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ORIGINAL PAPER

Significance and suitability of *Aeromonas hydrophila* vs. fecal coliforms in assessing microbiological water quality

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Abstract We examined the significance and suitability of *Aeromonas hydrophila* versus fecal coliforms in assessing microbiological water quality. For this, we used the membrane filtration method to simultaneously estimate the abundance level of *A. hydrophila* and fecal coliforms in waters from the Mfoundi river watershed at Yaoundé, and compared how fluctuations in *A. hydrophila* abundance matched those observed with fecal coliforms index as an indicator of water quality in the system under study. Our results revealed that waters were not safe according to the standards for water quality established by the Word Health Organization (WHO). They also indicated the prevalence of *A. hydrophila* as compared to fecal coliforms, and suggested that water from the Mfoundi River and its tributaries could be classified as hypereutrophic based on the density of *Aeromonas*. Moreover, the spatial distribution of fecal coliforms and *A. hydrophila* exhibited similar trends within the different water bodies investigated, suggesting that *A. hydrophila* can be used as indicator of water quality in highly polluted waters. We concluded that waters from the Mfoundi River watershed at Yaoundé represent a great potential risk of infection for users, and foresee that the next challenge will be to determine, among other factors, the physico-chemical factors influencing the observed spatial distribution.

Keywords Fecal coliforms · *Aeromonas hydrophila* · River · Stream · Water quality

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Introduction

The primary goal of water quality management from a health perspective is to ensure that consumers are not exposed to levels of pathogens that are likely to cause disease. Among existing methods, the detection and enumeration of indicator bacteria is the basic microbiological technique used in water quality monitoring. The coliform group (fecal and total coliforms) of bacteria are used as the principal indicators of quality of water for domestic, industrial and other uses. However, the interpretation of these classical indicators is known to be limited (Guillaud et al. 1997; Medema et al. 1997; Springthorpe et al. 1997) and several authors have proposed supplementing the coliform index of water quality with *Aeromonas* levels (Seidler et al. 1980; Monfort and Baleux 1990, Miranda and Castillo 1996; Okpokwasili and Akujobi 1996).

Bacteria species of the genus *Aeromonas* are frequently isolated in aquatic environments including sewage, seawater and freshwater ecosystems. *Aeromonas* is considered as a secondary or opportunistic pathogen and special attention has been given to *Aeromonas* since it has been shown to be associated with human infections (Seidler et al. 1980; Daily et al. 1981). *Aeromonas* is now recognized as the primary cause of several gastrointestinal diseases, ranging from self-limiting diarrhea to acute, persistent dysentery (Janda and Abbott 1998). Microbiological based risks remain associated with many aspects of

water use, including domestic tasks, drinking water, reuse of treated wastewater for irrigation and recreational water contacts. Through all these aspects, individuals with underlying chronic diseases and immunosuppression are likely exposed to potential pathogens such as *A. hydrophila* and are at an increased risk for developing gastroenteritis, respiratory diseases, ear, eye and skin ailments.

During this study, conducted in the Mfoundi River watershed at Yaoundé, Cameroon, the abundance of *A. hydrophila* and fecal coliforms were simultaneously estimated using the membrane filtration method. In conjunction with examining the level of *A. hydrophila* in waters from the Mfoundi river watershed at Yaoundé, this study was performed to examine how fluctuations in *A. hydrophila* abundance correlated with those observed with fecal coliform as indicators of water quality in the system under study.

Materials and methods

Study site and sampling

The watershed under study is the Mfoundi River located in Yaoundé, the capital of Cameroon (Central Africa) (Fig. 1). The climate in the region is temperate sub-equatorial and termed "type Yaoundéen" (Suchel 1987), with four seasons of alternating rainy and dry. High rainfall (annual mean of 1576 mm) and low temperature variations over time (annual mean = 24 ± 2.5°C) are two other characteristics of the climate in the region.

