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Application of real-time PCR to *Pseudomonas aeruginosa* monitoring in a public swimming pool ☆

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ABSTRACT

Pseudomonas aeruginosa (*Pa*) is an opportunistic pathogen that can be found in recreational water, often in form of biofilm, thus more resistant to disinfection procedures. Microbiological testing for *Pa* is included in monitoring practices recommended by WHO to swimming pool managers as internal control. Since a persistent contamination with low levels of *Pa* was found in a local indoor swimming pool, the efficacy of a method in real-time PCR was evaluated, in comparison with the reference method UNI EN ISO 16266:2008, to promptly locate the contamination site within the water treatment system and proceed with disinfection. Results indicated the higher sensitivity of the molecular detection, able to give definite results with a consistent reduction of analysis time (2 days vs 4–5 of the reference method). In conclusion, real-time PCR can be a useful, rapid and sensitive tool, for the control of microbiological risk in public swimming pool.

1. Introduction

Despite the well recognized health benefits associated with the attendance of swimming pools, the possible presence of pathogenic microorganisms in recreational water may constitute a public health risk. Therefore, monitoring and management practices have been recommended by WHO, intended to be used as a basis for the development of international and national approaches for the control of hazards, to ensure water safety to pool users [1,2], that have been converted into locally appropriate standards. Other Agencies, such as the South Australia Environmental and Public Health Service [3] and the United Kingdom Health Protection Agency [4] have established standards for routine monitoring of public and semi-public pools and hot tubs for microbial parameters.

In the US, the Centers for Disease Control and Prevention (CDC) is working about safety and prevention in recreational water environments, also developing recommendations for pool inspectors to optimize the effectiveness of the recreational water illnesses prevention efforts. Since pool codes are created by local public health agencies, there are no uniform national standards. However, the Model Aquatic Health Code (MAHC) of CDC's Healthy Swimming program is a resource providing uniform guidelines [5].

The present study has been carried out in Italy, where the responsibility is shared between local sanitary authorities (the so-called "official control") and swimming pool managers, who should apply self-monitoring programmes ("internal control"), including microbiological testing [6] in compliance with EU Regulations [7].

Pseudomonas aeruginosa is an opportunistic species that can be commonly found in recreational water [8,9], also accumulating in form of biofilm and therefore becoming more resistant to disinfection procedures. These bacteria may be responsible for otitis, conjunctivitis and pneumonia and are particularly harmful to susceptible population subgroups, including people with reduced immune function (immunocompromised people, pregnant women, the elderly, disabled people, cystic fibrosis patients). Microbiological criteria for *P. aeruginosa* provide for acceptability limits of ≤1 cfu (colony-forming units) per 100 ml of pool water and 0 cfu per 100 ml of inlet water [7].

P. aeruginosa detection is usually carried out through culture-based protocols, such as the reference analytical method UNI EN ISO 16266:2008 (18/09/2008 – Water quality – Detection and enumeration of *P. aeruginosa* – Method by membrane filtration) or APHA [10]. However, the main drawbacks associated with this kind of methods rely on time needed for analysis (at least 48 h for the isolation of suspected positive colonies and additional time for confirmation tests) and their inability to detect viable but non-culturable (VBNC) forms. Therefore, some culture-independent approaches in PCR and real-time PCR have been recently proposed for the detection of *P. aeruginosa* [11] or other microorganisms and parasites [12–17] in water. The molecular method previously optimised by our research group [11] takes advantage of a reduction of analysis time while maintaining sensitivity and specificity at very high levels and offering

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the additional capability of VBNC detection. Furthermore, it showed statistical substantial agreement with the reference method UNI EN ISO 16266:2008.

Hence, since a persistent contamination with low levels of *P. aeruginosa* was found in a local indoor swimming pool, this protocol has been used, along with the cited reference method, with the aim to locate the contamination site within the water treatment system and proceed with further disinfection practises.

2. Materials and methods

2.1. Water samplings

Water samples (1.5 L) were collected from various potential critical points of the entire circulation system (water supply, inlet piping, pool water near the drain grid, water from pool, pre-filters 1 and 2, sand filters 1 and 2) (see Fig. 1 for a schematic representation of the circulation system with sampling sites), for a total of 24 samples, resulting from three monthly samplings (sampling no. 1: 26th January 2011; no. 2: 23rd February 2011; no. 3: 28th March 2011) from each of the 8 sampling sites. Water was taken in sterile bottles containing 10% sodium thiosulfate. Water temperature, pH, free and total chlorine were recorded at the moment of sampling, to assess their compliance with national guidelines [6].

