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Development of the laboratory prototype "CavyPool" for assessing treatments and materials for swimming pools

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Parole chiave: Igiene delle piscine, sorveglianza, disinfezione, prototipo, biofilm

Abstract

Background. *Hygiene and surveillance in swimming pools are established by WHO Guidelines and national laws. Progress in water management and pool construction is revolutionizing the field, introducing new materials, systems, disinfection procedures or monitoring markers. Innovation advances challenge the upgrading of safety and quality in pools and the appropriate implementation of guidelines.*
Study design. *In order to provide a device for laboratory test, a prototype was realized and applied to study and compare swimming pool materials and treatments.*
Methods. *A pool scale-model was engineered and evaluated by computational fluid dynamics algorithms. An automated real time monitoring assured steady state. Critical control points along the water circuit were made accessible to allow the placing of different biocides or water sampling. Simulations were safely performed in a standard hood. Materials for pool surfaces and pipelines were evaluated for biofilm formation under different disinfection conditions. Adherent microorganisms were assayed by mfDNA analysis using real time PCR.*
Results. *The prototype reached the steady state within 5-25 hours under different conditions, showing chemical, physical and fluid-dynamic stability. A method was optimized for testing materials showing their different response to biofilm induction. Several innovative PVC samples displayed highest resistance to bacterial adhesion.*
Conclusions. *A device and method was developed for testing swimming pool hygienic parameters in laboratory. It allowed to test materials for pools hygiene and maintenance, including biofilm formation. It can be applied to simulate contaminations under different water treatments or disinfection strategies. It may support technical decisions and help policymakers in acquiring evidences for comparing or validating innovative solutions.*

Introduction

Hygiene measures in swimming pools have been clearly underlined by the WHO Guidelines (1). The classification of health risks and the development of strategies for their prevention played a pivotal role in extending safe access to swimming practice for the population of any age, impacting on health promotion as well as on technology and water sciences (2, 3). In the wake of public health progress, swimming pool

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construction and management had a powerful evolution, affecting lifestyles and being itself influenced by social issues (4). Even if archaeologists described a pool dating 3,000 BC in the Indus valley or *Gaius Maecenas* (68 BC-8 BC) already constructed a heated pool - as reported by *Cassius Dio*, it was only during the last century that swimming pools developed into the current settings, as evidenced by different examples such as the "Foro Italico" complex realized in Rome in the 1930s with selected materials and an impressive facility for water treatment.

Nowadays, innovation is continuously improving the swimming settings, showing the positive impact of technologies on safety and quality of water and environments, both indoor and outdoor. However, since progress in this field is so rapidly evolving, the public health authorities have to face additional challenges in evaluating new disinfection, monitoring or treatment strategies and, eventually, allow their official introduction under safe conditions (5-7). Since no specific European legislation, related to swimming pools, spas and similar environments is available, several countries developed different regulations and preventive strategies (8-10). In Italy, the current law is presently under revision, and several indicators are questioned or proposed *de novo* (11, 12). This updating of guidelines, surveillance indicators or treatment methods is usually questioned based on data available from the scientific literature or in field studies while an intermediate laboratory-step for testing specific alternatives is lacking. The availability of a scale model can allow evaluations by stressing different conditions and simulating contaminations or outbreak risks under controlled variables. Moreover, this approach would allow simultaneous comparison of different technological alternatives or provide additional information for validation before their approval. Otherwise, in clinical trials, several tests *in vitro* as well as *in vivo* - on small animals, e.g. mice or guinea pigs - can be scheduled to provide the Health Authority with preliminary evidences to support approval or rejection (13). Laboratory models are currently used by the pharmaceutical companies to identify priorities before implementing or commercializing a new drug (14). Some swimming pool models or laboratory systems are already available and could perform several kinds of manufacturing tests, but they are often too large for public health laboratories, and/or too specific or inappropriate for routine applications (15, 16). Furthermore, progress in water management and pool construction is evolving rapidly, introducing new materials, systems, disinfection procedures or monitoring markers. Innovation challenges guidelines and imposes new solutions for comparison, validation or implementation of technological alternatives, before the large-scale deployment. A handy laboratory model can be useful to test disinfectants, perform simulations with pathogens or pre-testing effectiveness and feasibility of hygiene procedures, under safe and controlled conditions. Moreover, its application in preliminary tests can support the updating of industrial parameters, e.g. the Italian UNI 10339 or UNI 10637 regarding swimming pool requirements or other indications provided by national standardization bodies and the Council on European standardization (17). In particular, the UNI 10637 covers the requirements of circulation systems, filtration, disinfection and chemical treatment of the pool water, specifying design requirements, construction and operation of water treatment plants, and providing guidance concerning the procedures to ensure adequate water quality for bathing (17). The rule, which dates back to 1997, already underwent several revisions, most recently in 2015, updating the standard to new technologies with regard to the engineering component. An important area on the cutting edge involves traditional as well as innovative materials used in swimming pools, assessing their safety and

resistance in water to chemicals or biofilm formation.