The Mfoundi River watershed is the principal natural water network irrigating Yaoundé. Its catchment area is diversely populated and occupied by communal habitations as well as commercial and open space. There are limitations in the ability to monitor all areas within the entire watershed, so four representative tributaries were monitored closely during this study. These tributaries were: Abiergué, Ekozoa, Olézoa and Biyéme. The primary uses of water from the Mfoundi and its tributaries by the near shore population are multiple and include: laundry, car washing, bathing and watering of crops which are eaten raw. In certain parts, youth also use this stream for swimming. Considering all these factors, an overview of the microbiological quality of the Mfoundi river watershed appears to be a major public health issue, and to assess this quality, the river and its four representative tributaries were sampled.

Samples were collected biweekly over a period of 1 year, at three different points in the Mfoundi River and its four main tributaries named above. Sampling sites (or water bodies) were selected within the downtown area, since the risks to the near shore population may be high in this zone. For each river, an upstream (X_1), midstream (X_2) and downstream (X_3) sample was taken, except in Ekozoa where only upstream and midstream samples were taken (Fig. 1). Samples were manually collected in 250 mL sterile Pyrex bottles, immediately stored in a dark refrigerated box and transported to the laboratory for analyses. The delay between the sample collection and laboratory analyses was in all cases <6 h.

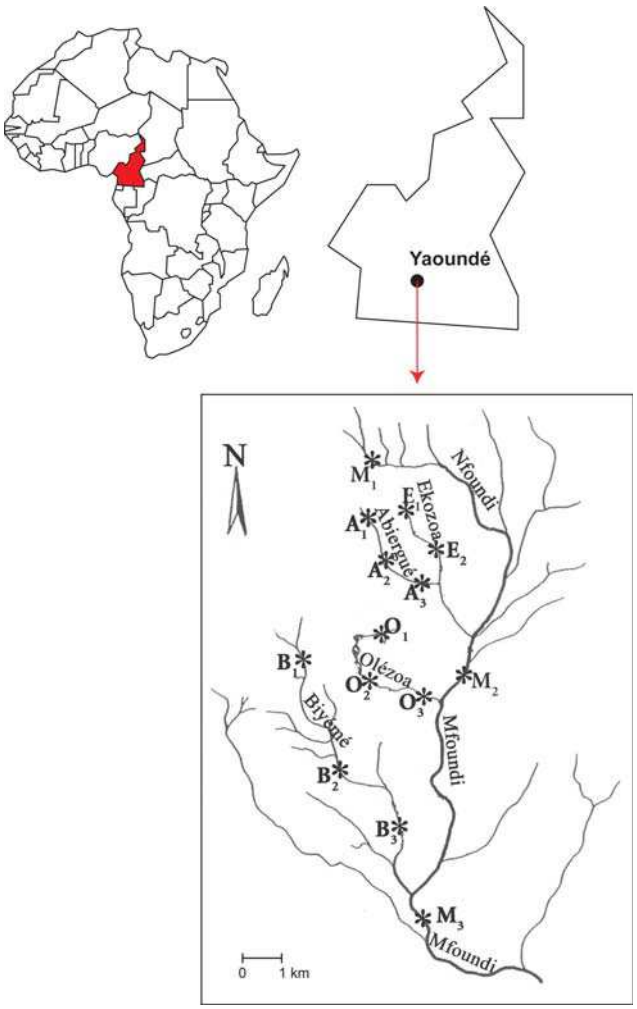

Fig. 1 Map of the Mfoundi river watershed and its geographical location at Yaoundé in Cameroon within the African continent. Sampling points (stars) are designated by the first letter (in capital) of the name of the sampling site, followed by subscript 1, 2 and 3 to indicate the upstream, midstream and downstream sampling points, respectively

Analytic and counting methods

In the laboratory, sub-samples from each station were analyzed for fecal coliforms (FC) and *Aeromonas hydrophila*. These bacteria were enumerated by the membrane filtration technique, utilizing sterile gridded cellulose filters of 0.45 μm nominal pore size (47 mm diameter) (APHA

