effective bacteriological monitoring of shellfish and shellfish waters and beds. Today, viruses are the major problem associated

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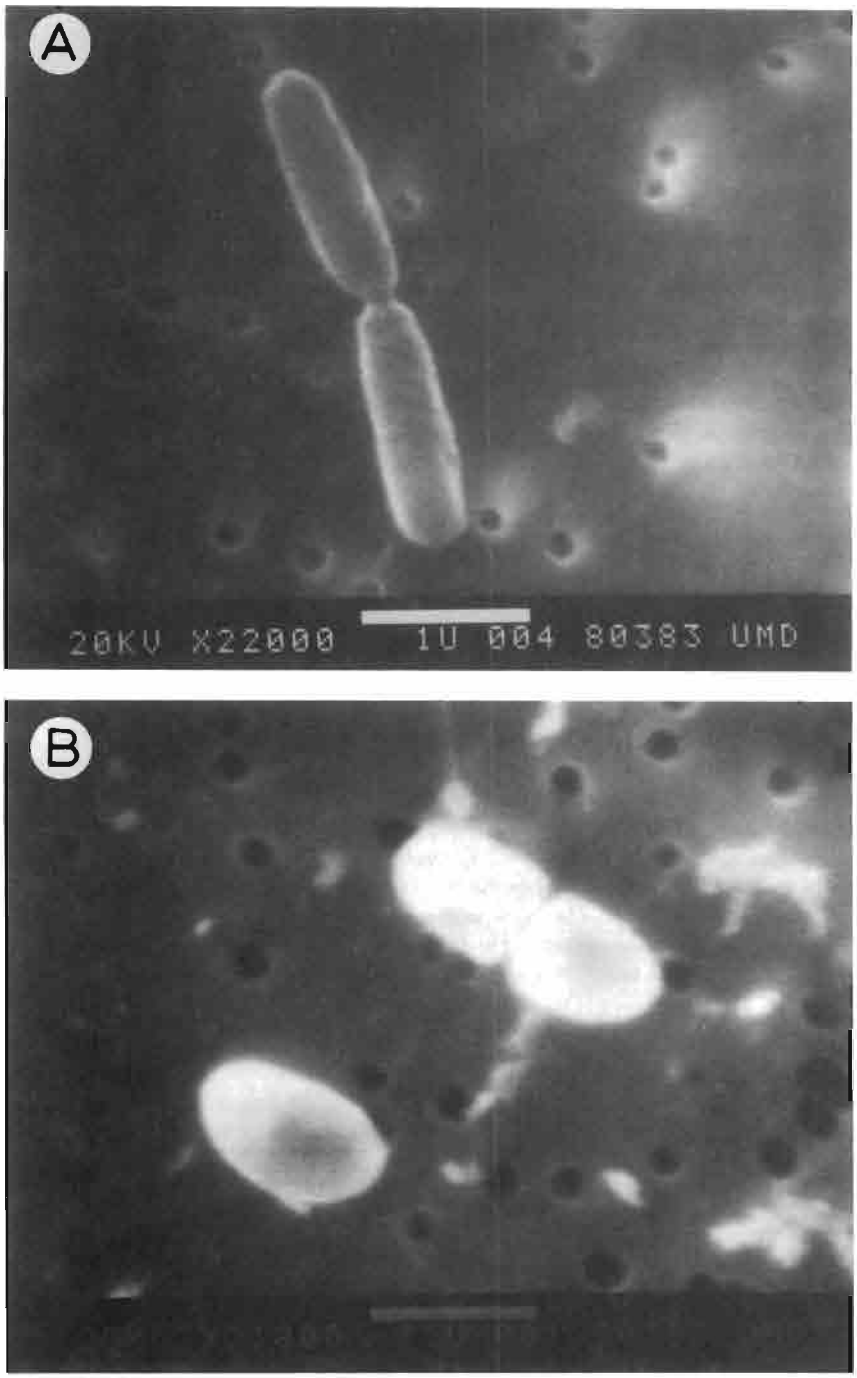

Figure 1.
Scanning electron micrographs of (A) *Vibrio carchariae* (× 22,000) and (B) *Listonella damsela* (× 21,000) grown in MSWC broth (see Grimes et al. 1984a).

Table 1.
Human pathogens frequently associated with shellfish.

