Chapter 4
Human Bacterial Diseases from Ocean

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Glossary

Allochthonous	Exogenous, alien or nonindigenous; arising from another source or medium.
Ambient	Being of the surrounding area or environment.
Archaea	One of three domains on Earth, including the *Bacteria* and *Eukarya*. *Archaea* are prokaryotes that do not have peptidoglycan cell walls; they lack membrane-bound organelles (e.g., nucleus, mitochondria, endoplasmic reticulum, chloroplasts), possess 70 S ribosomes and have ether-linked lipids in their membranes.
Autochthonous	Indigenous, native, arising from within.

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Bacteria	One of three domains on Earth, including the *Archaea* and *Eukarya*. *Bacteria* are prokaryotes that possess peptidoglycan cell walls; they lack membrane-bound organelles (e.g., nucleus, mitochondria, endoplasmic reticulum, chloroplasts), possess 70 S ribosomes and have ester-linked lipids in their membranes.
Biodiversity	The richness or complexity of life forms in an ecosystem, biome or on Earth itself.
Commensal organism	An organism participating in a symbiotic relationship in which one species derives some benefit while the other is unaffected.
Ectotherm	An organism that controls body temperature through external means.
Endotoxin	The lipid component (lipid A) of the outer membrane lipopolysaccharide (LPS) of all gram-negative bacteria. Endotoxin is released into a host or the environment when the cell lyses and its outer membrane breaks up.
Epidemic	The incidence of disease above the normal or endemic incidence.
Eukarya	One of three domains on Earth, including the *Bacteria* and *Archaea*. *Eukarya* possess membrane-bound organelles (e.g., nuclei, mitochondria, chloroplasts), histones associated with their DNA and 80 S ribosomes in their cytoplasm. Plants and animals are eukaryotic.
Exotoxin	Any toxin that is secreted into the cell's immediate environment. Most exotoxins are proteins, and they are made by both gram-negative and gram-positive bacteria.
Facultative organism	An organism that is capable of growth both in the presence and absence of oxygen.
Foodborne disease	A disease that is caused by the ingestion of pathogens conveyed by food.
Food intoxication	Illness caused by the ingestion of food that contains a toxic substance.
Hemolysin	A proteolytic enzyme that lyses red blood cells.
Lysogenic conversion	Insertion of bacterial virus (bacteriophage) DNA into the chromosomal DNA of its bacterial host thereby conferring one or more new traits on the host.
Nosocomial	Infections (and disease) that are acquired in clinical settings (e.g., hospitals, outpatient clinics, emergency rooms, physician offices).
Opportunistic pathogen	Any pathogen that accidently acquires entrance to a host and then only causes disease if one or more risk factors are present in the host.
Pandemic	An epidemic of world-wide proportions.

Pathogenesis	The production or development of a disease, specifically the cellular reactions and other pathologic mechanisms occurring in the progression of the disease.
Pathogenic	The ability of a species to cause disease. However, because pathogenesis is typically caused by one or more than one virulence factors produced by one or more genes, any given pathogenic species will often display different degrees of pathogenesis.
Pathogenicity island	A cluster of virulence genes (and sometimes cryptic genes and other small genetic elements) flanked by direct repeats, insertion sequences or tRNA genes such that the clusters are easily transmitted to other bacteria via a process called *horizontal gene transfer*.
Plasmid	A circular, double-stranded DNA molecule containing specialty genes that, in general, are not essential for survival of the host bacterium or genes that are cryptic (unknown). Plasmids can replicate autonomously or integrate into and replicate with the chromosome. Plasmids are smaller than the chromosome, on average 5% the size of the chromosome.
Point source	A single, identifiable localized source of something.
Quorum sensing	A chemical mechanism used by bacteria to measure their population density. When the chemical signals reach a certain level, special genes are expressed.
Sentinel	An indicator whose presence is directly related to a particular quality in its environment at a given location.
Sequences	The order of nucleotides in a specific length of DNA or RNA.
Virulence	The degree of pathogenicity. Virulence is a compilation of toxins, hemolysins, proteases and lipases that may not be possessed by all strains of a pathogenic species.
Waterborne disease	A disease that is transmitted by water.
Zoonosis	An animal disease transmissible to humans under natural conditions or a human disease transmissible to animals.

Definition of the Subject and Its Importance

Several bacteria that cause human disease can be found in the ocean. The actual incidence of bacterial disease that results from seawater or seafood is not precisely known but is thought to be relatively low in the USA, although some diseases are

on the rise. Bacterial disease from the ocean is more prevalent worldwide, especially in developing countries and in countries that derive most of their protein from seafood. Compared to the viruses, bacteria account for a much lower incidence of disease emanating from the ocean, both in the USA and worldwide. However, it is important to understand and mitigate bacterial disease from the ocean, because of such environmental pressures as global warming, antibiotic resistance, pollution, breakdowns in sanitation (e.g., Haiti after the earthquake) and tourism.

Introduction

Myriad bacteria reside in the ocean and most (>99.9%) have not been isolated and are known only by their unique molecular signatures (e.g., 16 S rRNA sequences) [35, 59]. These bacteria belong to the domain *Bacteria* which exhibits extensive *biodiversity*, only exceeded by the biodiversity within the domain *Archaea* and the viruses. The vast majority of bacteria in the ocean do not cause disease and Archaea are not known to cause any human disease [39]; however, a very small number (percentage) of bacterial species found in the ocean cause *pathogenesis* in plants and animals. Some of these pathogens are indigenous to the ocean and are defined as *autochthonous*. Others (*allochthonous* bacteria) are of exogenous or terrestrial origin and are introduced to the ocean via surface runoff, rivers and streams, atmospheric fallout and ocean disposal of wastes (intentional or accidental); the chapter on enteric viruses (See S.NO.12, J. Woods, Waterborne Diseases of the Ocean, Enteric Viruses, this volume) has a nice discussion of allochthonous sources. This chapter will focus on those allochthonous and autochthonous bacteria that cause *waterborne* and *foodborne* disease in humans. Some also cause disease in marine animals (and a few in marine plants) but nonhuman diseases will not be addressed in this chapter.

Autochthonous human pathogens have evolved in the ocean and for largely unknown reasons have the ability to infect and cause disease in humans. Almost all of these diseases result from ingesting seawater, eating seafood, or broken skin contact (swimming, wading, or working in seawater). The allochthonous human pathogens are transients in the ocean with varying abilities to survive in seawater; all of the allochthonous pathogens infect humans via the same routes as autochthonous pathogens, i.e., contaminated seawater, seafood or broken skin contact. There are a few bacteria that may fit both definitions, and there is no current scientific consensus about the place of these bacteria. Examples of these ubiquitous bacteria include the enterococci [159], *Staphylococcus aureus* (see Allochthonous Pathogens in this chapter) and *Pseudomonas aeruginosa* [114].

In the following pages, allochthonous and autochthonous marine bacteria that cause disease(s) in humans will be discussed in terms of their biology, ecology, pathogenesis, and epidemiology. Disease treatment will also be discussed but not in clinical detail.

Autochthonous Pathogens

Autochthonous pathogens generally cause one or more diseases in marine animals and if the disease(s) is transmissible to humans it is classified as a *zoonosis*. It some cases, humans can transmit disease to marine animals.

Vibrio

Vibrios, and specifically *Vibrio cholerae*, were first observed by Pacini in 1854 and later isolated in pure culture from cholera patients by Robert Koch in 1883 [8, 27]. Today, the Vibrios comprise a large genus (>80 species) and belong to the *Gammaproteobacteria* [47]. They are gram-negative rods, often slightly curved (e.g., *V. cholerae*) and most are motile in liquids by means of one or more polar flagella; on solid surfaces they are motile by means of lateral flagella. Unlike most bacteria, Vibrios possess two circular chromosomes (one large and one small) which are of relevance to this chapter because of the distribution of *virulence* genes on the two chromosomes. The large chromosome is usually referred to as Chromosome 1 (Ch1) and it tends to contain housekeeping genes (DNA replication, transcription, translation, flagellar synthesis, metabolic pathways). Chromosome 2 (Ch2) tends to contain accessory genes (pathogenicity, antibiotic resistance, host defense avoidance, survival in adverse environments). Most of the Vibrios can metabolize a large number of organic compounds, including sugars, amino acids, fatty acids, carbohydrates, proteins, lipids, alcohols, and selected aliphatic and aromatic hydrocarbons. Indeed, prior to the development of molecular biology methods, the Vibrios were largely identified and classified by these diverse and extensive metabolic traits [22].

