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INVITED FEATURE ARTICLE

The role of wetland microinvertebrates in spreading human diseases

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Abstract The increasing loads of anthropogenic pollutants, compounded with climate change events, are likely to induce environmental changes in many wetlands with impacts on the native microinvertebrates and pathogens causing increased occurrence of water-borne diseases, which affect millions of people each year. In wetlands bacterial pathogens are actively preyed on by many protozoa and filter-feeding organisms but this predation can be compensated by the nourishment and protection offered by certain microinvertebrates, acting as hosts, e.g., chitinous rotifers, copepods and cladocerans. The complex interactions of ecological, biological, and genetic components mediate disease-causing organisms to exploit microinvertebrate hosts to occupy diverse niches, obtain nutrition, and withstand physico-chemical stresses. The persistence of the human pathogens in wetlands is often enabled by their association with microinvertebrates and also depends on their quorum sensing mediated colonization, biofilm formation, switching into dormant stage, and horizontal transfer of adaptive genes. The symbiosis with microinvertebrates is facilitated by the pathogen's immune evasion and fitness factors, e.g., Type-IV pili, capsular-polysaccharides, nutrient transportation, virulence and binding proteins, proteases, chitinases, and secretion systems. Spatio-temporal variation in the population of copepods and aquatic eggs/larvae of mosquitoes and midge flies, which act as vectors, can influence the outbreaks of cholera, diarrhea, malaria, dengue, filariasis and drucunculiasis. Changes in climatic factors (temperature, salinity, cyclones, rainfall, etc.) and anthropogenic pollutions (sewage, fertilizer and insecticide) may modify the abundance and biodiversity of microinvertebrates, and thus possibly exacerbate the persistence and dispersal of water-borne pathogens. Thus there is a need to adopt ecohydrological and eco-friendly interventions for managing wetlands while conserving them.

Keywords Microinvertebrate · Pathogen · Chitin · Biofilm · Trophic regulation · Risk factors

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Introduction

The majority of the aquatic microbes are harmless, and some are even beneficial, contributing to nutrient turnover, e.g., degradation of complex organic matter, polyaromatic hydrocarbon, nitrogen fixation, etc. (Thompson et al. 2004). However, some strains of a pathogenic microbial species are infectious and responsible for millions of illnesses and tens of thousands deaths globally each year. An estimated 82 million of the disability-adjusted life year (DALY) per annum are attributable to waterborne diseases (Pruess et al. 2002). Various types of aquatic invertebrates may provide rich organic substrates to the human pathogens favoring their growth and survival. Although wetlands and their fringes are often highly utilized or impacted by humans, the interactions of pathogenic microbes and their natural hosts in wetland environments are not well explored.

Waterborne disease outbreaks can vary due to hygiene and sanitation practices, extent of the use of wetland surface water for household purposes, and the type and location of the wetlands, e.g., freshwater or estuarine wetlands, located near poultry or livestock farms, etc. It is known that microbial occurrences in aquatic environments are regulated by temperature, salinity, rainfall, turbidity, and other environmental factors (Lara et al. 2009; Neogi et al. 2011; Johnson et al. 2012; Batabyal et al. 2014). The predicted scenario of climate change indicates the occurrence of acritical rise in sea level, elevated temperatures, more frequent cyclone events, increased rainfall, etc., which may have substantial impact on water borne diseases due to the modifications of aquatic ecology and hydrodynamics, especially in the low-lying coastal areas (IPCC 2007). Wetlands exposed to anthropogenic inputs (e.g., sewage, industrial pollutants, insecticides and fertilizers) are likely to experience changes in the seasonality and diversity of native microinvertebrates, which may have impacts on pathogenic microbial populations persisting there and eventually human health (Reguera and Kolter 2005; Ravva and Korn 2007).

The interaction of human with wetland ecosystems may promote disease emergence in a number of ways including the consequences of the management of aquatic resources (e.g. wetlands drainage or creation, aquaculture, dam construction), effects of water pollution (chemical, microbiological, radioactive, thermal) and the impact of global changes affecting climate and hydrological cycles (e.g. habitat degradation, warming, increased rainfall, storms) (Lara et al. 2012). An in-depth knowledge of their impact on the environmental distribution and dynamics of aquatic microinvertebrates is important, largely because many species may act as reservoirs as well as antagonists of potential human pathogens. Indeed, there is considerable interest for public health and socio-economical reasons in understanding the factors controlling the abundance of waterborne pathogens (Stewart et al. 2008). Various kinds of wetland microinvertebrates are considered as important sources of infection in humans, especially among the children, indicating their significance on public health. According to the WHO (2004), diarrheal diseases including gastroenteritis, mostly by water-borne pathogens, are responsible for the deaths of 1.8 million people every year. On the other hand, $\sim$300–600 million cases of malaria, caused by certain protozoan (*Plasmodium*) species, with over one million deaths occur each year (WHO 2014c).

Use of untreated surface water for household or recreational purposes, and eating of raw and improperly cooked foods containing aquatic organisms, are the basic causes of the spread of waterborne diseases. Many of the causal pathogens develop symbiotic association with fish, shrimp, mussel, oyster, clam, etc., and a lot of efforts have been made to understand the interactions of pathogenic microbes with these economically important aquatic organisms along with the environmental changes that impact on waterborne diseases (Miller et al. 2005; Johnson et al. 2012; Tuševljak et al. 2012). However, the role of diverse microinvertebrate communities, which are often overlooked due to their small size, in facilitating the survival, growth and evolution of human pathogens deserves more attention. This paper focuses on describing how the environmental changes, both natural and human-induced, modify the wetland microinvertebrate communities and influence the occurrence, adaptation and evolution of aquatic pathogens. This understanding enables us to estimate the risks of waterborne diseases and suggest steps for intervention strategies. Thus we provide a synthesis of the known ecological relationship and regulation of pathogenic microbes and aquatic microinvertebrates, and the related biological, genetic and environmental fitness of the pathogens, while also describing some

processes regulating pathogenic microbial dynamics. We first focus on the reported pathogens and consequent diseases related to wetland microinvertebrate dynamics. Then we describe the underlying mechanisms of the host-pathogen association and influences of environmental and anthropogenic determinants. Finally, the probable ecological succession among competitive organisms, the hypothesized health risk factors, and suggested management strategies are also discussed.

In this article, microinvertebrates are defined as organisms having body length < 5 mm and mostly 5–500 μm, which exclude the economically important larger invertebrates such as shrimps, squids, oysters, etc. Aquatic microinvertebrates, e.g., protozoa, rotifers, nauplii (crustacean larvae), copepods, cladocerans, certain insect larvae, small nematodes, etc., serve as critical link between primary producers (algae) and higher trophic fauna like fish, amphibian and avian species. The establishment, composition and persistence of invertebrate-associated bacterial communities are influenced by the host responses including immunity as well as different environmental factors of the wetland (Chiavelli et al. 2001; Trosky et al. 2004; Lara et al. 2011; Seed et al. 2012). Temporal changes in aquatic microbial pathogens may be associated with the availability and conditions of wetland microinvertebrates serving as their hosts, antagonists or predators.

Human pathogens related to wetland microinvertebrates

The following section provides a brief of the important human pathogens known to be associated with aquatic microinvertebrates and related epidemiological features.

Bacterial pathogens

The major bacterial diseases which can be influenced by wetland microinvertebrates include cholera, gastroenteritis including diarrhea, septicemia, necrotizing fasciitis, wound infections, typhoid, *Escherichia coli* infection, campylobacteriosis, legionellosis, and pneumonia.

Vibrio

Vibrios are gram-negative highly motile curved bacilli that occur naturally in marine, estuarine, and freshwater ecosystems. These halophilic bacteria can be found in association with a variety of aquatic organisms including fish, shrimps, squids, phyto- and zooplankton, mollusks, coral, etc. (Thompson et al. 2004). Epidemiologically, the most important species is *Vibrio cholerae* which caused seven pandemics of cholera since 1823. During 2010 a total of 317,534 cholera cases including 7,543 deaths were reported from 48 countries, mostly in Africa followed by Asia (WHO 2014a). Among other important vibrios, *V. parahaemolyticus* is one of the leading seafood-borne pathogens, causing tens of thousands of gastroenteritis cases each year throughout the world including developed countries like USA and Japan (Nair et al. 2007). *Vibrio vulnificus* is another opportunistic pathogen causing gastroenteritis, ear infections, cellulitis, wound infections and necrotizing fasciitis, spreading into the blood-stream, with high (>50 %) fatality rate among immunocompromised patients (Jones and Oliver 2009). Apart from these important pathogenic species, *V. mimicus, V. fluvialis, V. alginolyticus* and five other *Vibrio* species are also reported to cause occasional gastroenteritis, wound infection and primary septicemia (Chakraborty et al. 1997).