Natural or synthetic materials are both used in the construction of a swimming pool environment. Evaluating their properties in presence of specific agents - e.g. chlorine, anti-algae - or specific pathogens - e.g. *Pseudomonaceae* or *Legionellaceae* - can provide evaluable information for guaranteeing consumers, producers and final users. Several materials are indeed exploited to realize components, such as filters, pipelines, compensation tanks or overflow channels. Besides, the internal coating of the pool plays a key role in ensuring the necessary safety requirements, including adequate resistance to mechanical or chemical hints, in addition to all the necessary skid-proof properties. Surfaces in contact with the water can be coated with several products such as polyvinyl chloride (PVC), ceramic, stainless steel (18). A major concern is related to the capability of bacteria to adhere to the surface and induce biofilm formation. Different materials show a different response to bacterial adhesion. Evaluation of this property in pools requires knowledge of operating conditions including the hydraulic requirements (e.g. flow, pressure), aggressiveness of water (e.g. pH, alkalinity), temperature, pipe materials, disinfection and treatment conditions, response to different microorganisms (19). In order to provide a new tool for laboratory test, the prototype "Cavy-Pool" was realized and its stability at the steady state was tested. It was then applied to compare materials for swimming pool, showing differences on response to bacterial adherence.

Materials and methods

System design, experimental set-up

System design considered the functional scaling of an Olympic Pool, as defined by the Fédération Internationale de Natation (FINA) and standards rules (17, 20), considering the fluid dynamics. The project scheme focused on the identification of critical points along the recirculation system, to allow an open structure for testing - in series and/or in parallel - different tools. These include: materials or plant components, filtration, disinfection or other treatments, electronic devices for real time monitoring and automated management of the prototype pool itself (Figure 1A) (21). Standard issues for environment and operator safety were considered (22). Compact dimensions (below: 86 cm x 55 cm x 78 cm) were adopted to allow its simple location in any public health laboratory and/or under a chemical or microbiological hood. The design, development and construction of a laboratory prototype to simulate critical points was performed in collaboration with the R&D departments of A&T Europe S.p.A. and the MDD University Spin Off (Italy), that identified components, provided their assembly and preliminary test. CFD (Computational Fluid Dynamics) and the dye test were performed to evaluate the water circulation system and mimic the scaled conditions for water velocity in an Olympic swimming pool (on average about 0.1 m/s). The dye test was executed, based upon UNI 10637 and EN 15288-2 (Figure 2a and b). The system was tested in independent laboratories and located in a hood for assuring safety when performing contamination experiments. Following the requirements for a public swimming pool, it was supplied (40 L) with potable water from mains (loading range: 25-50 L). Settings followed current Italian law values (11): Cl_2 (0.7-1.5 mg/L Cl_2), pH (6.5-7.5), T (24-30°C). In particular, experiments on materials were performed using a "Cavy-Pool" in a sand-filter configuration, with Chlorine as disinfectant ($Ca(OCl)_2$: CAS n. 7778-543; CE 231-908-7; UN2880), free Chlorine levels 0.3-1.5 mg/L at pH 7.3; pool water temperature: 25 °C, pH correctors: $NaHSO_4$-H_2O (CAS n. 7681-38-1; CE 231-665-7) and

Na_2CO_3 (CAS n. 497-19-8; CE 207-838-8). No flocculants or anti-algae were used. After starting, the system main parameters were monitored to verify the equilibrium. Once reached the steady state, the exposure experiments started. Monitoring was carried out in real time by the prototype sensors (Aquasafe multi, Gloobe) and manually by water resistant thermometer (Blubios, Italy). Cl_2 and pH were also periodically verified by manual kit (Pooltester pH-Cl, Lovibond Water Testing, Germany). Water replacement due to evaporation was surveilled following a level indicator, about 1-2 l every 24-72 h based on water temperature and environment microclimate.