In addition to metabolic diversity, the pathogenic Vibrios also possess an array of *exotoxins*, proteases, transport proteins, attachment mechanisms, and lipases that act as virulence factors. Since Vibrios are gram-negative, they also possess *endotoxin* which differs in toxicity from strain to strain. Indeed, the Vibrios are well equipped to cause disease in their hosts – accidental or otherwise. In general, the Vibrios are opportunistic pathogens – for both humans and marine animals – and they cause systemic infections, skin lesions and gastroenteritis. In fish, the infections often lead to hemorrhagic skin lesions (known as vibriosis) and pathology in the liver, spleen, and kidney. In humans, the diseases arise from contamination of cuts and other skin lesions with seawater (and to a lesser extent marine animals, e.g., stingray barbs and barnacles) and from ingestion of raw, undercooked or cooked but re-contaminated seafood and seawater. Depending on the species, skin infections can remain localized or become systemic and ultimately fatal. Oral ingestion of seafood and seawater leads to various degrees of gastroenteritis and, in some cases (e.g., *V. vulnificus)* life-threatening systemic infections. Although 12 *Vibrio* spp. are considered to be

Table 4.1 Number of *Vibrio* illnesses (excluding toxigenic *V. cholerae*) in the USA, 2008[a]

Species	Patients	Deaths	Isolates	Specimen[b]
V. alginolyticus	99	1	99	Wound and other[c]
V. cholerae (nontoxic)	50	2	50	Stool and blood
V. fluvialis	29	3	29	Stool and wound
V. hollisae[d]	4	1	4	Stool and wound
V. metschnikovii	1	0	1	Blood
V. mimicus	32	0	32	Stool and other
V. parahaemolyticus	270	4	270	Stool and wound
V. vulnificus	85	24	94	Blood and wound
Species not identified	23	0	24	Stool and other
Multiple species	6	0	13	Wound and stool
Total	**599**	**35**	**616**	**Stool most frequent**

[a]Data reported to the Cholera and Other *Vibrio* Illness Surveillance (COVIS) system maintained by the CDC (see http://www.cdc.gov/nationalsurveillance/PDFs/Jackson_Vibrio_CSTE2008_FINAL.pdf)

[b]The predominant specimen types with the most predominant listed first. Additional specimens, including actual numbers, can be seen in the COVIS report[a]

[c]"Other" consists of ear, sputum urine, and other specimens

[d]Reclassified to *Grimontia hollisae* in 2003 [137]

human pathogens, the predominant human pathogens are *V. cholerae, V. parahaemolyticus* and *V. vulnificus*. Table 4.1 presents a recent compilation of *Vibrio* diseases in the USA. In addition to the eight species listed in Table 4.1, the other human pathogens include *V. cincinnatiensis, Photobacterium damselae* (initially named *V. damsela*), *V. furnissii*, and *V. harveyi*; these four Vibrios, along with *G. hollisae* and *V. metschnikovii*, will not be discussed in this chapter, due to their low incidence of disease in humans.

All of the Vibrios require NaCl to grow properly, although this requirement is minimal for some species, e.g., *V. cholerae* and *V. mimicus*. Most if not all Vibrios use a sodium motive force to drive their polar flagella [94, 157] and at least a few species appear to use a sodium motive force to make ATP [37, 138]. Lateral flagella are produced by Vibrios when they grow on solid surfaces; these flagella are driven by a proton motive force [94] and are responsible for swarming. In addition to a requirement for sodium, most Vibrios are mesophiles and, therefore, do best in warmer waters. Recently, it has been suggested that global warming might increase the incidence of Vibrios worldwide [25, 28]. Interestingly, laboratory-confirmed infections with *Vibrio* spp. began to exceed those from *Salmonella*, Shiga toxin-producing *Escherichia coli* O157, *Campylobacter* and *Listeria* in late 2000 and this US trend has increased through 2008 [17]. In 2009, the overall rate of foodborne disease caused by Vibrios was 0.35 per 100,000 population [18]. The relative rates of the other four pathogens have either remained level (*Salmonella*) or shown a decrease (*Shigella* rates decreased 40% and STEC O157 decreased 25%) while Vibrio rates have increased by 47% [17]. Some have suggested that because this trend began just before the El Niño years 2002–2003 and continued through the El Niño years 2006–2007, it was caused by global warming. Intriguing as it may

Table 4.2 Temperature and salinity preferences for the pathogenic Vibrios[a]

	Temperature		Salinity	
Vibrio species	Optimum	Range	Optimum	Range
V. cholerae	25°C [24, 26]	17–40°C [15, 31]	2–14 ppt [26, 31]	<1–60 ppt [15]
V. parahaemolyticus	38°C [27, 33]	8–45°C [16, 33]	17–23 ppt [19, 27, 44]	<1–96 ppt [33]
V. vulnificus	30°C [39]	9–40°C [21, 39, 40, 106]	5–10 ppt [25]	5–35 ppt [25, 40, 106]

[a]Bracketed numbers are supporting citations found in the *Primary Literature*

be, this observation is circumstantial and cannot be confirmed. However, in 1991 there was an outbreak of cholera in Peru, a country that had not seen this disease for over 100 years [136]. The source of the 1991 outbreak was never determined and two more outbreaks occurred in 1993–1995 and 1997–1998. Gil et al. [56] very nicely demonstrated that the Peru outbreaks in summer 1998 correlated (linear regression, $P < 0.001$) with the sea surface temperature peak associated with the strong El Niño that year. Colwell et al. [25] and [28] have made similar observations for the Bay of Bengal. In 2004, an outbreak involving cultured raw oysters and *V. parahaemolyticus* occurred aboard a cruise ship in Prince Edward Sound [96]. Mean water temperatures had been increasing in Prince William Sound (0.21°C per year) since 1997, and in 2004, for the first time, mean daily temperatures in the sound did not drop below 15°C [96]. Johnson et al. [74] reported that when temperatures drop below 15°C *V. parahaemolyticus* and *V. vulnificus* are no longer culturable from water and sediment in Mississippi Sound; similar observations have been reported by others, suggesting that 15°C is a limiting temperature for many of the Vibrios. Clearly, it is beginning to look like increasing ocean temperatures are increasing the incidence of Vibrio disease and as more data become available this Vibrio incidence-climate link may be substantiated. Temperature and sodium preferences for the three principal pathogenic Vibrios are shown in Table 4.2. Finally, in the past decade, scientists are using remotely-sensed satellite data, including temperature and salinity, to predict human health risks from pathogenic vibrios in water and oysters [90, 117].

Vibrio alginolyticus

This *Vibrio* is very common in estuarine and marine waters worldwide; in fact, it is unusual not to isolate *V. alginolyticus* when culturing coastal and estuarine water samples. It is known for its ability to swarm on isolation media [141] and it often overgrows desired isolates. Recently, it was demonstrated that *V. alginolyticus* is capable of quorum sensing and that QS is responsible for biofilm formation (swarming is a prelude to this phenomenon) and development of virulence factors involved with fish disease [64].

The most common disease caused by *V. alginolyticus* in humans is wound infection. Of the 99 infections reported to the CDC in 2008 (Table 4.1), 64 isolates came from wound infections, 5 from blood, 3 from stools and 27 from other specimens. Most infections are mild and self-limiting, although *V. alginolyticus* has been demonstrated in a few cases of severe necrotizing fasciitis that involved patients at risk because of cirrhosis [66]. Cases of gastroenteritis caused by *V. alginolyticus* are rare [140], as indicated in Table 4.1. This Vibrio has also caused eye infections [86].

Vibrio cholerae

During the early cholera pandemics of the 1800s, *V. cholerae* was responsible for millions of deaths and was feared as the most dangerous waterborne human pathogen known [27, 76]. It still causes several thousand deaths annually [153] and a small number of cases occur in the USA each year (see COVIS report cited in Table 4.1). The World Health Organization recently reported that in 2009 the number of cases worldwide (45 countries reporting) was 221,226 with 4,946 deaths (2.24% case fatality rate) [153]. The number of cases in the USA in 2008 was nine and none of these patients died (see COVIS report, Table 4.1). Although molecular detection is very effective and widely used [102] and the genomes of several strains have been fully sequenced, for documentation and epidemiology purposes isolates are still serogrouped by means of their O antigens. The predominant serogroups causing human disease worldwide are O1 and O139; in the USA, the CDC tests for serogroups O1, O75, O139, and O141 (COVIS report cited in Table 4.1).