1992). For each sample, funnels and supports for the vacuum filtration system were sterilized before use by autoclaving for 20 min at 120°C and were decontaminated between samples by flaming. Appropriate sample dilutions for each sampling site were performed in triplicate, depending on the bacterial concentrations. For this, 10 mL of a series of decimal dilutions in sterile Ringers solution were filtered using gridded membranes, which were then placed on a specific sterile medium contained in 55 mm diameter sterile petri dishes and incubated at an appropriated temperature in an inverse position. FC were grown on Endo agar (Biomerieux) at 44°C for 24 h, and only green metallic sheen colonies were counted and recorded as FC. Indeed, preliminary tests including Gram coloration and identification using API 20E commercial gallery (Bio-Mérieux), indicated that under our cultural conditions at 44°C, almost all the CFU that presented these physical aspects were FC. *A. hydrophila* was grown on Ampicillin, dextrin Agar Media (Havelaar et al. 1987) at 37°C. Suspect colonies of *A. hydrophila* on this media are yellow (threhalose positive). There were further examined for manitol fermentation by an "in situ" test. For that, the membrane with suspected colonies were transferred to the manitol medium and incubated again at 37°C for 2–3 h. Only the colonies remaining yellow (manitol positive) were scored positive. A blank was routinely examined to control for contamination of equipment and the stock media.

Statistics

Descriptive statistics such as box plots were used to present the central tendency and distribution of *A. hydrophila* and FC collected in different streams. Log_{10} transformations were applied to the bacterial concentration data in order to improve the homogeneity of variance. One-way analysis of variance was used to compare data points from the same stream and *t*-tests for FC and *A. hydrophila* means comparison within a given stream. JMPIN software (Sall et al. 2001) was used for data analysis.

Results and discussion

For all examined samples, counts of FC ranged from 4.00×10^0 to 3.10×10^7 CFU/100 mL, while those of *A. hydrophila* fluctuated between 3.00×10^0 and 4.80×10^8 CFU/100 mL with a mean ± SD ($1.28 \times 10^7 \pm 4.84 \times 10^6$ CFU/100 mL) about two fold higher than that of FC (mean ± SD of $6.77 \times 10^5 \pm 2.42 \times 10^4$ CFU/100 mL). These recorded values of FC and *A. hydrophila* fall within the range of those previously published for highly polluted rivers and streams that in most cases were subjected to wastewater discharge from sewage treatment systems (Monfort and Baleux 1991; Goñi-Urriza et al. 1999; Jugnia and Nsimé-Ngando 2001; Griesel and Jagals 2002). Thus, waters from the Mfoundi River watershed can presumably be considered unsuitable for their multiple primary uses by the near shore population. This corroborates the results of our previous study on the microbiological water quality of this system, as inferred by bacterial indicators of fecal contamination (Djuikom et al. 2006). Moreover, according to the water quality standard established for FC (<100 CFU/100 mL) by the Word Health Organization (WHO 1998) these waters are not safe for primary contact.

Overall mean values of *A. hydrophila* and FC fluctuated between sampling sites (Fig. 2). However, *A. hydrophila* proved to be quantitatively the most important as evidenced by their significantly higher overall mean values (*t*-test, $p < 0.05$) than those of FC in all of the different water bodies investigated (Fig. 2). This was somewhat not surprising since even though both fecal coliforms and *A. hydrophila* are ubiquitous in the environment (Austin et al. 1996), most FC decay as soon as they leave the host (guts of warmed-blooded animals) (Howell et al. 1996), in contrast to *A. hydrophila* that can multiply in aquatic systems under appropriate conditions (adequate temperature, nutrient availability, etc.) (Rippey and Cabelli 1979). This also explains why the water quality, with respect to FC varied largely, while with *A. hydrophila* the concentrations were more stable as evidenced by the variation in the concentrations of FC and *A. hydrophila* shown in Fig. 3. Indeed, from this representation, the distinguishing feature between FC and *A. hydrophila* densities is that FC had larger abundance fluctuations all year round than *A. hydrophila*.

