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# Distribution comparison and risk assessment of free-floating and particle-attached bacterial pathogens in urban recreational water: Implications for water quality management

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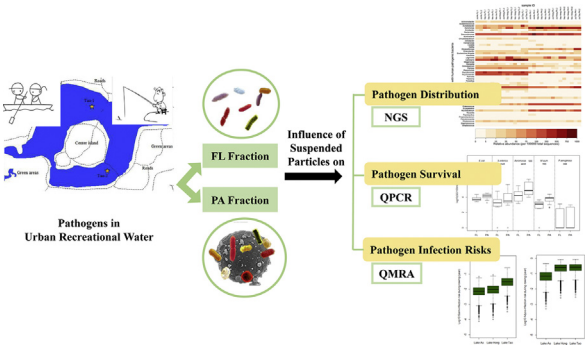
## HIGHLIGHTS

- Next generation sequencing and qPCR methods were combined to reveal pathogen risk.
- Stable pathogen distribution between particle-attached and free-floating fractions
- Most target gene levels were higher in particles compared with surrounding water.
- Pathogen health risks showed correlations with lake nutrients and particle levels.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The risk of pathogen exposure in recreational water is a concern worldwide. Moreover, suspended particles, as ideal shelters for pathogens, in these waters also need attention. However, the risk caused by the pathogen-particle attachment is largely unknown. Accordingly, water samples in three recreational lakes in Beijing were collected and separated into free-floating (FL, 0.22–5 μm) and particle-attached (PA, >5 μm) fractions. Next-generation sequencing (NGS) was employed to determine the diversity of genera containing pathogens, and quantitative PCR (qPCR) was used to assess the presence of genes from *Escherichia coli* (*uidA*), *Salmonella enterica* (*invA*), *Aeromonas* spp. (*aerA*), *Mycobacterium avium* (*16S*) and *Pseudomonas aeruginosa* (*oaa*). The NGS results showed stable pathogen genera composition distinctions between the PA and FL fractions. Some genera, such as *Aeromonas* and *Mycobacterium*, exhibited higher abundances in the PA fractions. qPCR revealed that most of the gene concentrations were higher within particles than were FL fractions. Some gene levels showed correlations with the particle concentrations and lake nutrient levels. Further quantitative microbial risk assessment (QMRA) of selected strains (*S. enterica* and *M. avium*) indicated a higher health risk during secondary contact activities in lakes with more nutrients and particles. We concluded that suspended particles (mainly composed of algae) in urban recreational water might influence the pathogen distribution and could serve as reservoirs for pathogen contamination, with important management implications.



## 1. Introduction

Many human pathogens exist outside their primary hosts (Berg et al., 2005; Liao et al., 2015), and pathogens in aquatic systems pose a severe threat to public health worldwide (Sherchand, 2012).

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Recreational lakes and rivers in urban areas are perceived as beneficial elements to citizens for their aesthetic and ecological services (Sales-Ortells et al., 2015). Nevertheless, people may frequently come into contact with waters containing such pathogen hazards owing to the input of sewage discharge and animal feces or other fecal materials to water (Arnone and Walling, 2007). Thus, pathogen prevalence in recreational water has attracted much attention from researchers worldwide (de Man et al., 2014; Fewtrell and Kay, 2015; Oster et al., 2014; Sales-Ortells and Medema, 2014). Moreover, urban recreational water typically contains a high level of suspended particles (*e.g.*, detrital materials or algae) (Byappanahalli et al., 2015; Parveen et al., 2011). Some pathogens could attach to these particles and might further mature into surface biofilms, thereby gaining considerable advantages such as abundant nutrient assimilation and increased resistance to adverse conditions (Droppo et al., 2009; Percival et al., 2013). Particles could be a critical factor for the fate and survival of pathogens in water (Liao et al., 2015). Thus, it is necessary to assess the associations of pathogens with particles in recreational waters.

Generally, bacteria can exhibit two life states in water: free-floating (FL) or particle-attached (PA) (Fletcher, 1991). In recent decades, attached or biofilm-related pathogens have gained much attention, particularly in the medical hygiene field (Hall-Stoodley et al., 2005; Shirtliff and Leid, 2009) and in water distribution systems (Jjemba et al., 2010; Wang et al., 2012; Liu et al., 2013; Falkinham et al., 2015). These studies have indicated the persistence of attached pathogens and their high health risks. However, knowledge concerning pathogen attachment in urban lakes or rivers is limited. Several reports have indicated the increased survival of fecal indicator bacteria (FIB) attached to suspended particles or sediments (Bai and Lung, 2005; Haack et al., 2015), and several other studies have described the existence of enteric pathogens (*e.g.*, *Salmonella* or *Campylobacter*) on suspended matrices (Oster et al., 2014; Byappanahalli et al., 2015). Three main factors leading to bacterial attachment to matrices have been summarized (Liao et al., 2015; Hall-Stoodley et al., 2005): bacterial properties, particle features and environmental conditions. These factors might interact at natural sites, driving pathogens to exhibit different distribution states in water. However, the occurrence and distribution of pathogens associated with particles have not been investigated in detail, and the risk caused by pathogen-particle attachment in recreational waters has not been addressed.