The classic disease caused by *V. cholerae* is a rapidly developing, profuse, watery diarrhea that is usually accompanied by severe dehydration. The cholera diarrhea is often called a "rice-water" stool because as the disease progresses the frequent stools are little more than water containing flecks of mucous abraded from the intestinal mucosa. When death occurs, it is because of the severe dehydration (water loss) and acidic coma (loss of sodium bicarbonate to the diarrhea). Historically, the death rate often exceeded 50% and death could result in as few as 24–36 h after onset of symptoms. As noted above [153] the death rate for cholera is now below 5% and most cases of cholera today are little more than a transient diarrhea. In addition to classic cholera, *V. cholerae* can also cause self-limiting gastroenteritis and wound infections; it has also been implicated in fish disease (eels and ayu). An interesting and illustrative human case occurred after Hurricanes Katrina and Rita and involved a Louisiana couple who consumed properly cooked (boiled) shrimp that had been placed on the ice used by them to transport the raw shrimp. The husband developed a severe case of cholera that caused renal, pulmonary and cardiac failure. He was given ciprofloxacin and aggressive rehydration therapy and he did not die. Of interest is that the husband had a history of common Vibrio risk factors – history of high blood pressure, alcoholism, diabetes, brain tumor, and renal failure that required frequent dialysis. His wife had mild diarrhea and was treated (ciprofloxacin and rehydration) as an outpatient. *V. cholerae* O1, serotype Inaba, biotype El Tor was isolated from both patients [15].

The main virulence factor associated with cholera is an exotoxin known as the cholera toxin (CTX). CTX is not produced by all strains of *V. cholerae*, and it is a protein that is composed of two subunits – one A subunit and five B subunits. The *ctx* genes that encode for these two subunits, *ctx*A and *ctx*B, are actually genes in a virus (a bacteriophage or phage) that infects *V. cholerae* and establishes a lysogenic relationship with its host [148]. The CTX phage that infects *V. cholerae* is a lambdaphage; and instead of producing more phage when it infects its host, this phage inserts its DNA into the chromosome (Ch1) of the host which then allows the host to produce CTX – a process called *lysogenic conversion.*

V. cholerae produces other virulence factors, including the toxin-coregulated pilus (TCP) produced by the *tcp*A gene, repeat-in-toxins (RTXs), and a heat-stable enterotoxin (NAG-ST) that is related to the heat-stable enterotoxin produced by *E. coli* [135]. The TCP is thought to be necessary for intestinal colonization by *V. cholerae* and may also serve as an attachment site for CTX phage [148]. Type 2 secretion systems are present in *V. cholerae*, and these systems provide a physical conduit for the bacteria to secrete toxins into their host cells (see secretion system discussion in "*Vibrio parahaemolyticus*" section).

In closing, prevention and treatment of cholera deserves mention. Cholera has a very low incidence in developed countries, primarily because of good sanitation, but this is not the case in developing countries [68]. In both developed and developing countries, cholera is usually a self-limiting disease requiring minimal treatment other than rehydration and electrolyte replacement; antibiotics are not usually administered. In developing countries where safe drinking water is not readily available, filtration of drinking water is very effective in preventing cholera. A very simple but highly effective filtration procedure was pioneered in Bangladesh [26]. The procedure involves using folded sari cloth (eight layers of old sari) to filter the drinking water; this procedure removes zooplankton to which the cholera bacilli are attached [26].

Cholera in Haiti

In January, 2010 Haiti was ravaged by a major earthquake that had an epicenter approximately 25 km from the capital city of Port-au-Prince. On October 21, 2010 cholera was confirmed in Haiti by the CDC; the causative agent has been identified as *V. cholerae* O1 serotype Ogawa. As of October 14, 2011, the Hatian Public Health Ministry reported that over 473,649 people have been infected and 6,631 patients have died. The disease peaked in early 2011 but cases and deaths continue. Interestingly, Haiti had not reported a cholera case for over a century and the source of the outbreak was, early on, not known. Clearly, the lack of hygiene, safe drinking water and safe food that followed the earthquake and continues to exist has contributed to the onset and continuation of cholera in Haiti. However, recent molecular evidence reported in the CDC journal "Emerging Infectious Diseases" demonstrated that a UN peacekeeping force from Nepal introduced the cholera strain into the Meille River.

Vibrio fluvialis

The incidence of disease caused by *V. fluvialis* is thought to be very low, both in the USA (Table 4.1) and worldwide. This *Vibrio* primarily causes enteric disease but can cause wound infections and, rarely, other extraintestinal infections.

Vibrio mimicus

This *Vibrio* is similar to *V. cholerae* in many ways and early on was thought to be an atypical *V. cholerae* – hence the species name "mimicus." The primary disease caused by *V. mimicus* is gastroenteritis and some strains carry *ctx* genes as well as other virulence factors found in *V. cholerae* and other Vibrios.

Vibrio parahaemolyticus

First isolated in Japan from a gastroenteritis outbreak traced to the consumption of shirasu, a popular fish product (272 cases and 20 deaths; [54]), *V. parahaemolyticus* is the most common cause of foodborne disease in countries that consume high quantities of seafood [61]; in the USA, it is the most common cause of seafood-borne disease (see Table 4.1). As is the case with all the Vibrios that cause human disease, *V. parahaemolyticus* causes gastroenteritis and wound infections, and it is the most common cause of Vibrio disease in the USA (Table 4.1). *V. parahaemolyticus* can be isolated from most forms of seafood but is most commonly associated with shellfish [12].

The first *V. parahaemolyticus* pandemic began in 1996, and it continues to involve three major serotypes – O3:K6, O4:K68, and O1:K untypable [23]. Recent outbreaks are described in Table 4.3.

All *V. parahaemolyticus* strains possess the *tlh* (thermolabile hemolysin) gene, and this gene is frequently used to rapidly detect and confirm identification of

Table 4.3 Recent outbreaks and cases of *V. parahaemolyticus* gastroenteritis

Date	Location (ref.)	Source	Cases/deaths	Serotype	El Niño
1997	Pacific North West [12]	Oysters	209/1	O1, O4, O5	Yes
1998	Gulf of Mexico, NE, Pacific NW [32]	Oysters	416/?	O3:K6	Yes
1998	Chile [62]	Shellfish	~300/?	O3:K6	Yes
1998	Japan [61]	Seafood	12,318/?	O3:K6	Yes
2004 and 2005	Chile [62]	Seafood	~5,100/?	O3:K6	No
2004	Alaska [96]	Oysters	62/0	O6:K18	No
2006	New York, Oregon, Washington [16]	Oysters	177/0	O4:K12	Yes
2006	Chile [62]	Shellfish	900/?	O3:K59	Yes

the species. However, *tlh* is not unique to *V. parahaemolyticus*; *V. harveyi*, *V. alginolyticus*, and *V. fischeri* have also been shown to contain a *tlh* homologue or a related hemolysin gene [72, 134, 149].

When first discovered, it was shown that *V. parahaemolyticus* had the ability to hemolyze red blood cells in a special culture medium called Watsumaga agar; it was later shown that this hemolysis, called the Kanagawa reaction, was mediated by a hemolysin called thermostable direct hemolysin TDH [67]. Most pathogenic *V. parahaemolyticus* strains possess the *tdh* gene and/or the *trh* (thermostable related hemolysin) gene [73], although some pathogenic strains contain neither *tdh* nor *trh* [98]. The *tdh* genes are located in a *pathogenicity island* on Ch2 [91]. In general, environmental and food isolates do not contain tdh and trh ([73] and [74]).

Gram-negative bacteria possess a fascinating injection apparatus called secretion systems. These allow bacteria to inject various substances into the cells of *Eukarya.* As is the case for many bacterial virulence factors, especially those associated autochthonous pathogens, such as the Vibrios; microbiologists understand how these factors function in human pathogenesis but do not know the function of these factors in the environment. To date, six secretion systems (T1SS, T2SS, T3SS, T4SS, T5SS and T6SS) have been described. In 2003, the sequenced genome of *V. parahaemolyticus* RIMD2210633 was shown to contain two different T3SS genes – T3SS1 and T3SS2 [91]. T3SS1 is located in a pathogenicity island on Ch1, T3SS2 is in a Ch2 pathogenicity island, and it is now known that there are two different T3SS2 – T3SS2α and T3SS2β [103].