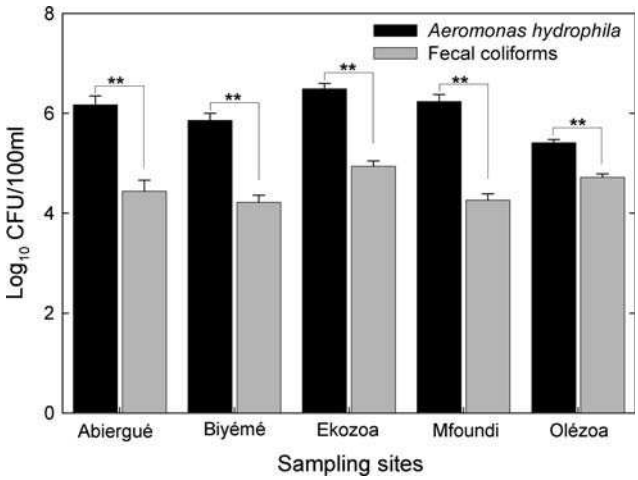

Fig. 2 Overall mean (±standard error) of *A. hydrophila* and fecal coliforms in the different sampling sites (water bodies) studied. Significant differences between FC and *A. hydrophila* for a given water body are also shown (**t-test = $p < 0.05$)

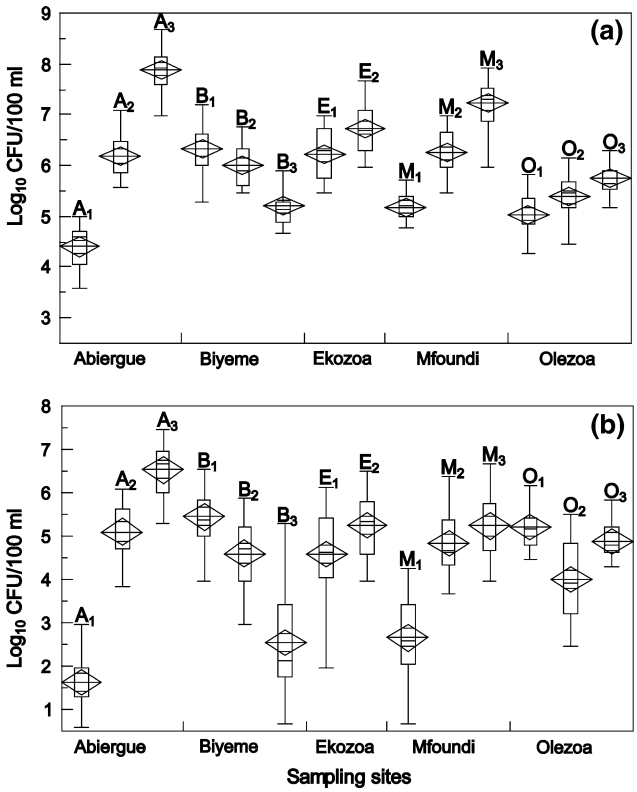

Fig. 3 Box and whisker plots of the spatial distribution at Yaoundé of (a) the level of *Aeromonas hydrophila* and (b) fecal coliforms at the different sampling points within each of the different sites (water bodies) examined. Data are presented as boxplots of first quartile (25th percentile), median value (50th percentile) and third quartile (75th percentile). Vertical bars on either side of the boxplots represent the 5th and 95th percentiles, while diamond are means diamonds with overlap marks. Two means diamonds are significantly different (ANOVA test, $p < 0.05$) when their overlap marks don't overlap

A high number of *A. hydrophila* have been found associated with sewage pollution, higher nutrient concentrations (Rippey and Cabelli 1985), and higher temperatures (Seidler et al. 1980). According to Araujo et al. (1989), *Aeromonas* tend to flourish in aquatic habitats of highly trophic nature, due to the presence of organic matter of various origins. It has been proposed for cold temperate aquatic systems that an evaluation of the degree of eutrophication be based on the density of *Aeromonas* (Table 1) (Rippey and Cabelli 1989; Rippey et al. 1994). This evaluation was modified by Canosa and Pinilla (1999) for tropical waters, following their study of four Columbian dams where densities of *Aeromonas* appeared 100 fold greater than those from water bodies in temperate zones (Table 1). *Aeromonas* densities recorded during this study were 100–10,000 fold greater than the above mentioned cold temperate systems and tropical waters, respectively. Accordingly, waters from the Mfoundi river watershed at Yaoundé could be assigned to the hypereutrophic level based on the density of *Aeromonas*. Furthermore, our

Table 1 Classification of the trophic state based on the densities of *Aeromonas hydrophila* 100 mL^{-1} (Canosa and Pinilla 1999)

