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Review

Toward a better guard of coastal water safety—Microbial distribution in coastal water and their facile detection

Yunxuan Xie^{a,1}, Ning Qiu^{b,1}, Guangyi Wang^{a,*}^a Tianjin University Center for Marine Environmental Ecology, School of Environmental Science & Engineering, Tianjin University, Tianjin 300072, China^b South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

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ABSTRACT

Prosperous development in marine-based tourism has raised increasing concerns over the sanitary quality of coastal waters with potential microbial contamination. The World Health Organization has set stringent standards over a list of pathogenic microorganisms posing potential threats to people with frequent coastal water exposure and has asked for efficient detection procedures for pathogen facile identification. Inspection of survey events regarding the occurrence of marine pathogens in recreational beaches in recent years has reinforced the need for the development of a rapid identification procedure. In this review, we examine the possibility of recruiting uniform molecular assays to identify different marine pathogens and the feasibility of appropriate biomarkers, including enterochelin biosynthetic genes, for general toxicity assays. The focus is not only on bacterial pathogens but also on other groups of infectious pathogens. The ultimate goal is the development of a handy method to more efficiently and rapidly detect marine pathogens.

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1. Introduction

Ocean tourism has been one of the most promising enterprises of global tourism (Hall, 2001; Sánchez-Quiles and Tovar-Sánchez, 2015; Morrison, 2012; Scott et al., 2012). In the United States, approximately 62 million people swim in the nation's coastal water (US Department

of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service). Coastlines along the Atlantic Ocean in Western Europe and North America attract nearly 40% of global tourists. As ocean tourism remarkably contributes to global tourism income, heavy environmental loads from tourists and other anthropological activities along the coastline are challenging the coastal ecological conditions. Maintaining a clean beach is the key to ensure public health and the viability of local economy for many coastal cities. Major threats to coastal sanitation include municipal sewage discharges, industrial discharges, ineffective wastewater treatment, and production of toxic chemicals.

* Corresponding author.

E-mail address: gywang@tju.edu.cn (G. Wang).¹ These authors contribute equally to the work.

The industrial and municipal efflux systems contribute disposed water with complicated chemical compositions to coastal oceans, creating hotbeds that harbor harmful microorganisms that cause various ecological hazards (Cleaveland et al., 2001; Cotruvo et al., 2004; Fayer et al., 2004; Bauer et al., 2010; Fleming et al., 2011). The highly diversified chemical nature of coastal water imposes technological hurdles for predicting the amplification of a specific pathogen and the resulting consequences to human health. Some of the well-studied cases included the mechanism for the outbreak of harmful algae blooms (Fleming et al., 2001; Backer et al., 2003; Backer et al., 2005; Judd et al., 2004; Von Glasow et al., 2013). In other cases, pathogenic microorganisms in coastal areas were discovered to contribute to the outbreak of acute infections among tourists, including gastroenteritis, dermatitis, otitis, and upper respiratory illnesses (described in Microbe Case Study). Preliminary studies on the outbreak of these infections called attention to a variety of microorganisms such as *Salmonella*, *Staphylococcus*, and *Vibrio*. Negative health effects that are caused by the massive proliferation of harmful microbes usually result in economic consequences such as medication costs and temporal loss of labor, calling for the intelligent management of marine resources and considerations of how we explore the ocean.

The significance of identifying the potential health risks with the use of coastal water for recreational and dietary purposes led to a proposal calling for global and regional collaborations. To acknowledge the presence and the diversity of pathogenic microorganisms worldwide, a group of distinguished experts from both marine and health research fields gathered in Sardinia for a discussion over the presence of marine-based public health risk, in which an “Oristano Declaration” form was summarized to call for collaborative efforts to be made on resolving the potential health risks posed by pathogenic microorganisms (Bowen et al., 2006; Fleming et al., 2006). As one of the major contamination signature microorganisms, fecal *Escherichia coli* residing both in the water column and in the sediments has been extensively characterized in contaminated coastal water, and standards for warning the public were also prepared to address the contamination status contributed by *E. coli* (Pachepsky and Shelton, 2011). Possible genetic markers for identifying different *E. coli* strains were described. Comprehensive examination of the distribution profile of pathogenic microbes in recreational beach waters suggested a further thorough investigation to discover appropriate molecular markers to address the presence of other potential pathogens and accurate identification of individual microbial species from complex backgrounds. Molecular detection approaches such as quantitative polymerase chain reaction (qPCR) offer a rapid and sensitive detection of microorganisms from a variety of backgrounds. The combination of survey studies with the reported cases using qPCR to characterize coastal water pathogens will aid in water sanitation assays with improved detection accuracy. Studies have been conducted to identify pathogenicity factors for different microorganisms. Furthermore, efforts are needed to search for general genetic markers for pathogenicity description, particularly those associated with in vivo toxicity, to simplify the detection procedure. A comprehensive analysis of universal secondary metabolic networks has been shown to be effective in revealing the characteristic metabolism of pathogenic microorganisms because secondary metabolism is highly responsive to environmental stimuli and features the environmental adaptability of different microorganisms. An overview of potential secondary metabolic pathways in pathogenic microorganisms suggests the presence of biosynthetic genes for siderophore—a chelating compound—in pathogenic microorganisms that can be used as a good candidate for pathogenicity description (Scholz and Greenberg, 2015; Mercado et al., 2008, Holmes et al., 2005, Flo et al., 2004; Miller et al., 2009; KEGG: Kyoto Encyclopedia of Genes and Genomes).