Aeromonas

Aeromonas spp., capable to withstand a broad range of salinity, pH and temperature, are considered as an emergent water-borne human pathogen, with estimated cases contributing up to 13 % of reported gastroenteritis in the United States (Kühn et al. 1997; Senderovich et al. 2008). Among 21 known species, the most important and commonly isolated human pathogens are *A. hydrophila*, *A. caviae* and *A. veronii* biovar sobria, and infrequently *A. salmonicida*, *A. schubertii*, *A. trota*, and *A. media* (Janda and Abbott 2010).

Salmonella

Salmonellae are widely distributed in nature, particularly among wild and domesticated animals. The major serovars of pathogenic *Salmonella enterica* are Typhi, Paratyphi, Choleraesuis, Typhimurium, and Enteritidis, of which the first two cause typhoid fever, choleraesuis is associated with systemic infections,

whereas the last two cause gastroenteritis. Typhoid fever causing salmonellae, which have no significant animal reservoirs, infect approx. 450 per 100,000 children (<5 years) in South Asian countries, whereas an estimated 93.8 million salmonella-gastroenteritis cases occur worldwide, with 155,000 deaths, each year (Sánchez-Vargas et al. 2011).

Pathogenic Escherichia coli

Although *E. coli* are mostly commensal flora of human and animal intestines, some strains belonging to diverse serotypes (>150) display a variety of pathogenicity, e.g., enterotoxigenic (ETEC), Shiga-like toxin-producing (STEC) or enterohaemorrhagic (EHEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enteroaggregative (EAggEC), and extraintestinal-/uro-pathogenic (ExPEC/UPEC). *Escherichia coli* sometimes cause severe illness leading to hemolytic uremic syndrome (HUS), especially among the infants and elderly persons (Nataro and Kaper 1998). *Escherichia coli* O157:H7 (STEC) are reported to cause large-scale morbidity and many fatal cases worldwide, with an increasing trend in some regions, e.g., Europe (Muniesa et al. 2006). In the United States approx. 265,000 STEC infections per annum has been estimated (Scallan et al. 2011). Recently, some evidences point out the aquatic persistence of *E. coli* strains with high biofilm forming capacity (Ishii and Sadowsky 2008). However, the risk of pathogenic *E. coli* from natural surface water and relevant food-chain has been grossly underestimated.

Campylobacters

Campylobacteriosis, caused mainly by *Campylobacter jejuni* and *C. lari*, is an important enteric infection worldwide. *Campylobacter jejuni* and *Campylobacter coli* are also associated with septicemia, reactive arthritis and Guillain-Barré syndrome. However, other campylobacters, e.g., *C. fetus, Arcobacter cryaerophilus*, *A. butzleri* etc. have been isolated from humans with gastroenteritis and septicemia (Yamasaki et al. 2010). Ecological surveillances suggest that campylobacters, which predominantly occur in poultry and livestock animals, can persist for certain time in the aquatic environment (Maugeri et al. 2004).

Listeria monocytogens

This facultative intracellular gram-positive bacterium, which is found widely distributed in nature including aquatic organisms, can cause listeriosis, mostly in immunocompromised individuals, manifested by septicemia, meningitis, gastroenteritis, pneumonia and spontaneous abortion in pregnant women, with high fatality. Over the past two decades tens of thousands of food-borne listeriosis incidences have been reported all over the world including USA, Japan, and China (Warriner and Namvar, 2009; Yan et al. 2010).

Legionella

Legionellosis is a pneumonia-like disease predominantly caused by inhalation of aerosolized water containing the aquatic bacterium *L. pneumophilla*. Although majority of the infections may remain non-symptomatic a high rate of hospitalization with Legionellosis are known, e.g., up to 20,000 patients each year in the United States (CDC 2014a).

Clostridium

Clostridium perfringens is a spore-forming Gram-positive anaerobic bacterium occurring in many environmental sources. Some of its strains cause diarrhea and abdominal cramps. In the United States, it *is* one of the most common causes of foodborne illness affecting nearly a million people with thousands of deaths each year (Scallan et al. 2011).

Microbes associated with nosocomial infections

Although Klebsiella spp. are commensal flora of human intestine these bacteria often cause infections, especially pneumonia, among the hospitalized patients with other diseases. Unfortunately, in the recent years, majority of the nosocomial *K. pneumonia* have grown multidrug resistance *and frequently cause fatal infections among the immunocompromised patients* (CDC 2014b). *Acinetobacter* spp., which are ubiquitous in soil and water and mostly harmless, accounts for about 80 % of nosocomial infections including pneumonia and a variety of systemic infections (Peleg et al. 2008). Another group of usually harmless bacteria widely occurring in the aquatic environment and agricultural products is *Pseudomonas* spp. However, *P.*

aeruginosa is considered as an important pathogen in hospital environments, causing respiratory and systemic infections with enormous morbidity and mortality (Strateva and Yordanov 2009).

Nematodes

Dracunculiasis, caused by *Dracunculus medinensis* (Guinea worm), is initiated by the ingestion of microinvertebrate vector called copepods (water fleas) harboring the larvae of this parasite. Although the disease was once prevalent among a huge population (3.5 million per year during eighties, particularly in many African countries), at present only few hundreds of cases are reported in Chad, Ethiopia, Mali, and South Sudan. *Another type of pathogenic nematodes requiring copepods as the primary intermediate hosts is Gnathostoma* spp. which can cause gnathostomiasis, with painful swellings under the skin, throughout the world, especially in tropical and subtropical Southeast Asia including Japan (CDC 2014c).

Roundworms belonging to *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* cause lymphatic filariasis, which is mostly non-symptomatic, affecting ~120 million people in 73 countries throughout the tropics and sub-tropics. The disease is spread by the biting mosquitoes which acquire parasitic larvae (microfilariae) from the blood of infected person. The common mosquito vectors are *Anopheles* spp. in Africa, *Culex quinquefasciatus* in the Americas, and *Aedes* and *Mansonia* spp. in the Pacific islands and in Asia (WHO 2014b).

Trematodes

Fasciola hepatica and *F. buski* infections, causing damage to liver and intestine, respectively, are known as fascioliasis, which affect at least 2 million people in >50 countries. On the other hand, Schistosomiasis (Bilharzia disease), an important tropical disease symptomized by rash or itchy skin, is caused by *Schistosoma haematobium, S. japonicum,* and *S. mansoni.* The eggs of *Schistosoma*, shedded through patient's stool, hatch in the freshwater environment to release larvae to infect snails (intermediate host), where the infectious metamorphic form develop. Schistosomiasis is initiated by the larval penetration of the skin whereas fascioliasis is transmitted via contaminated drinking water and aquatic plants (CDC 2014c).

Protozoan pathogens

Plasmodium falciparum, P. vivax, P. ovale, and P. malariae can utilize human blood cells to cause malaria among a huge population, e.g., 207 million episodes including 627,000 deaths during 2012, mostly (>90 %) in tropical countries. *Plasmodium* spp. are transmitted through the bites of *Anopheles* mosquitoes, which breed and spend larval stages in aquatic environments, especially wetlands. In the endemic countries, *P. falciparum* malaria is more prevalent among the pregnant women contributing to 8–14 % of low birth weight which may severely affect child health (WHO 2014c).

Giardia intestinalis synonym *G. lamblia cause a kind of diarrhea called Giardiasis*. The flagellated trophozoite stage, formed inside the intestine, is encysted within a shell while reaching the colon. The cyst, can survive long term in aquatic environment and tolerate chlorination treatment. Giardiasis is a global problem affecting 2–8 % and up to 33 % populations in developed and developing countries, respectively (CDC 2014c).

Cryptosporidium parvum causes an acute short-term diarrhea called cryptosporidiosis that can be severe for the children and immunocompromised individuals. *Cryptosporidium parvum* is associated with majority (~60 %) of the diarrheal outbreaks caused by water-borne protozoan parasites in many regions including Mexico, Brazil, Australia, Africa, Asia and Western Europe (Baldursson and Karanis 2011).

Humans, animals and many invertebrates can be intracellularly infected with *Microsporidia* parasites that produce spores, which can tolerate extreme environment for a long time. A diverse clinical manifestation, with diarrhea as most common followed by infections in eye, respiratory and urinary tracts, are known. Microsporidiosis is recognized as an opportunistic emerging disease, especially among immunocompromised patients (CDC 2014c).

Among the amoeboid protozoans several species of *Acanthamoeba*, including *A. culbertsoni*, *A. polyphaga*, *A. castellanii*, *A. lenticulata,* etc., are capable of causing infections of the eye, skin, and rarely nervous

system. *Acanthamoeba* can be transmitted through contact lens and skin wounds or by being inhaled into the lungs (CDC 2014c). *Entamoeba histolytica* and *Blastocystis hominis* are diarrhea causing parasites associated with 8–10 % of worldwide outbreaks caused by water-borne protozoans (Baldursson and Karanis 2011).