Makino et al. [91] identified at least 50 other genes in *V. parahaemolyticus* that may be involved with pathogenesis. Some of these additional virulence factors include urease [112], attachment mechanisms [60, 130], ToxR [30], and RTX toxin [91].

Vibrio vulnificus

One of the more recently discovered pathogenic Vibrios [46, 123], *V. vulnificus*, causes serious diseases in humans and is thought by some Vibrio biologists to be the most virulent bacterium now known (death rate >50% in patients at risk [104]; the overall case fatality rate reported by CDC [97] for 1992–1997 was 39%). *V. vulnificus* is thought to cause three human diseases: primary septicemia (caused by the ingestion of raw or undercooked shellfish – especially oysters), gastroenteritis (caused by the ingestion of raw or undercooked shellfish), and wound infections (caused by contact with water, barnacles, fish barbs, and other marine objects). The literature documenting simple gastroenteritis is scant and, accordingly, some experts question this disease having a *V. vulnificus* etiology (J.D. Oliver, personal communication). Primary septicemia is a rapidly developing disease that can result in death in less than 48 h after consuming seafood containing the bacteria. The bacteria move from the intestinal tract into the blood stream and, from there, set up serious infections in tissues, especially the extremities. Wound infections also most frequently involve extremities and will cause bullas (Fig. 4.1). When the infection

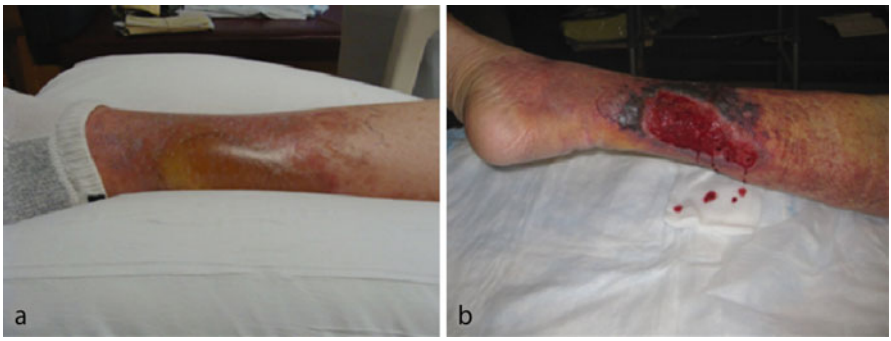

Fig. 4.1 Wound infection of unknown etiology, caused by a minor scratch from a barnacle. Within 36 h the scratch had developed into a bula (**a**). The bula was debrided (**b**) and did not progress into necrotizing fasciitis (Grimes DJ, Ekenna O unpublished data)

progresses into deep tissue and begins to cause death of that tissue, the disease is called necrotizing fasciitis. The infection shown in Fig. 4.1 did not develop into necrotizing fasciitis but is a good example of what often happens when patients are placed on aggressive antibiotic therapy to prevent progression of the infection. In this case, the patient was given three different antibiotics (clindamycin, levafloxacin, and doxycycline) prior to culture of the lesion, and these antibiotics probably caused the etiological agent to become nonculturable, a phenomenon observed and discussed by others [55, 126].

In general, *V. vulnificus* infections in healthy individuals are often not serious, but this is not always the case. Serious and fatal infections can occur in all patients and especially in those at risk; risk factors include: preexisting liver dysfunction or disease, diabetes, alcoholism, poor circulation, and immunosuppressive drug therapy. It is imperative that such individuals at risk are properly counseled about contact with seafood and seawater, so that they do not become infected with any *Vibrio* capable of causing disease in humans.

The virulence factors associated with *V. vulnificus* are not well understood. Known factors include *V. vulnificus* hemolysin (VVH) and RT [104]. There are two genotypes of *V. vulnificus* – clinical (C) and environmental (E) – and only C causes disease in humans. All strains of *V. vulnificus* have powerful iron sequestration ability (siderophores) which allows them to out-compete other species (including humans and fish) for essential iron. Capsular polysaccharide (CPS) formation is important and only encapsulated strains are virulent [104]. In addition, endotoxin (the lipoidal moiety of LPS) is very important and may be the most important cause of shock and death from *V. vulnificus* infections [104]. The complete genomic analysis of *V. vulnificus* YJ016 revealed the presence of RTX genes and they along with siderophore genes are located on Ch2 [21]. Type IV pilins, used as adherence mechanisms by many *Gammaproteobacteria* including *V. cholerae*, are a consistent feature of *V. vulnificus* and function in biofilm formation, attachment to epithelial cells, and possibly in the colonization of oysters [109, 110]. Other putative virulence factors have been described but their role in pathogenesis is unclear.

Aeromonas

Members of the genus *Aeromonas* are primarily freshwater bacteria that cause disease in both humans and aquatic animals. The most common isolates from human clinical specimens are *A. hydrophila, A. caviae, and A. sobria*, with *A. hydrophila* being the most common. Although *Aeromonas* spp. are frequently isolated from estuaries and the coastal ocean, they may not be truly autochthonous, and human disease from *Aeromonas* does not normally result from seawater or seafood. In fact, there is controversy about the ability of *Aeromonas*, a well-known pathogen of fish and amphibians, to cause disease in humans [48]. If one accepts the literature in support of human disease, it is believed that most human disease occurs in the form of gastroenteritis resulting from the ingestion of fish, shellfish, red meats, and contaminated water [48]. *Aeromonas* gastroenteritis presents either as a cholera-like disease with watery stools or a dysenteric-like illness that can include bloody stools [48]. Wound infections can also result from contact with contaminated water, either freshwater, seawater or brackish water [14, 75, 144].

Three recent reports of *Aeromonas* infections are illustrative of this seafood-borne and waterborne pathogen. In the aftermath of the 2004 tsunami that devastated eight countries and caused an estimated 225,000 deaths, the Thai Ministry of Public Health quickly began meeting health care needs in Thailand. From December 26, 2004 (the day of impact) to January 11, 2005, 1,237 cases of acute diarrhea, 356 wound infections, 177 febrile illnesses, and 156 respiratory illnesses were reported to the MOPH; only two deaths (both from aspiration pneumonia) resulted [14]. The most common isolates from the wound infections were *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp. *E. coli*, and *A. hydrophila* (two isolates); surprisingly, no Vibrios were isolated, as was the case with Hurricanes Katrina and Rita.

Eighty-two strains of presumptive *Aeromonas* spp. were isolated from 250 frozen freshwater fish (Tilapia, *Oreochromis niloticus niloticus*) intended for human consumption and purchased in local markets in Mexico City. The isolates were identified with standard molecular techniques (16 S rRNA) and 88.3% were placed in two species – *A. salmonicida* (67.5%) and *A. bestiarum* (20.9%). The remaining isolates were identified as *A. veronii* (5.2%), *A. encheleia* (3.9%), and *A. hydrophila* (2.6%). The authors noted that this was one of the first major *Aeromonas* studies conducted in Mexico, and further noted that their results demonstrated the need for concern over putative pathogens with antimicrobial resistance and known virulence factors being present in food meant for human consumption [9].

Finally, a study of the prevalence of *A. hydrophila* in marketed seafood (fish and prawns) was conducted in land-locked city in South India by Vivekanandhana et al. [145]. Random samples of seafood (536 fish and 278 prawns) were collected from several vendors in a popular seafood market, and fish showing visible spoilage, injury or disease were avoided. Overall, 180 fish samples (33.6%) and 49 prawn samples (17.6%) contained *A. hydrophila*. The authors attributed the incidence to

temperature abuse, fly contamination from a nearby sewage treatment plant, and the ability of *A. hydrophila* to grow at refrigerator temperatures (4–7°C); and they further noted that *A. hydrophila* is a pathogen of emerging importance [145].

Aeromonas Soft-Tissue Wound Infection

In late summer of 2010, a 7-year-old boy suffered a large and complex laceration injury to the right calf while recreational boating on a coastal river. He was brought within 2 h to the hospital, after initial first aid in the field. The wound was thoroughly washed and cleansed at surgery and closed with over 30 fine stitches. He was discharged the next day in stable condition and on oral antibiotics.