Norwalk virus	*Salmonella* spp
Picornaviruses	*Vibrio cholerae*
Aeromonas hydrophila	*Vibrio parahaemolyticus*
Campylobacter jejuni	*Vibrio vulnificus*
Clostridium spp.	*Gonyaulax* spp.
Escherichia coli	*Ptychodiscus brevis*
Plesiomonas shigelloides	*Pyrodinium monilatum*

with shellfish, and disease incidence derived from consumption of virus-contaminated shellfish is on the increase. Table 2, adapted and updated from Goyal (1984), lists the more recent outbreaks of shellfish-borne disease. Clearly, most cases have been caused by viruses.

Bony fish also carry microorganisms which are potentially pathogenic for humans. However, when human pathogens are detected in bony fish, they are usually transients, present on the skin or in the gut; very seldom are

Table 2.
Recent outbreaks of disease derived from shellfish[a]

Shellfish	Agent[b]	Location	No. of cases	Citation
Oysters	Norwalk	Australia	2,000	Murphy et al. 1979
Oysters	Norwalk	Australia	150	Grohman et al. 1981
Mussels	HAV	England	41	Bostock et al. 1979
Shrimp	Vp	Louisiana	600	CDC 1972
Crabs	Vc	Louisiana	10	CDC 1978
Oysters	HAV	Alabama and Georgia	10	CDC 1979
Oysters	Norwalk	Florida	6	Gunn et al. 1982
Clams	Norwalk?	New York	150[c]	CDC 1982
Clams	Cj	New Jersey	18	Blaser et al. 1982
Clams	Cj	Japan	101	Itoh et al. 1982
Oysters	Vv	United States	24	Blake et al. 1979
Molluscs	Norwalk	New York	1,017[d]	Morse et al. 1986

[a] Adapted from Goyal (1984).
[b] HAV = Hepatitis A virus, Vp = *V. parahaemolyticus*, Vc = *V. cholerae*, Cj = *C. jejuni*, Vv = *V. vulnificus*.
[c] Represents 14 different outbreaks including cases of hepatitis.
[d] Represents 103 different outbreaks.

human pathogens found within deep tissues of bony fish and when they are detected the fish usually exhibit pathology.

Table 3 lists some of the human pathogens that are frequently encountered in bony fish specimens. It should be noted that most microorganisms on this list have a broad host range, in that they are not only pathogenic for humans and fish but also for other mammals, birds, and amphibians. The incidence of human morbidity caused by *Cryptosporidium* is becoming more frequent (Stibbs and Ongerth 1986). This protozoan appears to be widespread in nature, being present in fish (Dykova and Lom 1983; Pavlasek 1983). The resulting diarrhoeal disease is most common among immunodeficient persons (Stibbs and Ongerth 1986).

Marine mammals also carry human pathogens (Buck and Spotte 1986; Smith et al. 1978), some of which can cause serious disease in man and other terrestrial animals. Table 4 lists microorganisms frequently isolated from cetaceans and pinnipeds. Of the species listed, coagulase-positive staphylococci and *Vibrio* spp. are also ubiquitous in seawater, comprising over 1% and 60%, respectively, of randomly picked colonies isolated from seawater on marine agar (Grimes et al. 1984b). It is well documented that *Vibrio* spp. are autochthonous to marine and estuarine environments (Grimes et al. 1984b) and it appears that staphylococci are as well (Gunn and Colwell 1983). A 1987 outbreak of disease among marine mammals involved the deaths of over 300 bottlenose dolphins, *Tursiops truncatus*, from the northern Atlantic coast of the United States. Results of necropsies on several specimens demonstrated a secondary *Vibrio* involvement and findings that parallel our studies in sharks, e.g., skin lesions and septicemia.

Table 3.
Human pathogens frequently associated with bony fish.

Aeromonas spp.	*V. fluvialis*
Clostridium botulinum	*V. furnissii*
Erysipelothrix rhusiopathiae	*V. parahaemolyticus*
Mycobacterium marinum	*Listonella damsela*
Plesiomonas shigelloides	*Clonorchis sinensis*
Pseudomonas aeruginosa	*Heterophyes heterophyes*
Salmonella spp.	*Diphyllobothrium latum*
Vibrio cholerae	*Cryptosporidium*
V. alginolyticus	

Table 4.
Human pathogens frequently associated with cetaceans and pinnipeds.