Pathogenic viruses

Dengue and Japanese encephalitis (JE) are the major viral diseases which spread through some mosquitoes. Dengue fever, caused by a RNA *Flavivirus* (DENV-1, DENV-2, DENV-3 and DENV-4 serotypes), is characterized by measle-type skin rashes, sometimes turns into life-threatening dengue shock syndrome and hemorrhagic fever with low blood pressure and platelet counts, respectively. The global incidence of dengue, which occurs mostly in urban and semi-urban areas of the tropics and sub-tropics, has grown dramatically in recent decades infecting up to 100 million every year (WHO, 2009). JE *Flavivirus* are prevalent in the irrigated areas throughout Asia and Western Pacific infecting 5–50 per 100,000 children per year. JE has 20–30 % case-fatality ratio while among the survivors 30–50 % develop serious neurologic or psychiatric diseases. In Australia, *Aedes* mosquitoes are known to spread the Ross River virus (*Alphavirus*) causing debilitating arthritis (Lindsay et al. 2007).

Fitness factors of microbial pathogens for aquatic persistence

Fitness factors for the survival of a microbial pathogen in the aquatic environment include factors associated with nutrient acquisition, competition, survival in unfavorable biotic and abiotic conditions, ability to colonize and grow in hosts, avoid immunological inhibition, virulence and transmission to a new host. During competitive existence struggle in the aquatic environment a microbial pathogen, capable of growing under a wide range of temperatures, salinities and other ecological conditions, may have better fitness. Pathogenic bacteria have evolved various adaptive mechanisms by switching related genes expression.

Motility

Nutrient sources in the environment are not uniformly distributed but occur as microscale patches, influenced by localized events such as cell lysis and waste excretion (Blackburn et al. 1998). Planktonic bacteria use motility to take advantage of such nutrient patches (Stocker and Seymour 2012). Bacterial pathogens possessing flagella, e.g., *V. cholerae*, are highly motile to competitively explore suitable environmental niche. Most vibrios possess a couple of flagella, one polar and the other lateral, for *swimming* in the aquatic environment and swarming on solid, respectively (McCarter 2004). Motility of pathogenic bacteria is influenced by the environmental conditions, e.g., in *V. cholerae,* changes in temperature, pH, osmolarity, etc. can regulate the expression of ToxR protein, which modulates the production of cholera toxin as well as other virulence regulatory genes including motility and colonizing factors (Skorupski and Taylor 1997).

Colonization and invasion

Lectins, flagella, pili, capsular polysaccharides, toxin corregulated pilus (TCP), and secretion systems like Type 3 secretion system (T3SS) are vital for bacterial chemotaxis, attachment and invasion in host organisms (Grimes et al. 2009). The occurrence of multiple colonizing factors may be an evolutionary genetic fitness for pathogenic bacteria to adapt with ecological changes and successful colonization on wetland microinvertebrates. Several amino acids (serine, arginine, and asparagine) serve as attractants to induce chemotaxis of pathogenic bacteria while colonizing on microinvertebrates (Nishiyama et al. 2012). *Vibrio cholerae* up-regulates chemotaxis genes in response to chitin oligosaccharides, facilitating attachment to chitinous organisms (Meibom et al. 2005). An effector protein of T3SS2 aids in improved colonization of *V. parahaemolyticus* (Liverman et al. 2007). Type IV pili also induce colonization in some bacterial pathogens including *V. vulnificus* (Paranjpye et al. 2007). *Salmonella* pathogenecity islands encode genes for cytotoxins, fimbriae and resistance to bacteriocins to aid bacterial invasion of host animals. Virulence factors, most notably, actin binding proteins (SptP and SopE, expelled by T3SS), surface polysaccharides and flagella are essential for the invasion and intracellular life cycle of *S. enterica* (Jennings et al. 2012). The

expression of *mgtC* gene of a T3SS is shown to be required for *Salmonella* growth in the Mg^{2+} poor phagosomes (Thompson et al. 2011). *Vibrio vulnificus* global virulence regulator CRP (cAMP receptor protein) and exotoxins *vvpE*, *vvh*, and *rtxA* are important for colonization in nematode *Caenorhabditis elegans* (Dhakal et al. 2006). Vibrios secrete major proteases through type II and type VI secretion systems, which may cause cytotoxic effects in microinvertebrates like flagellates, amoebae, and ciliates (Matz et al. 2011). Moreover, secretion of outer membrane vesicles (OMVs) containing virulence factors and high amount of hydrolases (proteases, phospholipases, etc.) may contribute to vibrios' invasion and survival within aquatic microinvertebrates (Valeru et al. 2014). Increases in oxidative stress, as found in host internal organs, can enhance phase variation (smooth to rugose phenotype) in exopolysaccharide of vibrios to withstand changing environmental conditions and phagocytosis (Hilton et al. 2006).

Transformation into viable but non-culturable (VBNC) stage

In response to the frequently occurring unfavorable environmental conditions (low temperature, pH, salinity, nutrient, etc.) the persistence of many bacterial pathogens, including *Vibrio, Aeromonas, Legionella, Salmonella, Campylobacter* and *E. coli* O157:H7 (STEC), is facilitated by entering a dormant state in which it remains viable but becomes non-cultivable (VBNC) (Oliver 2005). In this state, bacterial housekeeping genes like 16S rRNA, *relA*, *rpoS*, etc. are expressed in reduced manner, while some other genes, especially those involved in regulatory functions and cellular processes, are induced (Asakura et al. 2007). During VBNC stage bacterial cells become reduced in size and often clump together like biofilm, which may revert to cultivable form after passage through host intestine, co-culture with eukaryotic cells and also by signaling through autoinducer molecules in response to environmental stimulli (Alam et al. 2007; Senoh et al. 2012; Bari et al. 2013). VBNC cells of *E. coli* O157:H7 continues to express toxin gene (*stx*1), supporting the hypothesis of their role in causing infection (Yarron and Matthews, 2002). Despite thriving with a reduced metabolism VBNC cells can withstand environmental changes better than the cultivable part (Sun et al. 2008).

Biofilm formation

Biofilms are basically microbial aggregates on organic and inorganic substrates where cells adhere to by extracellular polymeric substances. Aquatic *E. coli* isolates have been shown as superior biofilm formers than human isolates, suggesting that selective environmental factors may induce higher biofilm formation, which is controlled by conjugative pili, Type-1 fimbriae, and curli of the bacterium (Van Houdt and Michiels 2005; Moreira et al. 2012). During biofilm development pathogenic bacteria like vibrios first approach a surface, then attach and spread on it with the help of Type IV pili and flagellar motility, and finally produce biofilm by hypersecretion of exopolysaccharide (Watnick and Kolter 1999). Development of heterogeneous biofilms has been observed for many pathogens including *E. coli* (Camper et al. 1996), *L. pneumophila* (Murga et al. 2001) and *A. hydrophila* (Kirov et al. 2004). Changing of community composition in biofilm is a natural process due to interspecies competition, e.g., pathogenic *E. coli* may be outcompeted by *P. aeruginosa* which can utilize a wider range of organic nutrients (Brümmer et al. 2000). While residing in complex communities of biofilms, pathogenic bacteria develop resistance to many stressors, e.g., extreme temperature, low pH, dessication, etc., and, may survive for a long term, mostly in VBNC form (Watnick and Kolter 1999; Kamruzzaman et al. 2010). Biofilms grown on chitinous exoskeleton of aquatic microinvertebrates may act as adaptive inter-epidemic reservoirs of pathogenic vibrios (Alam et al. 2007). *Vibrio cholerae* cells within such aquatic biofilms accumulate (via Type VI secretion system) organic compounds inhibitory to protozoan grazing, while the bacterial elevated production of exopolysaccharide and subsequent conversion to rugose phenotype resist bacteriophage attack (Matz et al. 2005). The persister cells, which are phenotypic variants and metabolically quiescent cells among the biofilm population, may contribute to higher tolerance to multiple antimicrobial agents (Keren et al. 2004). Moreover, biofilm formation may facilitate the genetic transfer events of pathogenic bacteria, which may aid in their adaptation (Meibom et al. 2005).

Quorum sensing

Quorum sensing (QS) is an intercellular communication process in which accumulation of extracellular N-Acyl-Homoserine-Lactones (AHL) molecules, called autoinducers (AIs), triggers intracellular signal transduction cascade aiding in bacterial association and persistence with wetland microinvertebrates. For example, QS-mediated secretion of haemagglutinin protease (HAP) enables *V. cholerae* to degrade the gelatinous matrix of chironomid eggs to acquire nutrients. Interestingly, QS of *V. cholerae* can be influenced not only by its own AIs but also other bacterial species present on chironomid (Senderovich and Halpern 2013). The population-dependent variations in AIs can regulate bacterial expression of many virulence and adaptive mechanisms including biofilm formation on chitinous microinvertebrates and production of anti-protozoal factors, e.g., ammonium (Matz et al. 2005). As transformation of culturable to VBNC state often involves biofilm formation, it has been shown that QS play a pivotal role in VBNC conversion (Kamruzzaman et al. 2010). In a remarkable study, VBNC cells of freshwater ecosystem have been resuscitated by introducing natural or chemically synthesized AIs (Bari et al. 2013).