He failed to take the prescribed antibiotics because of nausea and vomiting (no diarrhea or fever). On readmission 6 days later, the wound was infected. Wound culture showed a rapid growth of a gram-negative rod (GNR) that was beta-hemolytic on blood agar, and also grew on MacConkey and chocolate agars. It was oxidase positive, mucoid, catalase positive, and motile.

The organism was confirmed to be *Aeromonas hydrophila*. It was found to be sensitive to second and third-generation cephalosoprins, quinolones, tetracycline, trimethoprim/sulfamethoxazole, and aminoglycosides. He responded well to intravenous ceftriaxone (a third-generation cephalosporin), local wound care, and later to applied skin graft to the injured calf. He made a full recovery.

The infection was most likely caused by wound contamination from the freshwater (river), and occurred because of a combination of factors: premature surgical closure of wound, and inability of the patient to take prescribed oral antibiotics to which the organism was sensitive.

Edwardsiella

There are three species of *Edwardsiella*, and the one that causes human disease is the opportunistic pathogen *E. tarda*. *E. tarda* causes a Salmonellosis-type enteritis in humans and typically derives from freshwater and freshwater animals (e.g., pet turtles). Infections from marine sources are unknown. *E. ictaluria* is a serious fish pathogen, often associated with septicemia in catfish (especially in aquaculture of catfish), but it is not known to cause disease in humans or marine fishes.

Yersinia

Several *Yersinia* spp. cause diseases in humans (Bubonic plague or Black Death, pseudotuberculosis, enteritis, extraintestinal complications) and in fish, including marine fish (salmonids). However, none of the marine fish pathogens are known to cause human disease and will therefore not be discussed here.

Brucella

Long known to cause disease in terrestrial animals and humans, these zoonotic bacteria are now known to exist in the ocean. The classic *Brucella* spp., *B. abortus, B. melitensis* and *B. suis*, cause brucellosis or undulant fever in humans and domestic animals. In domestic animals, the disease outcome is often abortion because the bacteria prefer to metabolize mesoerythritol which is found in the uterus and fetus of animals but not in humans. In humans, symptoms are general and include fever, chills, malaise, with heavy sweating, and high fever at night.

The three classic *Brucella* species have not been reported in marine mammals. Instead, it appears that marine mammals carry their own *Brucella* spp. [71], and they have caused three naturally acquired cases of human disease. Two cases were reported in Peru in 2003 (Sohn et al. 2003) and one in New Zealand [95]. The three *Brucella* human isolates were characterized with several molecular methods, and all three were found to share a common genotype with previously reported marine mammal *Brucella* spp. [152]. Representing 173 animals and one human patient were analyzed using a molecular method called multiple loci VNTR (variable number of tandem repeats), and the authors targeted 16 genetic loci (MLVA-16) that had been shown to be highly descriptive for *Brucella* spp. [93]. The study included two new species isolated from marine mammals, *B. ceti* and *B. pinnipedialis*, and concluded that these two species cluster into three distinct clades. Interestingly, the three isolates described by Whatmore et al. [152] did not cluster within the three clades but were, however, closely linked to the three marine mammal groups [93].

Enterococcus

Members of the genus *Enterococcus* are largely commensal colonizing organisms of the gastrointestinal tract of humans and warm-blooded animals and are commonly recovered in their feces [49]. These organisms are gram-positive facultative anaerobes that do not form spores but are capable of survival and growth in a wide variety of environmental conditions. These include tolerance of temperatures ranging from 10°C to 45°C, pH from 4.5 to 9.0 and high sodium chloride concentrations [63]. Although *Enterococcus* species have been found in many different marine and freshwater environments [85, 92, 124, 143, 158] as well as being associated with processed and fresh fish and seafood [31, 70, 99, 116, 154], these organisms are not usual pathogens for fish or marine mammals. *Enterococci* are known to be introduced into these environments by sewage contamination from known point sources, such as sewage treatment plants, and are used as indicator organisms for the probable presence of disease-producing pathogens in marine waters [147]. It is unlikely that point sources are the sole contributor of these organisms to an aquatic environment. Domestic and wild animals, water runoff

from storms or agricultural sources, wind-driven sediment resuspension events, and humans utilizing the waters have all been shown to contribute to the presence of *Enterococcus* species in aquatic environments. ([1, 33, 42, 43, 107]; Rebarchik DM, Grimes DJ unpublished data).

The principal pathogenic *Enterococcus* in humans, *Enterococcus feacalis* and *E faecium*, are among the *Enterococcus* species isolated from aquatic environments; however, *Enterococci* found in marine settings have not been linked directly to the onset of human enterococcal infections. In general, these organisms are primarily associated with serious, often fatal, nosocomial infections, including postsurgical wound infections, endocarditis, urinary tract infections, and sepsis; and they are currently emerging common pathogens [49]. Enterococci lack significant virulence factors associated with disease but are intrinsically resistant to many antibiotics currently in use. These bacteria, especially *E. fecium*, are known to easily acquire antibiotic resistance genes from other microorganisms encountered in their environment. In addition to its importance as an indicator organism, the significance of *Enterococcus* in a marine-water setting is the increased likelihood that the organisms will be exposed to other microorganisms from which they might acquire antibiotic resistance genes, thus adding to the difficulty of treating an already challenging infection.

Streptococcus

Numerous species of fish are susceptible to infection by members of the genus *Streptococcus*. Although these infections are not common, when they do occur, it is often in an aquaculture setting and can be responsible for significant mortality and large economic costs. One species that is responsible for such infections, *Streptococcus iniae*, is a primary pathogen for fish that can also infect humans. This organism was first isolated from infected freshwater dolphins in 1996 [113] and has subsequently been associated with sporadic infections in multiple fish species [41, 77, 80, 115]. It was recognized as a human pathogen in the mid-1990s with several documented infections in North America [11, 150], and later in Japan [84]. *S. iniae* infections in humans present as fever and cellulitis, often with bacterimia, and can be treated with intravenous penicillin and gentamicin [84].

S. agalactiae (Lancefield group B) is a significant human pathogen especially in newborn infants where it can cause sepsis, pneumonia, and meningitis; and in pregnant women where it is associated with urinary tract infections. This organism has been linked to disease outbreaks and some massive kills in several fish species [121]. Investigations performed to type the bacteria isolated from infected fish and environmental samples indicated that sewage contamination was a likely source for the infections in fish [121, 125]. There is no evidence linking fish or a marine environment with human disease.

Listeria

The genus *Listeria* consists of six species including two that are recognized as pathogens. *L. monocytogenes* is an important human pathogen, and *L. ivanovii* is an animal pathogen that may very rarely infect humans but is not associated with marine related infections. *L. monocytogenes* are short, motile, gram-positive rods that may appear as coccobaccili. They are hardy organisms that grow as facultative anaerobes in a wide range of temperatures (from 1°C to 45°C), pH (pH 4.3 to pH 9), and they tolerate high salt concentrations. They are ubiquitous organisms that are commonly found in soil and water, on vegetation, and decaying matter and excreted in feces of humans and animals [89]. *L. monocytogenes* is the causative agent of listeriosis, a serious but rare infection caused by eating food contaminated with the bacteria. Multiple types of food have been associated with *Listeria* infections, classically soft cheeses made from unpasteurized milk, meat and processed meat products, and fish and shell fish [87]. Food may become contaminated before, during, or after preparation; and the usual measures for prevention of growth of contaminating organisms, low temperatures, extremes of pH, and high salt, are ineffective against *Listeria*. Included among the marine sources implicated in food contamination are crab meats and dips, lobster and shell fish, and many varieties of fish, especially those smoked or processed [6, 36, 81–83]. In a recent report from the CDC addressing the incidence of infection with pathogens that are commonly transmitted via a food borne route, of the greater than that 17,000 culture-proven infections, Listeria accounted for less than 1% of cases with an incidence of 0.34 per 100,000 [18].