Actinomyces mallei	*Neisseria mucosa*
Caliciviruses	*Nocardia* spp.
Coagulase + staphylococci	*Pasteurella multocida*
Clostridium chauvoei	*Pseudomonas aeruginosa*
Clostridium perfringens	*Streptococcus pyogenes*
Klebsiella pneumoniae	*Vibrio* spp.
Leptospira interrogans	

Risk of Contracting Human Disease from Marine Animals

Factors necessary for initiation of infectious disease in humans are listed in Table 5. Mode of transmission is, perhaps, the most critical factor, since all other factors are usually present in a given situation. It is for this reason that shellfish, primarily bivalve molluscs, constitute the

Table 5.
Key factors of infectious disease.

1. Infectious disease agent
2. Reservoir or habitat
3. Mode of transmission to host
4. Ability of agent to survive transmission
5. Portals of entry and exit
6. Susceptible host

most important source of water-borne disease in the United States today. Humans consume raw bivalves and, consequently, contract diseases. Transmission of pathogens from bottom sediment or seaweed surfaces to humans is not highly probable, and, hence, there are few reports of diseases deriving from such sources. However, as consumption of raw fish, seaweed, "seasalt", and other emerging marine foodstuffs increases, transmission of disease will become more frequent.

Human Pathogens in Sharks

The hypothesis that elasmobranchs carry an autochthonous bacterial flora throughout their various tissues was first proposed by Grimes et al. (1985b). This hypothesis is now based on a sampling regime that has included over 50 healthy, free-ranging neritic sharks collected from relatively pristine waters off the island of Bimini, Bahamas. Buck (1984) arrived at a similar conclusion after bacteriological examinations of skin, teeth, and gill surfaces and intestinal contents from 12 elasmobranchs taken from the Gulf of Mexico off Sarasota, Florida. Table 6 lists the shark species sampled to date, and Table 7 lists the tissue types examined and average bacterial concentrations in the various tissues. In general, blood is free from detectable bacteria, unless the shark has been severely compromised (e.g., stressed during capture) or is in a frank state of disease (Grimes et al. 1985b). Potential human pathogens isolated from various tissues and organs are listed in Table 8. Data presented in this table are a composite of all sharks sampled to date. The significance of *Clostridium* spp. present in

Table 6.
Sharks sampled for autochthonous bacteria.

Common name	Scientific name	Capture site
Blacktip shark	*Carcharhinus limbatus*	Bimini, Bahamas
Sandbar shark	*Carcharhinus plumbeus*	National Aquarium, Baltimore
Tiger shark	*Galeocerdo cuvieri*	Bimini, Bahamas
Nurse shark	*Ginglymostoma cirratum*	Bimini, Bahamas
Lemon shark	*Negaprion brevirostris*	Bimini, Bahamas
Sharpnose shark	*Rhizoprionodon porosus*	Bimini, Bahamas
Spiny dogfish	*Squalus acanthias*	Gulf of Maine
Sixgill shark	*Hexanchus griseus*	Bermuda

Table 7.
Densities of bacteria in selected shark tissues and organs[a].

	Lemon shark			
Organ/tissue	A	B	C	Average
Blood	0[b]	0	0	0
Muscle	0	$<10^5$	$<10^5$	$<7 \times 10^4$
Stomach	7×10^2	2×10^2	6×10^3	2.3×10^3
Intestine	2×10^4	$>10^6$	$>10^6$	7×10^5
Spiral valve	ND[c]	1×10^4	$>10^6$	$>5 \times 10^5$
Kidney	1×10^5	1×10^6	3×10^2	4×10^5
Spleen	ND	2	8×10^2	4×10^2
Liver	7×10^4	0	0	2×10^4

[a] Heterotrophic spread-plate colony count per gram of tissue on Marine Agar 2216 (Difco).
[b] 0 values represent no developed colonies after spread-plating 1.0 mL blood or 0.1 mL of a 1:5 dilution of muscle or liver, with extended (>21 d) incubation.
[c] ND = Not determined.

Table 8.
Human pathogens isolated from healthy sharks.

Aeromonas hydrophila	*Listonella damsela*
Clostridium botulinum	*Plesiomonas shigelloides*
Clostridium perfringens	*Proteus* spp.
Clostridium sordellii	*Vibrio alginolyticus*
Clostridium tetani	*Vibrio furnissii*
Escherichia coli	*Vibrio parahaemolyticus*
Fusobacterium sp.	

shark tissue samples is discussed by Youngren-Grimes (1990). In progress, and not included in Table 8, is a numerical taxonomic study of 236 gram-negative, oxidase-positive strains of bacteria isolated from sharks captured in the coastal waters of Bimini (5 lemon, 1 blacktip, 1 sixgill, 4 nurse, and 4 tiger; see Table 6). The various phena are still being evaluated, but one interesting identification was that of a strain of *V. parahaemolyticus* isolated from a forearm shark bite wound sustained by one of the scientists while assisting with blood collection from a lemon shark (Grimes et al., in prep.). Such an infection was predictable, based on the findings of Buck et al. (1984). Other potentially pathogenic species identified in this numerical study were *V. alginolyticus*, *V. fluvialis*, *V. vulnificus*, and *L. damsela*.