Horizontal gene transfer

In the wetland environment, toxigenic microbial populations can utilize the opportunity for horizontal gene transfer (HGT) of pathogenic genes among other non-pathogenic species, rendering them the potential to cause human diseases. HGT is a random event occurring among both intra- and inter-species, as exemplified by the plasmid transfer between *E. coli* and *Vibrio* strains (Paul et al. 1992). *Vibrio cholerae* fitness factors, e.g., genes for virulence, colonization, etc. can be horizontally transferred not only to closely related *V. mimicus* but also other distantly related species like *V. alginolyticus* (Boyd et al. 2000; Ren et al. 2013). Recombination events including the acquisition of plasmid containing virulence gene (*virA*) are beneficial for *Salmonella* spp. to invade the host immune system (Gulig et al. 1993). Genes responsible for multidrug resistance (MDR) in vibrios, *Salmonella*, *Klebsiella* and *E. coli* can be also carried by conjugative plasmids (Waldor et al. 1996). In parallel with conjugation and viral-transduction, the chitin induced DNA uptake event may facilitate HGT events in aquatic pathogens, e.g., transfer of cholera toxin gene among *V. cholerae* strains (Udden et al. 2008). HGT of T3SS genes has been also reported among bacterial pathogens which may promote their symbiotic interactions with aquatic microinvertebrates (Morita et al. 2013). Multiple environmental cues, including cell density, nutrient limitation, growth rate, physicochemical stresses, etc. can promote natural HGT by inducing a complex regulatory network linking QS, Type IV pilus and DNA binding protein of pathogen, and chitin of microinvertebrates to acquire exogenous genes useful in adaptation, virulence and MDR (Meibom et al. 2005; Blokesch 2012). Many pathogenic bacteria of freshwater and estuarine ecosystems have acquired integrating conjugative elements (ICEs) which carry genes for adaptive fitness including MDR. The self-conjugative transfers of ICEs, playing important role in bacterial evolution, can be triggered by DNA-damaging agents such as UV-light (Garriss et al. 2009). Comparative genomic analysis of toxigenic microbes indicate HGT as the major driving force in achieving adaptive fitness for pathogen-microinvertebrate interaction and disease transmission (Chun et al. 2009).

Immune evasion

Many bacterial pathogens have also evolved and acquired tools to avoid inhibition by host immune systems. Host immunological responses against a pathogenic microbe can include antibodies, macrophages, phagocytosis, lysozyme, hemocytes, and antimicrobial peptides (Bachère et al. 2004). The T3SS of many pathogenic bacteria including *S. enterica*, *E. coli* and vibrios, are known to inject immune-suppressing effector proteins into host cells which may lead to decreased production of host cytokine (Trosky et al. 2004). In *S. enterica* a two-component regulon (PhoP/Q) of T3SS facilitates bacteria to avoid intramacrophage phagolysosomal fusion (Thompson et al. 2011). Polysaccharides such as lipopolysaccharide (LPS) and capsular polysaccharide (CPS) can play a role in immune evasion by altering the cell surface epitopes, e.g., in pathogenic *V. cholerae*, the *manA* and *wbeL* genes mediated cell surface variations aid in intestinal colonizition (Seed et al. 2012). Flagellin phase variation may aid *Salmonella* in escaping the host defense system

(Stewart et al. 2011). Some virulence genes, e.g., *V. cholerae toxR* and *tcp*, may also inhibit host immune response like cytokines and antibodies (Parsot et al. 1991).

Nutrient acquisition

Proteases are important for bacterial nutrient acquisition because of their ability to degrade proteins into usable forms. Serine proteases and metalloproteases (e.g., PrtV, collagenase, vibriolysin) have been described as essential for vibrios survival in animal hosts due to their protease/gelatinase/glycosidase activity to meet metabolic demand during growth and virulence, especially, in the intestinal mucus (Shinoda and Miyoshi 2011). Nutrient acquisition by mucinase activities of some bacterial pathogens promote their association and survival with microinvertebrates, as reflected in bacterial degradation of mucilaginous matrix, mostly in the outer body covering, e.g., oral region and egg sac of microinvertebrates (Halpern et al. 2004). Iron is one of the most critically needed nutrients for bacteria. Vibrios secrete hemolysins (e.g., HlyA of *V. cholerae*) to lyse host cells and thus access nutrients including iron while persisting within the host nematode *Caenorhabditis elegans* (Cinar et al. 2010). Many pathogens, including *E. coli* and vibrios, have evolved siderophore-mediated iron uptake systems to enable persistence in low iron environments while some of these bacteria may possess receptor systems to utilize siderophores not only secreted by themselves but also other microorganisms (Wyckoff and Payne 2011).

Attachment to chitin, the most abundant biopolymer

Chitinase-producing bacterial pathogens, e.g., vibrios, aeromonads, etc., play a vital ecological role by degrading a huge amount of chitin ($\sim 10^{11}$ tons per year, produced from crustacean organisms) and thereby releasing organic nutrients utilizable to other aquatic communities (Chiavelli et al. 2001). Chitinase-mediating adherence of *V. cholerae* to crustacean microinvertebrates (e.g., cladoceran *Daphnia magna*, copepod *Acartia tonsa*, crustacean nauplii, etc.) is promoted by mannose sensitive haemaglutinin (MSHA) and a chitin (GlcNAc)-binding protein A (GbpA) (Chiavelli et al. 2001; Meibom et al. 2005). Notably, upon growth on chitinous surfaces, pathogenic bacteria enter a state of natural competence for the acquisition of exotic DNA, including virulence genes (Udden et al. 2008; Blokesch 2012). Presence of chitin causes a series of inductions in *V. cholerae,* firstly of a membrane-bound chitin-sensing histidine chinase (ChiS), followed by the extracellular chitinases (ChiA-1 and ChiA-2), a chitoporin, a type IV pilus (ChiRP) and later aggregation of a periplasmic competence protein (ComEA), which are regulated by QS (Blokesch 2012; Seitz et al. 2014). Bacterial attachment to chitin can be influenced by physico-chemical changes in water, e.g., nutrient levels, temperature, salinity and pH (Chiavelli et al. 2001). The symbiotic association with chitinous microinvertebrates facilitates the pathogenic bacteria in nutrient acquisition, resists grazing by protozoan predators and stress tolerance, including protection from stomach gastric acid, and thus aids in its survival (Pruzzo et al. 2008). Development of biofilm on chitinous microinvertebrates can boost the number of pathogenic bacteria and thus contributes an important role in disease outbreaks, e.g., an individual copepod is shown to harbor the requisite infectious dose ($\sim 10^4$ - cells) of *V. cholerae* for clinical cholera (Lipp et al. 2002).

Association with microinvertebrate hosts

Bacterial association with microinvertebrate host is often primed by their metabolic need in wetland environment, for example, attachment to and degradation of chitinous exoskeletons of copepods, cladocerans, rotifers and crustacean larvae encourages bacterial growth and colonization (Tamplin et al. 1990). In addition, aquatic microinvertebrates are hotspots for the growth of many pathogens, as food-borne organic matter is concentrated in their guts and excreted by faecal pellets. Obviously, a large variety of aquatic microinvertebrates provide survival fitness for the associated pathogens such as *V. cholerae* to withstand unfavourable physico-chemical conditions (Colwell et al. 2003; Pruzzo et al. 2008; Neogi et al. 2012).