Listeriosis occurs primarily in pregnant women, newborn infants, elderly patients, and patients who are immunocompromised, and in all but the newborns; the infections result in an initial mild influenza-like illness that may progress to sepsis and meningitis. In pregnant women, there is an increased risk of miscarriage, and in newborns the infection is associated with a high mortality, up to 40%, and long-term side effects [7, 120]. Adults and children acquire the disease after ingestion of contaminated foods, whereas newborns acquire infecting organisms either transplacentally or via an ascending route during labor and delivery. Onset of the disease varies with population and exposure routes, and it may be from days to weeks. The mortality rates for infections in these populations are high, up to 25%. *Listeria* are facultative intracellular pathogens, a characteristic that contributes to the severity of listeriosis. After being phagocytosed, the bacterium utilizes unique virulence factors to spread from cell to cell without an extracellular state, thus evading the humoral immune response. Included among these factors are internalins (inlA, inlB, inlC) that facilitate attachment to the cells. Once inside the cell, listerolysin O and phospholipase C enzymes act to release the bacteria to the cytosol where the bacteria utilizes a protein, ActA, to coordinate the assembly of actin into a "tail" that propels the bacterium across one cell and into the next. *Listeria* infections can be treated with common antibiotics, such as ampicillin, ciprofloxacin, linezolid and azithromicin.

Mycobacterium

The genus *Mycobacterium* consists of numerous aerobic, nonmotile, non-spore-forming, acid-fast rods that occur widely in nature. These bacteria range from soil-dwelling saprophytes to pathogens of humans and animals. Of the greater than 20 human pathogenic *Mycobacterium* species, few have been associated with infections from or in a marine environment [155]. *Mycobacterium marinum* (synonym *M balnei*) is the primary aquatic pathogen and is a free living organism found throughout the world in both fresh and marine water environments. It was first discovered as a pathogen for fish, causing skin nodules and ulcers, in the mid-1920s and has since been recognized as a natural pathogen for other *ectotherms*, such as frogs. Since the early 1950s [88] it has been recognized as a cause of human infections first described as causing superficial skin lesions, nodules referred to as "swimming pool granulomas," in children who swam in contaminated pools. Like most other human pathogenic Mycobacteria, *M marinum* grows slowly, having a typical incubation period of 2–4 weeks before cutaneous lesions appear, but occasionally may progress rapidly. Unlike other human pathogenic Mycobacteria, it optimally grows at lower temperatures, 30–33°C, and is usually inhibited at 37°C, helping to explain the typical location of the lesions on the extremities and the usual lack of systemic involvement.

Currently *M marinum* is the most common cause of atypical *Mycobacterium* infection in humans with a reported incidence of 0.27 cases per 100,000 adults [128] Infection with these organisms can occur at any age, but it usually occurs in adults who handle fresh- or saltwater fish or fish tanks, and is now rarely associated with swimming pools, as proper construction and chlorination has eliminated this source [40]. Human exposure now primarily comes through inoculation of existing skin abrasions while handling fish or their tanks, or directly through fish bites or puncture wounds from fins. Local trauma is an important factor in establishing *M marinum* infections and their sequelea. Infections obtained after inoculation of an existing abrasion or a direct puncture manifests as a localized nodule or granuloma at the site of bacterial entry that may develop into an ulcer or progress to involve nearby lymph nodes, sporotrichotic lymphangitis. In healthy individuals it rarely progresses to involve bones, joints, or other systemic sites. Immunocompromised states increase the risk for becoming infected and can be associated with more aggressive systemic disease [111, 151]. Diagnosis and treatment are often delayed because of a lack of suspicion for mycobacterial involvement versus more common bacterial pathogens and are contributed to by the long incubation period.

Treatment for *M marinum* is driven by the severity of the infection [122] and ranges from oral monotherapy with minocycline, clarithromycin, doxycycline, ciprofloxacin, and trimethoprim-sulfamethoxazole for superficial cutaneous infections with susceptible organisms to combination therapies for drug resistant strains. Severe infections, including those with a sporotrichoid distribution pattern, generally require combination therapy with rifampicin and ethambutol. Surgical debridement is not usually

recommended, however, other alternative topical therapies, such as cryotherapy, X-ray therapy, electrodesiccation, photodynamic therapy, and local hyperthermic therapy can be effective.

Allochthonous Pathogens

Escherichia

The first species of this genus, *Bacterium coli*, was first isolated in the late 1800s and it was proposed as an indicator of fecal contamination of water. The isolate was renamed *Escherichia coli* in 1919 and today remains in use as the fecal indicator recommended by the USEPA for freshwater [142]. Unfortunately, *E.coli* and the enterococci do not correlate with indigenous pathogens such as the vibrios [29] and enteric viruses (see s.no. 12, J. Woods, Waterborne Disease of the Ocean, Enteric Viruses, this volume). In addition to its worldwide use as a fecal indicator, *E. coli* is the most common cause of urinary tract disease in humans (ca. 90% of human UTIs): certain strains cause gastroenteritis of various degrees of severity (e.g., STEC or the Shiga toxin-producing *E. coli* O157 usually derived from meats and produce) and nonpathogenic strains such as *E. coli* K12 that laid the early foundation for much of what is known about metabolism and enzymology.

Despite extensive documentation of *E. coli* causing human disease from the consumption of contaminated raw and undercooked foods, oral–fecal transmission in public facilities such as nurseries and day care centers and contaminated drinking water, transmission from seafood and seawater is rare and does not occur in the USA with great frequency. The overall foodborne STEC O157 incidence in the USA for 2009 was 0.99 per 100,000 population, and the STEC non-O157 incidence was 0.57 [18] for many years. This disease agent will not be further discussed in this chapter, and readers interested in this important pathogen are encouraged to peruse other literature.

Shigella

Shigella spp. cause a significant incidence of foodborne disease worldwide, but they are not often acquired from the ocean. Some strains produce the powerful Shiga toxin which, like CTX, is a lysogenic conversion product. In the USA, shigellosis (bacillary dysentery) has a fairly high incidence (3.99 per 100,000) as a foodborne agent of disease [18].

Salmonella

The Salmonellae are important pathogens but are much like *E. coli* and *P. aeruginosa*, with regard to their importance as marine pathogens. There are two species of *Salmonella, S. enterica* and S. *bongori*, and these two species are comprised of many serovars and subspecies. Both *Salmonella enterica* serotype Typhi (formerly *S. typhi*) and the other gastroenteritis-causing *Salmonella* spp. are human pathogens that historically were frequently acquired from the ingestion of contaminated seafood (especially filter-feeding bivalves, such as raw or undercooked oysters) and seawater. With the advent of fecal indicator monitoring of seafood and seawater, refrigeration of seafood, sanitary surveys (especially surveys of molluscan shellfish beds), and sewage treatment in developed nations, the origin of these diseases from the ocean declined significantly. Today, diseases caused by Salmonellae are still frequent worldwide, most commonly caused by *S. enterica* subspp. *enterica*, and they are almost always foodborne in both developed and developing countries (see http://www.who.int/mediacentre/factsheets/fs139/en/; [97]). In the USA, CDC reported a Salmonella foodborne disease incidence of 15.9 per 100,000 population; this is the highest incidence of any foodborne disease, but there was no breakdown on food type, i.e., seafood incidence was not given [18].

Non-typhoid salmonellosis in humans is most commonly gastroenteritis although complications, such as septicemia, can occur; deaths are rare if the patients are kept hydrated and placed on appropriate antibiotic treatment. Virulence is largely determined by pathogenicity islands, and the non-typhoid Salmonellae contain at least 12 of these genetically mobile elements [65]. Human salmonellosis is usually acquired from food, although contaminated water can also serve as a vehicle for transmission. The Food and Agriculture Organization (FAO) of the United Nations recently published a report on the control of *Salmonella* in sustainable aquaculture, and it contains a very nice review of occurrence and survival in the aquatic environment [44].

The incidence of salmonellosis deriving from seafood in the USA is low but is probably far underreported. DePaola et al. [34] recently provided evidence that, even though the reported incidence is low, the potential for acquisition in the USA certainly exists. They conducted a 2-year study of market oysters collected twice each month from retail establishments (restaurants and raw bars, seafood markets, wholesale dealers) in nine states. In all, FDA collected 397 samples representing 258 establishments. Salmonella was detected in 8.6% of the market oysters, a rate only exceeded by *V. parahaemolyticus* and *V. vulnificus* [34].

In the FAO report [44], it was noted that many studies have shown *Salmonella* serotype Sneftenberg to be the major serotype in marine environments and raw seafood worldwide. The report further noted that *Salmonella* spp. have been isolated from many marine mammals.