2.2. Detection of Pseudomonas aeruginosa by culture method

P. aeruginosa detection and characterization was carried out by the standardized procedure UNI EN ISO 16266:2008: 100 ml water from each sampling site was filtered through a cellulose ester membrane (0.45 μm porosity, 47 mm diameter; Millipore, Billerica, MA, USA). Filters were then placed on Pseudomonas Agar Base with Pseudomonas CN Selective Supplement (Oxoid, Basingstoke, UK) and 1% glycerol (PAB–CN), and incubated at 36 ± 2 °C for 44 ± 4 h. Suspected positive colonies were confirmed both biochemically, in accordance with the reference method, and in PCR, by amplification of a 528 bp sequence of the ecfX gene (GenBank Accession No AE004091) (see oligonucleotides ECF1–ECF2 in Table 1) [15].

2.3. Detection of Pseudomonas aeruginosa by real-time PCR

One-L water samples from the same sampling units were also analysed by using a previously described molecular protocol [11]. Briefly, after filtration, membranes were grown for 6 h in 20 ml of Pseudomonas Selective Broth (Biolife, Milan, Italy) supplemented with 1% glycerol at

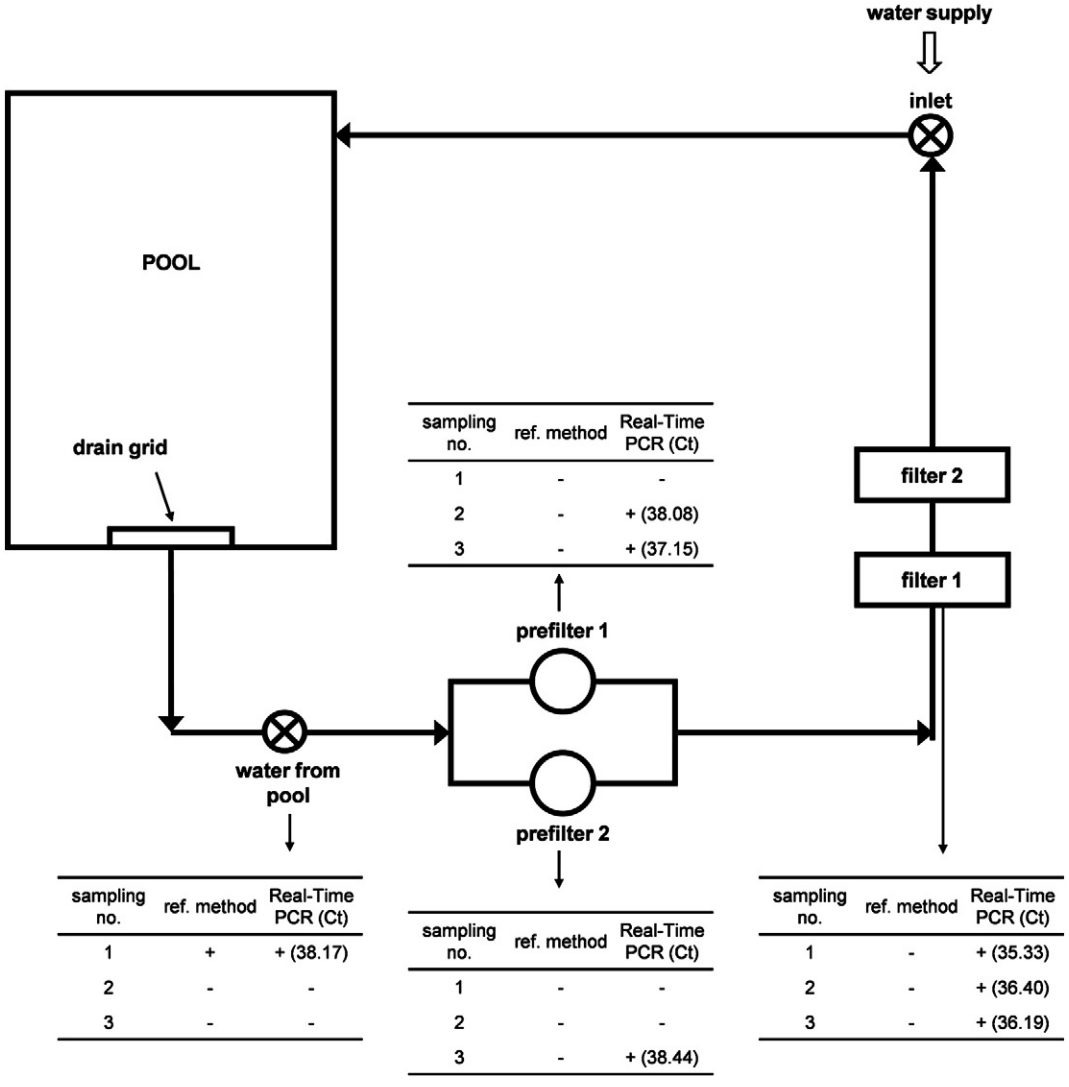

Fig. 1. Schematic representation of the circulation system with sampling sites. A comparison of results obtained with the reference method and the real-time PCR in each sampling is shown, except for samples that always tested negative (Ct, threshold cycle).