Filter feeding oral apparatus trapping nutritious food for microinvertebrates like crustacean zooplankton are preferable attachment sites for bacterial pathogens to meet their metabolic demand (Tamplin

Table 1 List of important aquatic microinvertebrates serving as hosts/vectors for different human pathogens

Human diseases	Causative agents	Microinvertebrate hosts/vectors
Cholera	*Vibrio cholerae*	Copepods (*Acartia*, *Eurytemora*), Rotifers (*Brachionus*), Cladocerans (*Daphnia*, *Bosmina*, *Ceriodaphnia*, *Diaphanosoma*), Protozoa (*Acanthamoeba*), Nauplii, Chironomids (eggs and larvae)
Gastroenteritis/diarrhea	*V. parahaemolyticus*, *Aeromonas* spp., *Clostridium defficile*	Copepods (*Acartia*), Nauplii, Chironomids (eggs and larvae)
Septicemia	*V. vulnificus*, *Pseudomonas aeroginosa*	Rhabditid nematode (*Caenorhabditis*) Chironomids (eggs and larvae)
Wound infections/Necrotizing fasciitis	*Aeromonas* spp., *V. vulnificus*, *V. parahaemolyticus*	Copepods (*Acartia*) Chironomids (eggs and larvae)
E. coli infection	*Escherichia coli*	Rhabditid nematode (*Caenorhabditis*)
Campylobacteriosis	*Campylobacter jejuni*, *C. coli*, *C. fetus*	Protozoa (amoeboid *Acanthamoeba*, cilliate *Tetrahymena*), Copepods
Legionellosis	*Legionella pneumophila*	Protozoa (amoeboid *Acanthamoeba*, cilliate *Tetrahymena*)
Listeriosis	*Listeria monocytogenes*	Protozoa (cilliate *Tetrahymena*), Rhabditid nematode (*Caenorhabditis*)
Salmonellosis (typhoid, gastroenteritis and systemic infections)	*Salmonella enterica*	Protozoa (amoeboid *Acanthamoeba*, cilliate *Tetrahymena*), Rhabditid nematode (*Caenorhabditis*)
Pneumonia (nosocomial)	*Acinetobacter* spp., *Klebsiella* spp., *Pseudomonas* spp.	Chironomid (eggs and larvae), Rhabditid nematode
Malaria	*Plasmodium falciparum*, *P. vivax*, *P. malariae*	Mosquitoes (*Anopheles*) (eggs and larvae)
Cryptosporidiosis	*Cryptosporidium parvum*	Rotifers (*Brachionus*), Cladocerans (*Daphnia*)
Dracunculiasis	*Dracunculus medinensis*	Copepods (*Cyclops*)
Gnathostomiasis	*Gnathostoma* spp.	Copepods (*Cyclops*)
Filariasis	*Wuchereria bancrofti*, *Brugia malayi*	Mosquitoes (*Anopheles*, *Culex*, *Aedes* and *Mansonia*) (eggs and larvae)
Microsporidiosis	*Microsporidium* spp.	Cladocerans (*Daphnia*)
Giardiasis	*Giardia intestinalis*	Cladocerans (*Daphnia*)
Japanese encephalitis	Flaviviridae viruses	Mosquitoes (*Culex*) (eggs and larvae)
Dengue	Dengue virus (DENV1 to 4)	Mosquitoes (*Aedes*) (eggs and larvae)

et al. 1990). Colonization of pathogenic bacteria on carbohydrates- and protein-rich mucosal matrices inside the gut or body covering of aquatic organism promotes bacterial growth and persistence (Rosenberg and Ben-Haim 2002). Microinvertebrates feeding on natural food particles would bring external bacteria into their gut, where an ingested pathogen may rapidly proliferate utilizing the nutrients from digested foods. *C. parvum* oocysts, *E. coli*, *Pseudomonas* sp., *S. enterica,* and *Listeria* spp. have been observed to survive and grow in the intestinal tract of nematodes (e.g. *Caenorhabditis*) (Chang et al. 1960; Huamanchay et al. 2004; Anderson et al. 2003). Microsporidia are intracellular parasites which can frequently invade the cladoceran *Daphnia* spp. (Weigl et al. 2012). *Salmonella enterica* and *C. jejuni* were shown to replicate within intracellular digestive vacuoles of some amoeboid protozoa, e.g., *Acanthamoeba* spp. (Tezcan-Merdol et al. 2004; Axelsson-Olsson et al. 2005). Similarly, ciliated protists like *Tetrahymena pyriformis* can support the persistence and growth of *Campylobacter* spp., *Leg. pneumophila* and *Lis. monocytogenes* (Barker and Brown 1994; Ly and Müller 1990; Snelling et al. 2005). Wetland rotifers have been observed to serve as vehicle for *Cryptosporidium* oocysts (Nowosad et al. 2007). A list of

aquatic microinvertebrates reported to provide shelter and support to a variety of microbial pathogens has been included in Table 1. Some of the wetland fauna like copepods, chironomid larvae, etc. act as hosts for several pathogenic species, while individual pathogen may also invade multiple microinvertebrates to widen the chance for environmental survival and thus become more potent to infect humans in the nearby communities.

Pathogenic bacteria capable of producing chitinase, e.g., *Vibrio*, *Aeromonas*, etc., have a competitive survival advantage in the presence of chitinous microinvertebrates. The chitin-rich hind- and mid-gut of crustacean zooplankton are preferable attachment sites for this kind of bacteria (Vezzulli et al. 2010). Nevertheless, *E. coli*, *Campylobacter* and *Arcobacter* spp., which do not secrete chitinase, can remain firmly associated with chitinous zooplankton (Maugeri et al. 2004). Among various microinvertebrate groups having chitinous exoskeleton, the copepods (e.g. *Acartia*, *Eurytemora* spp.) are implicated as prime vectors of *V. cholerae* (Huq et al. 1984). However, many other chitinous organisms including cladocerans (*Bosmina*, *Ceriodaphnia*, *Diaphanosoma*, etc.), crustacean nauplii and rotifera (e.g., *Brachionus* spp.) can also act as hosts for toxigenic *V. cholerae* (Tamplin et al. 1990). Apart from the beneficial colonization on live organisms, pathogenic bacteria also derive nutrition from the discarded chitinous exoskeleton during molting as well as degraded products from a decaying microinvertebrate (Tamplin et al. 1990). The flourishing of *Vibrio* in the benthic sediments of estuarine environment can be related to the input of organic matter from algal- and microinvertebrate-detritus (Williams and LaRock 1985). Thus, microinvertebrates are not only important as reservoirs in the water column but also fuel benthic seasonal cycles of the pathogens. The relevance of sediment resuspension to the increased occurrence of waterborne diseases deserves more attention (Lara et al. 2009).

Egg sacs of certain microinvertebrates, especially the copepods, can be heavily colonized by pathogenic vibrios (Huq et al. 1984). More notably, a variety of pathogens, i.e., *Vibrio, Aeromonas, Acinetobacter*, *Klebsiella* and *Pseudomonas spp.*, may remain colonized on the chironomid (non-biting midge flies) egg masses, which are widespread in different freshwater bodies and waste stabilizing ponds (Halpern et al. 2007). *Vibrio cholerae* can degrade the gelatinous matrix of chironomid egg masses and prevent hatching by secreting hemagglutinin/protease (Halpern et al. 2004). Clearly, bacteria attached to the egg masses have easier access nutrient-rich food compared to planktonic ones.

In general, larger organisms, e.g. fish, birds, mussels, etc., engulf aquatic microinvertebrates as food and thus their migratory behavior may aid in the spread of microinvertebrate-associated pathogens. Some aquatic microinvertebrates also act as vector for particular pathogens facilitating their dispersal (Fig. 1). For example, the higher abundance and wind-mediated migration of midge flies (chironomids) can coincide with concurrent increase in cholera incidences due to *V. cholerae,* which can remain associated with all life stages, i.e., eggs, larvae, pupae and adults of midge flies (Broza et al. 2005). The ecology of malaria is closely associated with the availability of wetlands because the vector mosquitoes have to pass pre-adult stages in the aquatic environment (Dale 2008).

Trophic regulation and resistance mechanism of pathogenic microbes

Among the microinvertebrate communities an increase in protozoan ciliates and heterotrophic nanoflagellates (HNFs) may regulate pathogenic microbial populations. Microcosm studies have shown the capacity of these microinvertebrates to eliminate *V. cholerae* through their grazing activities (Martínez Pérez et al. 2004). Various ciliated protozoa can limit many bacterial populations by their predatory activities, e.g., *Paramecium caudatum* can rapidly ingest the *Cryptosporidium* oocysts (Stott et al. 2001). Increased occurrence of protozoans (ciliates) in general also act as limiting factors for pathogenic *E. coli* and *Salmonella* spp. (Sinton et al. 2002; Chandran and Hatha 2005). Astonishingly, the protozoan ciliates as well as flagellates have grazing rates of up to 2,000 bacteria cell^{-1} h^{-1} (Macek et al. 1997).