Trophic state	(Rippey and Cabelli 1989) (CFU/100 mL)	(Canosa and Pinilla 1999) (CFU/100 mL)
Oligotrophic	<15	<1,500
Oligo-mesotrophic	15.1–65	1,510–6,500
Mesotrophic	65.1–325	6,510–32,500
Meso-eutrophic	326–575	32,600–57,500
Eutrophic	576–3400	57,600–340,000
Hypereutrophic	>3400	>340,000

observations indicate that: (1) *Aeromonas* densities in water are apparently greater in the tropics, presumably because of the more stable environmental conditions, particularly light and temperature as previously stated (Seidler et al. 1980; Payne 1986), (2) trophic levels based on the density of *Aeromonas* may vary from one region to another, most likely as a consequence of waste discharge from human activities.

Among the spatial distribution of the two groups of microorganisms considered, concentrations decreased significantly downwards between two consecutive sampling points in the Biyeme stream for both *A. hydrophila* and FC (Fig. 3a and b), and between O_1 and O_2 sampling points in the Olézoa stream for FC only (Fig. 3b). Otherwise, in all other cases, densities of the investigated bacteria generally increased significantly (ANOVA test, $p < 0.05$) downwards between two consecutive sampling points (X_1 # X_2 # X_3) (Fig. 3a and b). In view of this last trend for the different streams examined, one could suggest that, in addition to the stream flowing from a source and delivering a constant background supply of the bacteria to the mid and downstream sampling points, the stream receives additional bacterial loads from other sources. This has been previously suggested for bacterial indicators of fecal contamination in the Mfoundi River watershed (Djuikom et al. 2006). A similar observation has been reported from the Mingoa stream, which belongs to the Mfoundi River watershed (Jugnia and Nsimé-Ngando 2001), where cesspools, seepages and row waste from the sewage system were detected.

There is a high number of *Aeromonas* spp. in sewage, and it has been proposed that these bacteria could serve as pollution indicators in waters that receive human and industrial sewage (Seidler et al. 1980, Monfort and Baleux 1990, Miranda and Castillo 1996), and as hygienic indicators for potable water sources (Burke et al. 1984b, Kersters et al. 1995). Moreover, studies in mountain stream, ground and spring waters or in water samples that have been exposed to low levels of pollution found no correlations between aeromonads and fecal indicator organisms (Legnami et al. 1998; Hirotani et al. 1999; Chao

et al. 2003). To be useful in environmental monitoring as an indicator of water quality, it is essential that the bacterial group considered must consistently reflect water quality under a wide range of conditions. Our sampling points within the Mfoundi River watershed were chosen in order to sample waters from different origins, and the study lasted one year so as to cover different seasons. This provides our monitoring of *A. hydrophila* populations to meet this requirement. From the results, we observed a positive and significant ($p < 0.05$) correlation between the concentration of *A. hydrophila* and FC. An interpretation of this observation is that *A. hydrophila*'s quality as an indicator of pollution may be as good as FC, at least in our survey. More precisely, similar to FC, the distribution of *A. hydrophila* did reflect changes in water quality throughout the Mfoundi River watershed, despite the fact that the water quality with respect to FC widely varied, and *A. hydrophila* concentrations were higher and more stable. This conclusion is in accordance with results reported by Monfort and Baleux (1990) who found a correlation between *Aeromonas* spp. and water quality, and others authors who have reported similar behavior for the two bacterial groups (Seidler et al. 1980; Burke et al. 1984a; Monfort and Baleux 1991).

In conclusion, the spatial distribution of FC and *A. hydrophila* exhibited, in most cases, similar trends within the different water bodies investigated, with the concentrations of the latter being more stable and at higher levels. Besides the fact that there is a great potential risk of infection for users of water from the Mfoundi River and its tributaries at Yaoundé, we believe that in light of our results, *A. hydrophila* can be used as an indicator of water quality in highly polluted waters. The next challenge will be to determine, among other parameters, the physico-chemical factors affecting the observed spatial distribution. Moreover, following the study by Goñi-Urriza et al. (1999), the extent to which the presence and density of *A. hydrophila* may be used as an indicator of river pollution, and whether the represent a health hazard to the users of these waters, are questions to be addressed by further microbiological investigation and epidemiological surveys.

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