Detection methods play a major role in pathogen risk monitoring and assessment. The culture-dependent method of FIB counting has long been used as a surrogate of pathogens; however, these methods have been widely found to be inefficient because indicator occurrences cannot represent the levels of various pathogens (Girones et al., 2010; Aw and Rose, 2012). Recently, there have been remarkable advances in culture-independent methods, such as qPCR, microarray and genome sequencing (Aw and Rose, 2012; Lu et al., 2015). Regarding sensitivity, qPCR is one of the relatively reliable quantitative methods and has been widely used to monitor pathogens in urban water (de Man et al., 2014; Byappanahalli et al., 2015; Yamahara et al., 2012). However, qPCR and microarray target only specific pathogens. In contrast, sequencing methods have become promising tools because of their high-throughput features, and these methods have been applied to examine pathogen diversity in various environments (Ye and Zhang, 2011; Kumaraswamy et al., 2014; Tan et al., 2015). Therefore, sequencing and qPCR methods can be combined to screen potential pathogen diversity, quantify specific pathogens and thereby provide comprehensive information concerning pathogen distribution.

In the present study, we selected three typical urban recreational lakes in Beijing for *in situ* investigation. The aims of this study were to 1) show the potential pathogen distribution characteristics in water by analyzing the diversity of genera, including pathogens, within two fractions; 2) reveal the risks caused by pathogen-particle attachment by quantifying selected pathogen species and comparing the infection probabilities; and 3) discuss related implications for safe water practices based on the association of pathogen occurrences with environmental factors. Specifically, for recreational risk assessment, although international interest has been primarily in direct water contact (*e.g.*, swimming) (de Man et al., 2014; Oster et al., 2014; Sales-Ortells and Medema, 2014), such activities were forbidden in recreational water in Beijing because of public safety and health issues. Therefore, this study focused on secondary recreational contacts such as rowing, fishing and walking (Sales-Ortells and Medema, 2014). In addition, the water supplemented into the lakes in this study had received disinfection treatment and was not direct sewage discharge. Thus, the pathogen risk may be different from that in fecal-polluted waters in other regions. Overall, this study presents a detailed pathogen risk assessment in the recreational water and provides useful suggestions for water management.

## 2. Materials and methods

### 2.1. Study sites and sampling

Three recreational lakes in three different parks popular with tourists in Beijing urban areas were selected (Fig. S1). The three lakes were far apart, and the water bodies were not connected. Moreover, the lakes were all supplemented with reclaimed water each year to compensate for water loss (details in Table S1). The samplings were conducted in July, September and November 2014, representing summer and autumn seasons, when people most frequently visit these parks (approximately 20,000 people per day in each park). The sampling time of the day was approximately 10:00–14:00. Duplicate sampling sites in each lake were randomly selected (approximately three meters away from the shore) (18 total samples) (Fig. S1), and boats were used to reach the sampling sites. Without considering the lake depth or stratification, we focused mainly on the upper surface water, which was more related to pathogen exposure during recreational activities (*e.g.*, rowing, fishing). Samples were collected at a depth of approximately 15 cm below the surface using sterile bottles (12 L in total) and transported to the lab on ice within 6 h.

The water samples were initially filtered through a 5-mm nylon mesh to remove zooplanktons or large floating leaves. Depending on the suspended solid concentrations, 300 to 1000 mL of water was filtered using 5-μm-pore-size filters (SMWP04700, Merck Millipore, Country Cork, Ireland) followed by 0.22-μm-pore-size membranes (GSWP04700, Merck Millipore, Country Cork, Ireland) with a vacuum pumping system to ensure similar filtration rates. Currently, there are no standard protocols for the separation of PA from FL fractions. Filtration enables hierarchical and quantitative separation through membranes of a known pore size (Allgeier et al., 2005), and it has been widely used to separate bacterial communities into different fractions (Parveen et al., 2011; Rösel et al., 2012; Bižić-Ionescu et al., 2015). Different pore size filters (1–10 μm) have been used to separate PA bacteria (Liu et al., 2013), but there is still no consensus because the characteristics of suspended particles vary in different water systems. For example, Liu et al. (2013) used 1.2-μm filters to collect PA bacteria in drinking water distribution systems, and some studies (Mohit et al., 2014; D'ambrosio et al., 2014) introduced 3-μm membranes in marine/coastal systems. However, in most studies related to lakes/limnic systems (Parveen et al., 2011; Rösel et al., 2012; Bižić-Ionescu et al., 2015), PA bacteria were defined as fractions above 5 μm, based on the microscopic observations of various samples. Therefore, we selected the 5-μm filter to separate PA fractions in the present study. We further confirmed this size fractionation by particle size analysis (LS13320, Beckman, USA) (results ranged between 5 μm and 100 μm) and visual examination of PA/FL fractions using a scanning electron microscope (Quanta 200, FEI, USA) (data not shown). The filters were stored at −20 °C until DNA analysis.

### 2.2. Physicochemical analysis

Field parameters of electrical conductivity, pH and temperature were sampled on site. These parameters were determined using a