Morganella

Human disease from *Morganella morganii* is common (postoperative and other nosocomial) but these infections rarely emanate from the ocean. However, *M. morganii is* often associated with the decomposition of seafood; and if such seafood is consumed, scombroid fish poisoning can result. Scombroid results from histamine build up (and possibly buildup of other vasoactive amines) in the seafood as a result of histidine decarboxylation during the seafood spoilage process. This disease is a true food poisoning or intoxication, as opposed to a food infection, e.g., Salmonellosis and *Vibrio* gastroenteritis. Scombroid is probably caused by several enteric bacteria, including *Proteus*, spp. *Klebsiella pneumoniae, Hafnia alvei, Enterobacter* spp., *Serratia* spp., and *Citrobacter freundii;* in addition, *V. alginolyticus, Aeromonas* spp., and *Photobacterium* spp. are also histamine formers; and all have been isolated from spoiled fish [139]. The incidence of scombroid in the USA is thought to be common (http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogens NaturalToxins/BadBugBook/ucm070823.htm), and worldwide incidence is also common. However, because scombroid is not a reportable disease, documented cases are very low, e.g., only 103 incidents involving 4 people were reported from 1968 to 1980 (see above FDA URL). The most common fish involved with this intoxication are tuna, bonito, mackerel, and *mahi mahi*, and once the amines are formed in the meat, neither cooking, canning, or freezing lowers toxicity. Onset of this intoxication is rapid (often 30 min) and symptoms include a tingling or burning sensation in the mouth, rash on the upper body, and a drop in blood pressure; nausea, vomiting, and diarrhea may also present, and hospitalization may be required.

Pseudomonas

The most common human pathogen in this genus is *Pseudomonas aeruginosa*, and, like the Vibrios, this species is metabolically very diverse. *P. aeruginosa* is a very common cause of death in third-degree burn patients, it is a common nosocomial agent of disease, is frequently resistant to most clinically useful antibiotics, it is a cause of urinary tract infections, and it is a common (and often lethal) complication of cystic fibrosis. There have been numerous reports of human *P. aeruginosa* infections occurring from various types of freshwater contact (e.g., swimming pools, whirlpools, hot tubs, atomizers), but this literature is far too extensive to be summarized here. Although *P. aeruginosa* is frequently isolated from the coastal ocean [58], the authors are not aware of any literature documenting that disease caused by *P. aeruginosa* came from the ocean. It is primarily a freshwater bacterium [114].

Campylobacter

Campylobacter spp. are frequently isolated from healthy cattle, chickens, and birds [48], and they are also associated with several foods (unpasteurized milk, poultry, shellfish, fruits, and vegetables), freshwater ponds, and streams contaminated with fecal material [69]; Campylobacters are not normally isolated from seawater. While a few reports of seawater isolations exist [3, 58], most marine isolates come from shellfish [69]. The association with shellfish is similar to that of *Salmonella*, in that shellfish acquire *Campylobacter* spp., and usually *C. jejuni*, from filter-feeding in water contaminated with fecal material [2]. The incidence of foodborne *Campylobacter* disease in the USA is 13.02 per 100,000, second only to Salmonella [18]. However, seafood-borne disease is rare [2, 69].

Staphylococcus

The genus *Staphylococcus* is made up of at least 40 species of gram-positive, facultative anaerobic organisms that are found throughout the world. Most of these organisms exist as commensal colonizing organisms of animals and humans, but may also be found in soil, on surfaces and in untreated water. They are hardy organisms that grow in the presence of bile salts and NaCl (up to 6.5%), and they can survive on many types of surfaces for extended periods of time making them a challenge to eliminate in public environments, such as gyms, prisons and hospitals. *Staphylococcal* species are differentiated from other important gram-positive organisms by the presence of the enzyme catalase, and they are differentiated amongst themselves by the presence of the enzyme coagulase which is present in the more clinically relevant organisms.

Among the organisms that make up this genus are a large variety of coagulase-negative staphylococci (CoNS) that are the primary commensal colonizers of humans. CoNS are pathogenic primarily for compromised populations only, such as preterm infants or persons with implanted prosthetic devices. *Staphylococcus aureus* is coagulase positive, and this species is the principle pathogen associated with human infection. In humans, *S. aureus* strains are opportunistic pathogens that may colonize, without infecting, up to 40% of the population [24, 79, 146], but may occasionally gain access to the host, evade the immune response, and causes disease [52, 53]. In addition, these organisms have acquired resistance to most of the antibiotics used against them making treatment of infections challenging.

Most infections caused by *S. aureus* are limited to the cutaneous tissues and are caused by a person's own colonizing organisms. However, these bacteria are also capable of causing serious, life threatening systemic disease. In fact, *S. aureus* including the methicillin resistant *S. aureus*, MRSA, are among the leading causes of *nosocomial* infections [10, 78]. Furthermore, MRSA have

emerged as significant causes of community as well as hospital-associated infections [38, 78].

The diseases caused by *S. aureus* in humans and animals are often produced through the action of specific toxins or virulence factors that different bacterial isolates can produce. Specific toxins are associated with particular syndromes, such as toxic shock syndrome toxin and scalded skin syndrome. Many *S. aureus* are also capable of producing and secreting toxins responsible for staphylococcal food poisoning, termed enterotoxins, that only need to be ingested to cause intoxication and do not require the continued presence of the bacteria for disease. Consumption of seafood contaminated with *S. aureus* producing enterotoxins leads to staphylococcal food poisoning. Contamination of the food products often comes during processing as is seen in Listeriosis; however, organisms in the water and associated with marine life as seen in Peter the Great Bay, Japan, and Nha Trang Bay, South China, seas may also contribute to human disease [5].

S. aureus including drug resistant MRSA and CoNS have been isolated at recreational beaches, from marine ccc and temperate environments [5, 19, 50, 57, 133]. Adults and toddlers in diapers have been shown to shed *S. aureus* and the indicator organism *Enterococcus* into recreational marine waters and sand [118]. Persons using these recreational beaches may transmit and receive these organisms from the environment [42, 51, 131]. A retrospective epidemiological/microbiological monitoring study performed in Hawaii in subtropical marine waters and beaches found that persons were four times more likely to have staphylococcal skin infections if they had a history of seawater contact [20]. Whether there is a correlation of the microbial load in these environments and increased infections is yet to be verified; however, a recent study performed at a South Florida recreational beach did show a correlation between the average number of bathers in the water and the presence of *S. aureus* [119].

S. aureus and MRSA have also been isolated from marine mammals, including bottlenose dolphins (*Tursiops truncates*), seals, and walruses (blowholes, gastric fluids, fecal and anal cultures), both in captivity and in the wild, and have been associated with both colonization and disease [45, 105, 129]. In marine mammals in captivity, it is likely that the source of *S. aureus* and MRSA are colonized human handlers. The source of these organisms for the non-captive animals is not clear; however, colonized wild mammals were primarily identified in locations associated with human recreational use in the estuarine waters of Charleston, SC and Indian River Lagoon, FL [100, 129]. It has been suggested that some of the marine mammals, such as bottlenose dolphins, might serve as sentinels for transfer of resistant organisms from humans and animals into this environment, or simply indicate that the antibiotics are reaching this environment. To date, there are no confirmed cases of human infection from colonized or infected marine mammals.

***Staphylococcus aureus* and MRSA at Recreational Beaches**

Since the early 1990s, investigators in Hawaii have isolated *S. aureus* from the waters used for recreation and suspected that exposure of bathers to the organisms in this environment might put them at risk for staphylococcal infections. Recent investigations have also isolated *S. aureus* and methicillin resistant, MRSA, from recreational marine beaches at multiple locations, including Hawaii, Puget Sound, California, and South Florida. The sources of the bacteria in these environments are likely multiple and not yet completely appreciated. *S. aureus* are not known to have a marine reservoir; however, humans and other mammals that may be present at the beach are known to be colonized with the bacteria and are potential sources. In fact, humans have been shown to readily shed their colonizing methicillin sensitive *S. aureus* (MSSA) and MRSA into marine waters and sand.

A recent study completed at a South Florida recreational beach collected MSSA and MRSA from ambient water, water nearby bathers and sand and evaluated the bacteria present in these environments for their potential to be associated with infection by determining the virulence factors they could produce. The study showed that 30–37% of water samples had *S. aureus*; however, it was unable to show a correlation between exposure to *S. aureus* and reported illness. The majority, greater than 97%, of *S. aureus* found at this location were MSSA that carried few virulence factors known to be associated with infection. However; the MRSA isolated from this location were similar to the MRSA found in the community that are known to have the potential to cause serious infections. The lack of association between exposure to *S. aureus* and illness in this study, at this location, and the lack of an adequate number of participants (sample size) to establish an association with an organism that is present in only present 37% of samples overall is explained in part by the low percentage of potentially virulent MRSA. The populations and concentrations of MSSA and MRSA at other more crowded, recreational beaches would likely be as different as the human populations utilizing these beaches. Bathers exposed to greater numbers of more virulent organisms could be at increased risk for infections. Further studies are required to establish the true risk to bathers exposed to MSSA and especially MRSA in these settings.