In addition to direct isolations, substantial serological evidence of human pathogens in elasmobranchs has also been accumulated. Microagglutination experiments performed with heat-inactivated serum collected from healthy, free-ranging sharks and with members of the *Vibrionaceae* and *Aeromonadaceae* provided preliminary evidence of high antibody titers to several strains of pathogenic *Vibrio* spp. Pooled sera from six nurse sharks were then purified by DEAE (cellulose N, N-diethylaminoethyl ether) separation, followed by size exclusion with Sephadex G-200. The resulting purified protein had a molecular weight of >200,000, lost all agglutination activity upon treatment with 2-mercaptoethanol, and migrated through 10% polyacrylamide in a manner similar to human immunoglobulin M (IgM). These results strongly suggest that nurse sharks produce IgM in response to autochthonous pathogens such as *Vibrio carchariae* (Brayton et al., in prep.). Perhaps of greatest ecological significance was the observation that nurse sharks consistently possessed the highest antibody titers of all sharks (~1:1024), and that these high titers were frequently against *V. cholerae*, the classic agent of human cholera. Nurse sharks are bottom feeders, living on crustaceans and molluscs; both of these benthic invertebrates are natural habitats for *V. cholerae* (Hood et al. 1981; Huq et al. 1986).

It is now quite clear that elasmobranch tissues and organs, unlike those of bony fish, contain autochthonous bacteria, including potential human pathogens. Both direct isolation and serology support this hypothesis. Portals of entry have been previously discussed (Grimes et al. 1985b), and both the serological and direct isolation data support the hypothesis that sharks are continuously exposed to a variety of marine bacteria. These primitive fish have evolved an ability to co-exist with most of the potential pathogens, including the human pathogens. Only when sharks are compromised does this co-existence break down, and the sharks succumb to clinically evident disease.

Past studies with ^{14}C-labelled urea and anti-*Vibrio* antibiotics suggested that shark tissues are incapable of hydrolyzing urea. Instead, data revealed that the resident bacteria hydrolyzed urea, thereby benefiting their shark hosts by maintaining a relatively stable tissue urea concentration (Knight et al. 1988). Also of interest is a study of the ability of *V. carchariae* to decompose common shark biopolymers, especially connective tissue constituents (Grimes et al. 1989). The data collected showed that *V. carchariae* strains were capable of hydrolyzing chondroitin sulfate, hyaluronic acid, gelatin, and collagen and were also capable of using squalene as a pure carbon source. Obviously, such a hydrolytic repertoire would be of great benefit to *V. carchariae* as a colonizer and scavenger of shark tissue.

Conclusions

In conclusion, potential human pathogens are ubiquitous in marine animals, including elasmobranchs. These pathogens do not often cause disease in man, presumably because they lack a mode of transmission. However, increased reliance on the sea for food and recreation, combined with a concomitant increase in the disposal of anthropogenic wastes in the sea, increases the probability for human disease to occur, especially when one considers that fish are often consumed in a raw state, e.g., sushi, sashimi, and raw oysters. Obviously, consumption of raw fish, including raw shark meat, with an infective dose of a human pathogen (Table 8) could lead to gastroenteritis. Supportive of this statement are recent reports describing an increase in shellfish-borne diseases derived from the sea (Goyal 1984; Table 2). Clearly, continued introduction of human pathogens into the oceans of the world, along with nutrients to support growth of both introduced (allochthonous) and autochthonous microorganisms, will increase the probability of transmission (Grimes et al. 1986). Less clear is the role that these pathogens play after colonizing marine animals. Allochthonous human pathogens are probably transient, having little positive or negative effect on their temporary host. There are exceptions to this generalization of course, for example the calicivirus diseases of swine and marine mammals (Smith et al. 1978). Autochthonous bacteria capable of causing human diseases, on the other hand, fill a true niche in the marine environment. While the identity of this niche is not always clear,

in some cases it appears to involve degradation, e.g., degradation of invertebrate chitin (Huq et al. 1986; Wortman et al. 1986) or shark tissue urea (Grimes et al. 1985b; Knight et al. 1988) by *Vibrio* species.

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