Symbiosis with microinvertebrates may overcome protozoan grazing, aided by diversified mechanisms, and, thereby, enhance the survival of the pathogen (Fig. 1). The cells of *Legionella* spp., *V. cholerae* and *V. mimicus* can withstand digestion and grow intracellularly for prolonged periods in both cytoplasm and

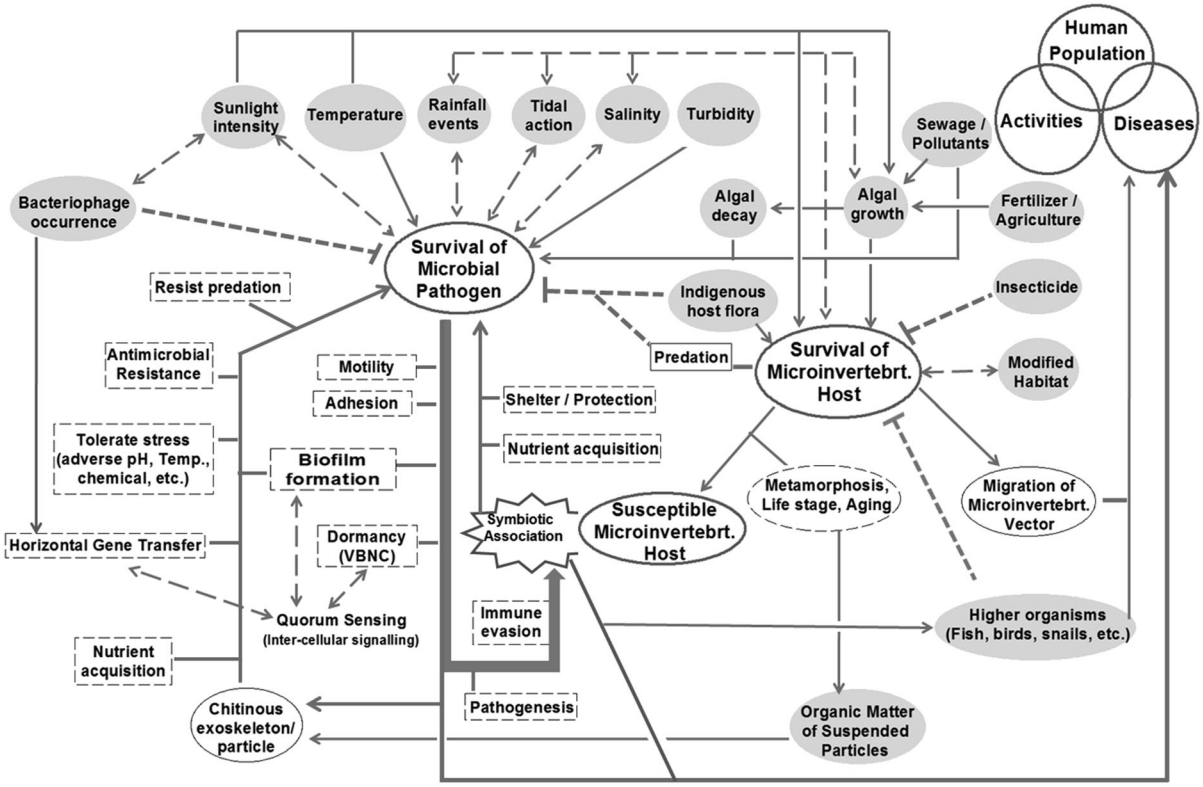

Fig. 1 A sketch of key ecological processes regulating the association of human pathogens and microinvertebrates in a wetland ecosystem. The relationships are indicated as follows: a positive influence by an *arrow* with *solid line*, a negative influence by a truncation with *dotted line*, while an interaction with either positive or negative influence by a *dotted line* with *arrows* at both ends. Important biological processes and trophic regulations influencing the survival and association of human pathogens and microinvertebrate hosts are also linked. Survival of the symbiotic organisms depends on a variety of interacting forces including climatic factors (temperature, rainfall, etc.), anthropogenic influences (habitat modifications, pollutions, etc.), algal growth, preponderance of bacteriophage and indigenous flora, etc. The degraded host products, including chitinous exoskeleton, may also promote the growth and survival of microbial pathogens. Human pathogens utilize motility, biofilm formation, dormancy, pathogenesis and immune evasion strategies during the symbiosis, which provide them with many adaptive benefits including nutrient acquisition, transfer of genetic materials, resist predation, tolerance of unfavorable conditions and chemical hazards, dispersion to new places, etc., and ultimately influence the disease outbreaks

cysts of amoeba protozoans (Rowbotham 1980; Abd et al. 2010). The effector proteins (VgrG-1, -2, and -3) secreted from the syringe-like T6SS and virulence factor, VasX, can support the survival of *V. cholerae* colonized on microinvertebrates and inhibit protozoan predators like *Dictyostelium discoideum* (Miyata et al. 2011). *Vibrio parahaemolyticus* secretes effector proteins via T3SS2 to attenuate bacterivorous nanoflagellate grazing (Matz et al. 2011). *Vibrio cholerae* cells may overcome intense grazing events by increasing their growth rate if environmental conditions are conducible, whereas their biofilm formation on wetland microinvertebrates may resist adverse conditions, e.g., antibiotic effect, low temperature, etc. and predation by amoebic protozoa, e.g., *A. castellanii* and the HNF, e.g., *Rhynchomonas nasuta* (Matz et al. 2005; Abd et al. 2010). However, the secreted AHL signal molecules, required for biofilm formation of pathogenic microbes, can be inactivated by the acylases, lactonases and phenetyhlamide compounds released from the commensal microbial flora of aquatic microinvertebrates (Natrah et al. 2011).

In contrast to their predatory activities, a variety of protozoan species may harbor certain pathogenic microbes and also aid in their survival. For example, *C. jejuni*, *Leg. pneumophila, Lis. monocytogenes,* etc. can resist the digestion of protozoans like amoeboid *Acanthoamoeba* and ciliate *Tetrahymena* (Ly and Müller 1990; Barker and Brown 1994; Axelsson-Olsson et al. 2005). *Salmonella* may act the same way

as *Legionella* resists digestion inside the food vacuoles of amoebae and human macrophages, i.e., by stopping the fusion of the phagosome to the lysosome (Brandl et al. 2005). Presence of some bacteriovorous amoebae can induce *L. pneumophila cells to persist in* biofilms, which may aid in their transmission to humans (Greub and Raoult 2004; Kuiper et al. 2004). Higher survival of *Campylobacter* was observed while persisting within protozoans *T. pyriformis* and *A. castellanii* than that of planktonic bacteria (Snelling et al. 2005). However, certain coliforms and mycobacteria can persist within the amoeba without multiplying (Barker and Brown 1994). Survival within protozoa, including amoebae, can protect the pathogenic microbes from hostile environmental conditions such as chlorination (King et al. 1988).

In drinking water systems, the presence of nematodes, especially of the *Rhabditidae* family, which can act as transport vehicles of pathogens, e.g., *E. coli*, *Pseudomonas* sp., etc., has been considered as a potential health risk since many years (Chang et al. 1960). Later studies have confirmed that nematodes like *Caenorhabditis elegans* can ingest, transport, and excrete viable and infectious *C. parvum* oocysts, *E. coli*, *S. enterica,* and *Listeria* spp. (Huamanchay et al. 2004; Anderson et al. 2003). *Vibrio* spp. are under high grazing pressure and top-down control by many nematodes (Vezzulli et al. 2009). However, in a laboratory study, Vaitkevicius et al. (2006) showed that ingested *V. cholerae* can kill certain nematodes like *C. elegans* by secreting the extracellular protease PrtV. Interestingly, PrtV is also required to prevent grazing of *V. cholerae* by the flagellate *Cafetaria roenbergensis* and the ciliate *T. pyriformis* via a QS regulatory mechanism (Matz et al. 2005; Erken et al. 2011).

Many microinvertebrates belonging to higher zooplankton taxa usually regulate the density and viability of human pathogens in wetlands. *Daphnia carinata,* a cladoceran species, was observed to reduce 99 % of *C. jejuni* population within 72 h in co-culture experiments (Schallenberg et al. 2005). Rotifers like *Philodina, Euchlanis, Epiphanes* and *Brachionus* spp., which are highly abundant in wetlands, have been observed to ingest and carry viable *Cryptosporidium* and *G. lamblia* cysts (Fayer et al. 2000; Trout et al. 2002; Nowosad et al. 2007). The grazing activity of some cladocerans, e.g., *Daphnia pulicaria,* may decrease the viability of these cysts, but predatory digestion may also induce the excystation process to release infectious trophozoites of *Giardia* (Connelly et al. 2007). Hence, although the predation of filter-feeding and ciliated microinvertebrates could potentially reduce pathogen abundance, some larger organisms of higher trophic level like copepods, rotifers, etc. may serve as their reservoirs and transmission vectors (Fig. 1). Notably, due to the low infectious dose of *Cryptosporidium* and *Giardia*, their persistence, even in low number within wetland microinvertebrates, is a grave concern from epidemiological view point. Several studies have indicated a complex relationship between *Vibrio* abundance and the structure and composition of the zooplankton community (Heidelberg et al. 2002; Möller et al. 2007; Lizarraga-Partida et al. 2009; de Magny et al. 2011). The attachment and biofilm formation on chitinous microinvertebrates, particularly with copepods, as well as utilization of the digestive by-products of host organisms can be considered as important survival strategies of pathogenic microbes (Matz et al. 2005). Nevertheless, other studies have detected greater concentrations of *Vibrio* in water compared to zooplankton (Lara et al. 2011; Neogi et al. 2011, 2012). Apart from the immune defense mechanisms, the autochthonous bacteria of many microinvertebrates may also impede the growth of pathogenic bacteria (Kirschner et al. 2011). Intriguingly, diet types may be a selective force for different bacterial communities inside the host's body to regulate the co-occurring pathogen population (Tang 2005). However, some pathogens like vibrios can produce secondary metabolites with antagonistic properties to outcompete other bacteria in aquatic microinvertebrates (Mansson et al. 2011). Association of the pathogenic bacteria with zooplankton may be also influenced by metamorphic life stages. For example, juvenile copepod can frequently molt and shed chitinous exoskeleton along with attached vibrios in the process (Tamplin et al. 1990).