General Treatment Principles

Many of the marine or ocean-dwelling microorganisms important in human disease are gram-negative bacteria. The diseases caused by these microorganisms run the spectrum, from septicemia (e.g., *V. vulnificus*), to gastroenteritis, wound infections, ear infections, and eye infections. It is not surprising that treatment is also varied from syndrome to syndrome.

Cholera (*V. cholera*) is the classic example of a cause of noninflammatory severe gastroenteritis (rice water stools). In cholera, the diarrhea is toxin induced. The pathogenesis of diarrhea in other organisms may be inflammatory (e.g., *Salmonella, Campylobacter* or *Shigella*). The nature or mechanism of the diarrhea may affect the primary choice of, or decision regarding, treatment.

As a general rule, severe diarrheal diseases require fluid replacement, given either by mouth (oral rehydration) or by intravenous means as the primary mode of treatment, while inflammatory diarrheas require, in addition to fluids, also antimicrobial therapy. The mode of fluid delivery will depend on how sick the patient is, the availability of which treatment, and the capacity or resources available to deliver the fluid replacement to the patient.

Sometimes, antimicrobial treatment may be detrimental in severe diarrhea. A good example is the bloody diarrhea caused by *E. coli* 0157:H7, which may lead to hemolytic-uremic syndrome, especially in children exposed to both the toxin-producing *E. coli*, as well as antibiotic therapy [156]. In such cases, withdrawal of antimicrobial therapy may sometimes help prevent further complications of renal failure [108]. Treatment in this circumstance is entirely supportive.

For toxin-producing diarrheas, antibody binding in situ presents an attractive and elegant option, but this type of therapy has not yet been developed (for all practical purposes) for most diarrheal diseases seen in the clinical setting [101].

Septicemia (e.g., due to *V. vulnificus*) requires aggressive management in the intensive care unit (ICU) setting, surgical debridement where necessary, as well as antimicrobial therapy. Severe wound infections like necrotizing fasciitis require primary surgical debridement, antimicrobial treatment, and sometimes hyperbaric oxygen therapy.

Most patients with severe wound infections or septicemia do not typically die or have other adverse outcomes due to or as a consequence of antimicrobial resistance, but complications result because they present too late to the hospital, have devitalized tissues that were not promptly debrided, or for other host factor reasons.

In our experience, most *Vibrio* organisms seen in clinical practice on the Gulf Coast are sensitive to the third-generation cephalosporins (sometimes also to second generation cephalosporins), quinolones, tetracyclines, and aminoglycosides. The same goes for *Aeromonas hydrophila*, with similar susceptibilities as above, in addition to usual sensitivity to trimethorprim/sulfamethoxazole.

Acute diarrheal illnesses require antimicrobial therapy usually only for a short period (5 days is typically enough); while septicemia (e.g., typhoid fever and *Vibrio* sepsis) would require longer therapy (2 weeks or longer), depending on the complications.

Severe wound infections of the necrotizing fasciitis type often require multiple surgical debridements, in addition to antimicrobial therapy and local wound care. In addition, skin grafting or plastic surgery is often required to cover defective skin or tissue.

The key to successful treatment of all of these disease entities is timeliness in starting treatment. The earlier appropriate therapy is started, the better the clinical outcome. The later treatment is started, the more complications one can expect.

Adjustments in antimicrobial therapy can and should be made after in vitro antimicrobial susceptibility studies are available. However, prompt treatment must be started very early, empirically (best guess or educated guess), before the laboratory susceptibility reports are available.

The Vibrios have predictable antimicrobial susceptibilities, more so than *Salmonella, E. coli*, or *Shigella*. Antimicrobial resistance to enteric pathogens reflects the pattern of use of antibiotics in a given environment, as well as the ease of antimicrobial drug availability and abuse in the area or locale where the infection was acquired. Typically for *Aeromonas*, resistance to ampicillin-like agents and first-generation cephalosporins is common; these agents should therefore not be used to treat infections due to *Aeromonas*.

In general, a gram-negative bacillus found in coastal or ocean water (outside and far away from a sewage drainage site) is likely to be free-living and, therefore, is more likely to be sensitive to multiple antimicrobial agents. Organisms causing disease acquired through human-to-human or foodborne transmission (e.g., *Salmonella*), on the other hand, are more likely to have been previously exposed to antibiotics (e.g., in animals or food products). Infections acquired through such contacts may therefore be more resistant to antimicrobials than free-living ocean, river, or brackish water bacteria [4].

The sensitivity of human-to-human or animal-to-human transmitted gram-negative bacteria (*E. coli, Salmonella, Shigella, Pseudomonas*, etc.) will usually reflect the pattern of local prevailing antimicrobial use in that community. The local hospital's antibiogram should provide the initial guide in the choice of empiric therapy, with necessary adjustments made after in vitro susceptibility studies are available.

Antimicrobial therapy for Brucellosis is often prolonged (up to 6 weeks) in order to prevent relapse. Often, combination therapy that includes a tetracycline plus rifampin, or an aminoglycoside, is required and recommended for complete cure of this debilitating disease [127, 132].

Acute gastroenteritis caused by food poisoning (e.g., staphylococcal preformed heat-stable enterotoxin) is often rapid in onset (within 1–6 h of food ingestion) and is also self-limited. The symptoms of severe nausea and vomiting occur usually within 1–6 h, and are usually over in less than 24 h [13]. Antimicrobial therapy is usually not required.

Treatment Considerations for Gram-Positive Organisms

Enterococci lack significant virulence factors associated with disease but are intrinsically resistant to many antibiotics currently in use. These bacteria are known to easily acquire antibiotic resistance genes from other microorganisms encountered in their environment. Treatment is guided by the determined antibiotic sensitivities of the infecting organisms and may be prolonged.

Streptococcus iniae infections usually present after exposure to fish with fever and cellulitis, often with bacterimia, and can be treated with intravenous penicillin and gentamicin [84]. *S. agalactiae* (Lancefield group B) remains sensitive to penicillin.

Listeria infections can be treated with common antibiotics, such as ampicillin, ciprofloxacin, linezolid and azithromicin. The delivery method of antibiotic is determined by the severity of diease.

Treatment for *Mycobacterium marinum* is driven by the severity of the infection [122] and ranges from oral monotherapy with minocycline, clarithromycin, doxycycline, ciprofloxacin, and trimethoprim-sulfamethoxazole for superficial cutaneous infections with susceptible organisms to combination therapies for drug-resistant strains. Severe infections, including those with a sporotrichoid distribution pattern, generally require combination therapy with rifampicin and ethambutol. Surgical debridement is not usually recommended however other alternative topical therapies such as cryotherapy, X-ray therapy, electrodesiccation, photodynamic therapy, and local hyperthermic therapy can be effective.

The majority of infections by *Staphylococcus aureus* are cutaneous infections limited to skin and soft tissues. Minor skin infections are usually treated with topical antibiotics, such as a nonprescription triple-antibiotic mixture or mupirocin. In some cases, oral antibiotics may be given for more severe skin infections. If abscesses are present, surgical drainage may be required and for smaller abscesses may be curative. More serious and life-threatening systemic infections are treated with intravenous antibiotics. The choice of antibiotic depends on the susceptibility of the particular staphylococcal strains, as determined by culture results in the laboratory. MRSA from the community may be sensitive to several antibiotics effective against MSSA; however, hospital-associated MRSA are usually resistant multiple antibiotics and may be challenging to treat. Vancomycin remains the drug of choice for multidrug resistant MRSA.

Future Directions

Clearly, most of what is known about waterborne human pathogens is based on laboratory and clinical observations, and this is especially true for autochothonous bacteria. The diseases and metabolic capabilities of these pathogens can be described in great detail, and in many cases the genomes of these bacteria have been completely sequenced. However, until their role or niche in the ocean and in freshwater habitats is fully investigated, it will not be possible to fully understand their ability to cause disease in humans. Accordingly, scientists need to continue asking the question, "how does this microorganism live in the ocean and yet invade humans to cause disease?"

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