Testing of pool materials

A dedicated tool (Figure 1A, n. 2) housed the samples (50x20 mm slides) within the water, allowing simultaneous exposure under similar fluid-dynamic conditions (Figure 2). Tested materials (M) include: austenitic stainless steel AISI grade 316 2B finish (M1), Glossy glazed porcelain tile (M2), acrylonitrile butadiene styrene (M3), plasticized polyvinyl chloride (PVC-P) membrane with biocides treatment (M4), hard PVC Myrtha-technology hot rolled on stainless steel in two different coatings (M5 and M6), plasticized PVC-P membrane with acrylic surface (M7), another different plasticized PVC-P membrane (M8), anti-slip resin (M9). The capability to resist to biofilm formation was evaluated using two traditional indicators for swimming pool waters: the gram-negative *Pseudomonas aeruginosa* and the gram-positive *Enterococcus faecalis*. Experiments used strain *P. aeruginosa* ATCC 9027 and *E. faecalis* ATCC 7080, respectively. A 1% culture (master culture 10^9 CFU/mL) was inoculated directly on the material surface (100 µL), loading about 10^6 cells on a 25 mm^2 area. Before exposing materials in the water of the prototype (*dynamic exposure*), each material was left to incubate at 37°C for 24 hours to allow the biofilm induction (*static exposure*) (23, 24). Additional experiments were performed inducing the biofilm for longer periods ranging from 1 to 14 days. During exposure, main parameters were continuously monitored as described. Before and after the exposure experiments, microbiological analysis following standard procedures was performed for all the microbiological indicators, by applying standard protocols (25-29) and by molecular methods (MicroPool Kit, GeckoBiotech, Italy) to monitor microbiological indicators and *Legionella* during exposure (6, 30, 31). After each exposure experiment, the Cavy-Pool was sanitized by replacing the water, cleaning and disinfection by hyperchlorination. After each experiment the prototype was sanitized by hyperchlorination, emptied, cleaned and rinsed with deionized water.

Molecular analysis of biofilm

The bacterial adhesion to each material was evaluated at time zero (t_0) before exposure and after 24 hours (t_{24}) of exposure in the prototype pool water. To evaluate the persistence of the biofilm on each different material, the DNA was purified using the GenElute Bacterial Genomic DNA Kit (Sigma–Aldrich, St. Louis, MO, United States) according to the Gram-Positive Bacterial protocol with minor modifications. Briefly, 200 µL of Lysozyme Solution were added directly on each material surface and incubated at 37°C for 30 min. Afterwards, according to kit manufacturer, 200 µL of Lysis Solution C and 20 µL of Proteinase K were added and the digested solution was transferred in a 1.5 mL tube and then incubated at 55°C for 10 minutes. Finally, DNA elution was performed in 60 µL of Elution Solution (10 mM Tris–HCl, 0.5 mM EDTA, pH 9.0). Real-time PCR was carried out according to Valeriani et al. (27, 28), using forward and reverse primers and probe specific for *E. faecalis* and *P.*

aeruginosa. Cycle thresholds (CT) where used as indicator of Genomic Units (GU), and the difference between C_T (exposed sample t_{24}) and C_T (control t_0) was measured to further asses the residual DNA (ΔC_T), as previously described (32).

SEM microscopy

Small blocks (<1 cm^2 coupons) were cut from selected materials (n=6) for microscopic analysis of surface structure by scanning electron microscopy (LEO1430VP, EDX detector, Roentec, Germany). The materials were dried in a vacuum evaporator and sprayed with chromium in a POLARON SC7620 sputter coater (Quorum Technologies, England). Three selected microscopic fields (20, 100, 270 μm) on the surface of each coupon were analyzed.

Results

A device and a method for testing materials in water within a microbiological contaminated environment were developed (Figure 1) and applied to samples (n=9) of different products used for swimming pools (Table 1). The Cavy-Pool scale model was successfully set up in a standard conformation using sand filtration and Chlorine as disinfectant, as confirmed in independent and inter-laboratory trials. The system was stabilized within 7-24 hours after setting the parameters in accordance with main international standards and current Italian law for swimming pools: Cl2 (0.7-1.5 mg/L Cl2), pH (6.5-7.5), T (24-30°C) (1, 11). The Cavy-Pool remained stable assuring the steady state for at least 50 days (Figure 3).