In the aquatic environment, pathogenic bacterial abundance can be limited by specific bacteriophages, e.g., *E. coli* by coliphages, *V. cholerae* O1 by O1 phages, etc. (Sinton et al. 2002; Faruque et al. 2005; Mookerjee et al. 2014). Seasonal dynamics of some water-borne diseases, e.g. cholera, are, therefore, limited to some extent by certain aquatic bacteriophages (Faruque et al. 2005). The population diversity of particular lysogenic phages also plays a key role in the evolution of pathogenic clones via HGT of

toxigenic genes, e.g., cholera toxin in *V. cholerae*, and Stx1 and Stx2 toxins in STEC/EHEC (Watarai et al. 1998; Boyd et al. 2000). The hypothetical key ecological events influencing the association of human pathogens with microinvertebrates in wetland ecosystem, with important biological processes and trophic regulations, have been depicted in Fig. 1.

Role of physicochemical, nutritional and climatic factors

It is important to understand how and to what extent the interaction between pathogenic bacteria and aquatic microinvertebrates is regulated by the changes in bio-physico-chemical conditions of wetland ecosystems. As shown in Fig. 1, the persistence and association of both the aquatic pathogens and their host microinvertebrates are influenced by a variety of interacting climatic factors, e.g., temperature, rainfall, salinity, tide, etc., and anthropogenic activities, e.g., habitat modifications, pollution by sewage, insecticides and pesticides, eutrophication promoting algal growth, etc.

Aquatic survival of microbial pathogens like *E. coli, Vibrio*, and *Salmonella* spp. depends on various physicochemical factors such as water temperature, adsorption and sedimentation processes, sunlight action and nutrients (Sinclair and Alexander 1984; Sinton et al. 2002; Neogi et al. 2011). Higher incidences of most water-borne diseases, including *Vibrio*-related gastroenteritis and septicemia, have been reported in the summer and early fall, when aquatic microinvertebrate populations are usually more abundant (Hlady and Klontz 1996). Temperature changes induce differential gene regulation by the histone-like nucleoid structuring (H-NS) protein in pathogenic *E. coli*, which may facilitate their aquatic adaptation (White-Ziegler and Davis 2009). *Escherichia coli* O157:H7 strains have evolved mechanism to withstand acidic conditions (pH as low as 2.5) by genetic regulations of the alternate sigma factor *rpoS* (Lin et al. 1996). Phytoplankton enrichment causing more availability of microinvertebrates, especially zooplankton, plays a vital role in the Eastern English Channel, whereas salinity influences vibrio dynamics in the Mediterranean coastal lagoons (Julie et al. 2010; Cantet et al. 2013). However, increased salinity mediated rise in total *Vibrio* population may coincide with a decline in *V. vulnificus* populations, as observed in the Eastern coast of USA (Blackwell and Oliver 2008). Better understanding of the environmental factors regulating the persistence and dispersal of pathogen populations may aid in developing region-specific prediction model based on measurable climatic factors, e.g., temperature, salinity, tide, chlorophyll, etc. Satellite-based remote sensing of these influencing factors has been proposed as a method to predict cholera outbreaks (Lobitz et al. 2000).

Seasonal dynamics of aquatic pathogens are often correlated with nutrient availability. Concentrations of particular sugars (glucose and mannose), phosphorus and nitrogenous compounds may influence the attachment and biofilm formation of *V. cholerae* cells (Pruzzo et al. 2008). The ability to store inorganic phosphorus (P_i) may protect *V. cholerae* against environmental stresses such as acidity, salinity, and the damaging effects of H_2O_2, as P_i is essential to induce RpoS, a major stress response regulator of the bacteria (Jahid et al. 2006). The phosphoenolpyruvate phosphotransferase system (PTS) and ABC transporters in pathogenic *V. cholerae* and *E. coli*, respectively, are transport systems for nutrients (amino acids and sugars) facilitating biofilm formation (Ihssen et al. 2007; Houot et al. 2010). Pathogenic *E. coli* strains exhibit a high degree of catabolic flexibility including growth in freshwater with very low organic nutrients (Vital et al. 2008). However, it is worthy to consider that small-sized invertebrates can compete with aquatic microbial pathogen for organic nutrients, e.g., particulate and dissolved organic C and N (Neogi et al. 2011).

Sunlight may induce the lysogenic CTX phage in *V. cholerae* cell to release phage particles and its subsequent integration into another susceptible cell (Faruque et al. 2000). Therefore, there is a high possibility of toxigenic conversion of aquatic *V. cholerae* strains during the summer season. As an adaptive strategy for UV resistance, *V. cholerae* may increase the production of a protective melanin pigment (Valeru et al. 2009). On the other hand, sunlight is an important deactivating factor for the survival of *E. coli* and *Salmonella* spp. (Sinton et al. 2002; Chandran and Hatha 2005). However, it may induce an STEC cell to release hundreds of *stx2*-bacteriophages, which may be integrated into many other susceptible *E. coli* cells (García-Aljaro et al. 2005).

Dengue viruses are primarily transmitted by *Aedes aegypti* mosquitoes, although *Ae. albopictus*, *Ae. polynesiensis* and *Ae. scutellaris* may also act as vectors (WHO 2009). *Aedes* and their larvae are principally found in the 'high' marsh areas where transient and stagnant water pools are formed in estuarine mudflats due to seasonal spring tides, rainfall/runoff and siltation or other blockage of the normal daily tidal flushing. The eggs of *Aedes* spp. can tolerate dessication until hatching upon availability of tidal or rain water even after several months (Dale 2008). The dynamics of dengue fever and other mosquito-borne diseases like malaria and JE are influenced by monsoon rains and irrigation practices, which regulate the aquatic survival of mosquito larvae (Pham et al. 2011). The occurrence of *Anopheles* and *Culex* mosquitoes, which are vectors for malaria causing *Plasmodium* spp. and JE virus, respectively, are mostly associated with permanent freshwater pools otherwise their eggs will desiccate. Salt marshes are also utilized for breeding purpose by the *An. atroparvus* and *An. camptorhynchus,* vectors of *P. vivax* and Ross River virus, respectively, which prefers to breed in the presence of abundant algae and microinvertebrates (Lindsay et al. 2007, 2010).

Risk factors and inducing conditions

It is predicted that, in some areas, climate change will increase temperature, sea level, rainfall and the frequency of cyclone events (IPCC 2007). These hydro-climatic changes may have substantial impact on wetland ecology including the abundance, diversity and seasonality of both the wetland microinvertebrates and pathogenic microbes. Many field-based investigations have observed a salient role of salinity on aquatic bacterial pathogens, e.g., *Vibrio* populations (e.g., Mahmud et al. 2008; Cantet et al. 2013). A positive influence of temperature increase (>20 °C) on the aquatic survival and growth of many pathogens, e.g., *Vibrio* spp., *E. coli, Giardia* and *Salmonella* spp., with subsequent rise in disease incidence, is well known (Hlady and Klontz 1996; Kevin and Deirdre 2005; Mahmud et al. 2008; Sánchez-Vargas et al. 2011; Neogi et al. 2011). In some estuarine ecosystems, the role of higher temperature (>25 °C) on pathogen abundance may be overshadowed by the influence of salinity (Wright et al. 1996). Global warming may induce a substantial rise in mosquito populations and subsequently dengue and malaria outbreaks (Githeko et al. 2000). Moreover, increase in rainfall, relative humidity and sunshine hours have been observed to instigate dengue incidences (Pham et al. 2011).

Organic matters including chitin, derived from the degraded microinvertebrates, usually accumulate in the suspended and benthic sediments, which provide vital nutritional support to aquatic microbes. Increased load of suspended particulate matters and associated chitin in surface water due to re-suspension of benthic sediments by cyclones, floodings, and turbulences may enhance the survival and persistence of pathogenic microbes, including *Vibrio* spp. (Lara et al. 2009). Frequent occurrences of post-cyclone waterborne diseases, including cholera, gastroenteritis and septicemia, have been reported in many parts of the world (e.g., Bhunia and Ghosh 2011). During the onset of summer and fall the occurrence of phytoplankton bloom and subsequent increase in microinvertebrate populations and enrichment of particulate organic matter can be related with the concurrent rise in waterborne diseases, e.g. cholera, gastroenteritis, legionellosis, etc. (Neil and Berkelman 2008; de Magny et al. 2008; Vezzulli et al. 2010).