Here, we present survey events to provide preliminary information over the type of pathogenic microorganisms that commonly occur in coastal waters and propose simplified molecular detection schemes using pathogenicity-associated genes as targets. In the first part, we

discuss the survey reports of different beaches worldwide to learn of the methodologies adopted in individual studies for sample collection and pathogen characterization. To further our discussion in developing uniform analysis for pathogen identification, individual cases reporting the examination of pathogenic microbial species using PCR have been summarized in the second part. This discussion suggests the vast utility of general molecular descriptors (mostly 16S ribosomal RNA) and individual descriptors that are mostly pathogenicity-coding genes. Toward the end, we discuss the feasibility of using enterochelin synthase as a new parameter for describing the pathogenicity for multiple pathogens to indicate the high diversity of genes related to pathogenicity. The ultimate goal of this review is to acknowledge the occurrence and distribution of pathogenic microorganisms and facilitate the development of rapid identification techniques for pathogenic microorganisms in coastal water.

2. Distribution of pathogenic microorganisms in the coastal region—anthropological and geographical assays

Contagious infections are problematic for many population-based public activities. Ocean-based tourism is among one of these enterprises that is easily challenged by infectious events during traveling seasons. Extensive research efforts have been devoted for characterizing individual pathogens that are potentially infectious and generally include three sequential steps: experience-guided preliminary judgments, forcing sampling strategies based on hydrodynamic tracking model, and experimental verification using either traditional plate assay or molecular biological assays (most preferably PCR) (Tables 1 and 2). For an efficient examination, correct sampling strategies are critical. In many case studies, sampling attempts are largely decided by the preliminary evaluation of the geographic and anthropological factors. It has been indicated that without point-source contamination, in which exact pollution mechanism can be traced, introducing contagious pathogens into coastal water by people visiting for recreational purpose is unneglectable (Elmir et al., 2007). For instance, the increased number of coastal water bathers during traveling seasons results in *Staphylococcus aureus* overload in sea water. To evaluate the effects of infectious *Staphylococcus aureus* on human bathers in coastal water, a systematic study was conducted to estimate the volume and characteristics of methicillin-sensitive *S. aureus* and methicillin-resistant *S. aureus* (MRSA) in sea water (Plano et al., 2011). A combination of selective growth media, biochemical studies, and PCR analysis was used to identify and characterize the different *S. aureus* strains isolated from water and participants. The results suggested that both adults and toddlers contributed colonizing pathogenic MRSA to recreational beaches during traveling seasons. To identify influential factors that affect the pathogenicity of southern California beaches, the concentration of common potential pathogenic bacteria including *S. aureus*, MRSA, and enterococci at three southern California beaches was analyzed (Goodwin et al., 2012). Using linear regression analysis, several significant variables were found to correlate with the number of *S. aureus* in seawater including the total number of *S. aureus* in sand, water temperature, enterococci in seawater, and the number of swimmers. To identify possible sources of *S. aureus* at Hollywood Beach, a comprehensive evaluation was performed to analyze *S. aureus* distribution in sand (Esiobu et al., 2013). A high density of *S. aureus* was detected in dry sand [3.46×10^5 colony forming units (CFU)/g] at Hobie Beach when human density was high.

In addition to direct contributions from recreational water users, near-shore anthropological activities, primarily sewage disposal and aquaculture, have significant effects on sea water microbial compositions. Municipal discharges, which are washed off into rivers that converge in the estuary sites into the ocean, can complicate the chemical compositions and microbial distribution of coastal water. A comprehensive microbial examination for potential pathogenic bacteria suggested the presence of numerous pathogens including *Enterobacteria*, *Vibrio*, *Pseudomonas*, *Coliforms*, *Salmonella*, *Shigella*, and heavy metal-resistant

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