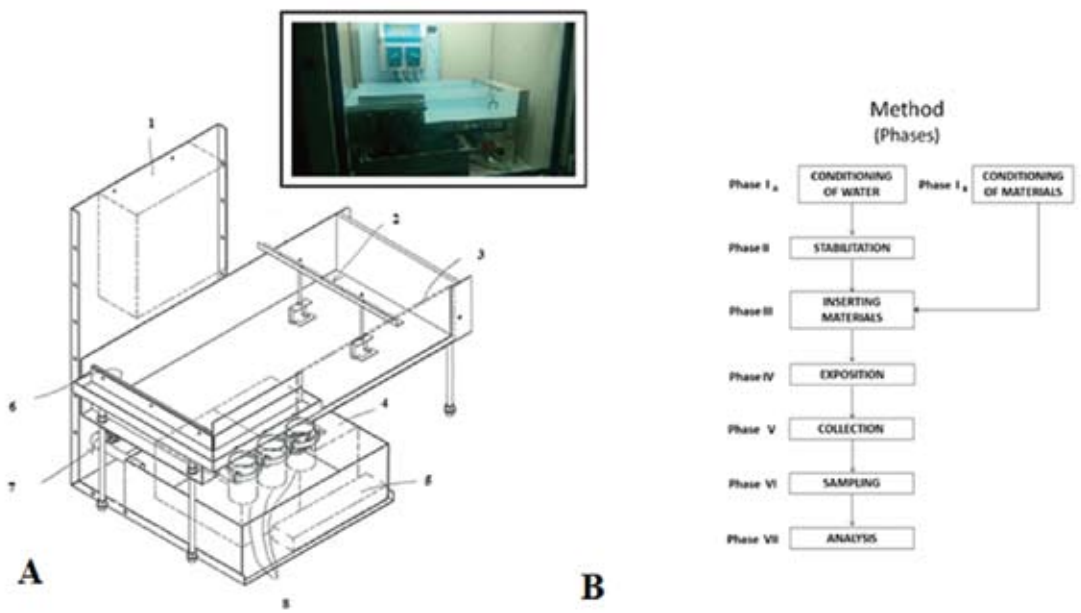

Figure 1 - **A: schematic structure of prototype**. 1) electronic control unit for parameter surveillance (pH, redox potential, other probes); 2) shelf and removable holder for sample exposure; 3) transparent methacrylate panel for inspection and water level, 4) compensation tank, 5) heater system; 6) overflow channel; 7) pipeline system; 8) filters and treatment drawers. **B: Flow chart of method phases**. Phases I-VII. I: conditioning of chemical and physical parameters of pool water (I_A) and preparation of materials for biofilm inoculum and maturation (I_B); II: stabilization of pool water to the steady state; III: location of materials in the dedicated holder; IV: dynamic exposure in recirculating water under controlled conditions; V: collection of samples from holders; VI: sampling by swab and classical microbiological tests or by direct DNA extraction; VII: detection of Colony Forming Units by culture or of Genomic Units by real time PCR.

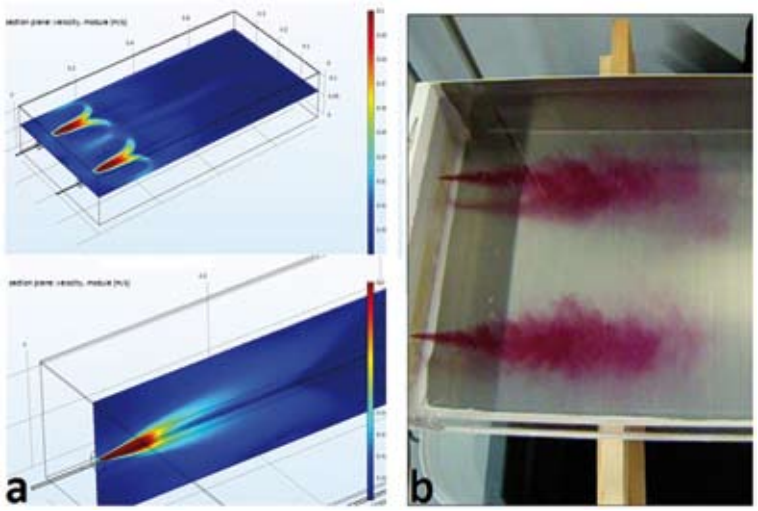

Figure 2 - **A)** Simulation tests of fluid dynamic and **B)** Exemplificative dye test, based upon UNI 10637 and EN 15288-2.