Many of the wetlands receiving agricultural and urban waste water effluents may contain large number of pathogens like *Acinetobacter, Klebsiella,* STEC, *Salmonella*, etc. Therefore, adjacent human population density, exposure of livestock or human sewage, leaky septic tanks, boat discharges, etc. can be considered as putative risk factors, which need to be managed properly. However, some pathogenic species may not withstand all kinds of wastewater samples, e.g., the acidic and alkaline extracts of wastewater from dairy lagoons may inhibit while the neutral aqueous extracts may enhance the growth of pathogenic *E. coli* (Ravva and Korn 2007). In this context, the retention time of introduced wastewater materials and availability of suitable microinvertebrate hosts providing survival advantage for the pathogenic organisms in wetland environment may determine the risk.

Rainfall driven input of fecal matter and sewage may increase the organic nutrient load in wetlands instigating population increase among some aquatic pathogens, e.g., toxigenic *Vibrio* and *E. coli* (Blackwell and Oliver, 2008; Batabayal et al. 2014).

Similarly, in seafood, a higher prevalence of *Cryptosporidium* and *Giardia* spp. is often observed in seafood samples during the wet season (Miller et al. 2005). However, in many temperate regions, despite precipitation-mediated nutrient input into wetlands, the prevalence of low temperature may hinder pathogen growth in wet months. Rainfall mediated changes in environmental conditions (e.g., pH, salinity, nutrient, etc.) may regulate bacteria–invertebrate interactions, possibly due to altered bacterial diversity and/or the invertebrate feeding dynamics. Cholera incidences in Bangladesh have conspicuous bi-modal peaks, before and after monsoon, which typically coincide with prior zooplankton blooms (Sack et al. 2003). On the other hand, in Africa, Guniea worm disease transmissionhas differential seasonal pattern, i.e., in arid climatic zone people generally get infected during the rainy season while the opposite scenario happen in regions with frequent rainfall (Cairncross et al. 2002). The variable influences of the probable risk factors should be estimated targeting individual pathogenic microbes and based on local environmental conditions.

The digestive vacuoles of predatory amoeba are often expelled as encysted microvesicles (<5 μ diameter), which may contain pathogenic microbes (Greub and Raoult 2004). Hence, the occurrence of amoebae may be considered as a risk factor in water systems. Some wetland microinvertebrates, e.g., insect larvae, may act as reservoirs, whereas its adult form may aid in the dispersal of human pathogens. Among the aquatic insects, chironomids can tolerate a wide range of conditions of fresh-, brackish- and waste-water. *Vibrio cholerae* aerial transfer by chironomid flies may play an important role in the spread of cholera outbreaks (Broza et al. 2005). Therefore, for better prediction of waterborne disease outbreaks the biological and seasonal cycle of aquatic invertebrates need to be considered as influential factors.

The widespread use of fertilizers leading to enormous input of nutrients may drive eutrophication and associated changes in the microinvertebrate communities in wetlands. An increase in anthropogenic runoff, can also lead to higher zooplankton blooms causing increased availability of host organisms as well as chitinous surfaces, which may spur a drastic increase in the numbers of the pathogenic microbes, e.g., vibrios, even though bacteriovorous predators may also become more abundant. Thus, eutrophication of estuarine ecosystem has been linked to increased cholera risks (Huq et al. 2005). Vector-borne diseases like malaria and schistosomiasis involve intermediate invertebrate hosts (mosquitoes and snails, respectively), whose growth and reproduction depend on the availability of suitable aquatic conditions and natural foods, e.g., algae. Eutrophication mediated increase in algal numbers and/or changes in species diversity may affect the density of intermediate hosts, with cascading consequences in disease dynamics (Johnson and Carpenter 2008).

Intervention strategies

The therapeutic use of antimicrobial agents has reduced the burden of waterborne diseases in many parts of the world. Unfortunately, through mutation and the selective pressure of antimicrobial agents, pathogenic microbes have increasingly become resistant to these drugs (Nair et al. 2010; Strateva and Yordanov 2009). Alternatively, contemporary studies are focusing on the use of natural compounds with antimicrobial properties, e.g., use of spices and herbs for pathogenic vibrios (Yamasaki et al. 2011). Moreover, utilization of the QS inhibitors as alternatives to conventional antibiotics is a latest strategy to fight the alarming threats of MDR in waterborne pathogens.

Since zooplankton has been shown to harbor the cholera bacterium and zooplankton blooms follow phytoplankton blooms, remote sensing can be employed to determine the relationship between cases of cholera and ocean chlorophyll concentration, as well as sea surface temperature, ocean height, salinity and turbidity (Lobitz et al. 2000; Johnson et al. 2012). An empirical model relating the number of cholera cases, rainfall and chlorophyll successfully reproduced cholera outbreaks in Bengal delta (de Magny et al. 2008). Application of sensitive, rapid, and simple molecular methods to quantitatively detect aquatic pathogens can aid not only in disease management but also predicting pathogenic populations in wetland samples to facilitate early intervention strategies (Neogi et al. 2010; Yi et al. 2014).

On the basis that aquatic microinvertebrates play a vital role in instigating disease incidences like cholera, Colwell et al. (2003) developed a simple filtration procedure whereby zooplankton were removed from natural surface water by cloth filtering apparatus

before household use. The utilization of this filtration procedure in rural Bangladesh yielded a 48 % reduction in cholera, compared with control areas not practicing filtration. Similar kind of intervention may reduce the incidences of some other waterborne diseases, e.g., the Guinea worm disease, which is directly influenced by copepod vectors.

In Japan, the number of *V. parahaemolyticus* infection has been substantially reduced by strictly adapting the hygiene guidelines (Hara-kudo et al. 2012). In fact, most waterborne diseases can be prevented by avoiding drinking unsafe water. Preventing the access of patients to wetland may be a possible social intervention to reduce secondary transmission. Vector-borne diseases like schistosomiasis can be controlled by social preventive efforts nearby the wetlands like reducing human-water contact, improvisation of sanitary and water supply facilities, changing irrigation practices from perennial (wet in all seasons) to basin type (dry out seasonally), introduction of concrete dam-based irrigation, regulating water flow and timely use of molluscicides (Gu et al. 2001; Appleton and Madsen 2012).

Personal intervention strategies, e.g. use of protective clothing, topical repellents, bed-nets, and avoidance of wetlands during particular times (dawn, dusk, and evening hours) are recommended to avoid mosquito-borne malaria, dengue and filaria. Government authorities, in addition, can lower the risk of infection, particularly during the mosquito seasons (summer months), by eliminating wetland mosquito populations with appropriate methodologies, e.g., by filling or draining (with renovated tidal channels) to remove habitats, modified water management with barrier construction and runneling, applying insecticides and introducing larvivorous fishes and genetically modified/sterile mosquitoes (Ostera and Gostin 2011; Griffin and Knight 2012; Rochlin et al. 2012). Other techniques are also available. For instance, for a safe way of controlling mosquito larvae, the *Bacillus thuringiensis israelensis* bacterium is relatively specific and kills a large portion of larvae when ingested (Woodrow et al. 1995). Recently, use of *Wolbachia*, a bacterium which infects the larvae and adults of *Aedes aegypti* mosquitoes, shortening their life span, is in the trial phase to control dengue (Ostera and Gostin 2011). Also a growth retardant insect hormone analogue called methoprene can be used in the integrated vector management program (Woodrow et al. 1995).

Conclusions

A rising trend in waterborne disease incidences is predicted due to climatic changes including global warming, salinity intrusion in coastal wetlands, increasing cyclone events, anthropogenic environmental alterations, all of which will also act together to change the ecosystem dynamics. These scenarios have prompted growing interests in understanding the risks of human health and proper use of the natural and constructed wetlands. The environmental survival of microbial pathogens, aiding their infective potential to humans, depends on the abundance of a variety of wetland microinvertebrates, acting as hosts and/or vectors, and interactive processes like colonization, biofilm development, transforming into dormant state, immune evasion and secretion of effector proteins, proteases and toxins. Moreover, the population dynamics of aquatic pathogens are also regulated by the multi-dimensional effects of abiotic (e.g., physicochemical propterties and climatic factors) as well as biotic (predators like ciliates and HNFs, bacteriophages, indigenous microflora, algal populations, etc.) components. Human activities around the wetlands, such as aquaculture, agriculture and wastewater management in both developing and developed countries may have salient impacts on the dynamics of human pathogens occurring in the wetlands. Therefore, if these wetlands are to be preserved, a management policy is needed that incorporates ecohydrological tools, e.g., sequestration of nutrients, regulating anthropogenic run off, etc., and other eco-friendly strategies to control microinvertebrate populations and waterborne pathogens. In addition, active surveillance is needed on the dynamics and diversity of aquatic microbial pathogens as well as microinvertebrates. Research is especially needed to understand how ecological processes in the wetlands regulate disease virulence, transmission and epidemiology of human diseases. An in-depth understanding will result of the predictable influences of wetland microinvertebrates as reservoir, predator or vector of waterborne pathogens, and this will increase our ability to adopt timely and effective intervention strategies to reduce the increasing burden of waterborne diseases.

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