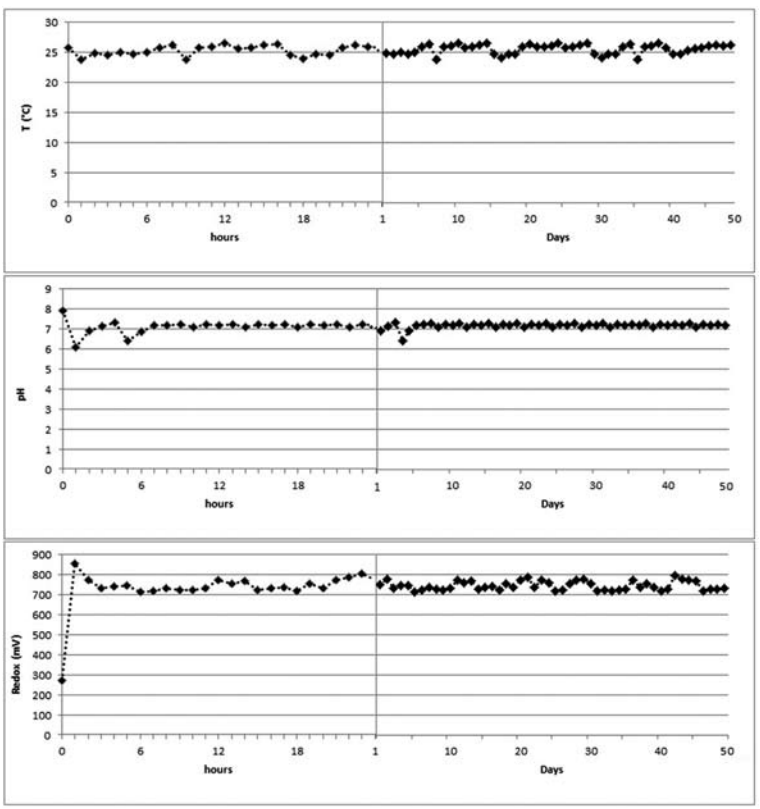

Figure 3 - Monitoring start up and steady state. The diagram shows the trend of Temperature, Redox, pH. The system was filled with mains water and settings followed standard conditions under Chlorine disinfection (Italia, 2003). Stability was reached in few hours (<10h) and maintained for at least a month.

Table 1 - Biofilm formation on several materials. Molecular analysis of residual bacterial DNA was measured in Cycle Threshold (C_T). (M1): austenitic stainless steel AISI grade 316 2B finish; (M2): glossy glazed porcelain tile; (M3): acrylonitrile butadiene styrene; (M4): plasticized poly-vinyl chloride membrane with biocide; (M5) and (M6): hard PVC Myrtha-technology hot rolled on stainless steel; plasticized PVC-P membrane with acrylic surface (M7), another different plasticized PVC-P membrane (M8), anti-slip resin (M9). Mean value from experiments in triplicate.

Materials	t_0 (C_T)	t_{24} (C_T)
M1	25.12	38.665
M2	19.04	38.47
M3	21.9	35.33
M4	20.93	39.8
M5	23.69	38.295
M6	23.69	39.7
M7	20.48	28.03
M8	22.35	39.2
M9	19.65	33.74

Once the system reached the equilibrium at the programmed settings, exposures were performed using different materials (Table 1) and applying the method summarized in Figure 1B. The capability to resist to biofilm formation was tested using two indicators included in most regulations for swimming pool surveillance: an environmental gram negative *P. aeruginosa* and a mesophilic gram positive *E. faecalis*. To assess residual adherent microorganisms a molecular method was used to identify and quantify bacterial genomes by real time PCR, allowing identification of all attached cells: viable, dead and viable but not cultivable (data not shown) (6)Interestingly, a different and consistent response was observed when samples from various materials were exposed simultaneously in the prototype under the same disinfection conditions (Figure 4 and 5). In particular, some PVC materials (M4 and M6) showed the best resistance to biofilm formation under the experimental settings when using *P. aeruginosa* (ΔC_T>20) and materials M4, M5, M6 when using *E. faecalis* (ΔC_T>10). Therefore, we repeated the experiments on selected samples, focusing on *P. aeruginosa* and setting the prototype at lower disinfection levels: below the law-reported threshold (<0.7 mg/L and <0.3 mg/L). Exposures confirmed the initial observation with the highest ΔC_T values for M4 and M6, even at the lowest disinfection levels: ΔC_T >5 at Cl_2 <0.3 mg/L (Table 2). An additional analysis by electron microscopy on

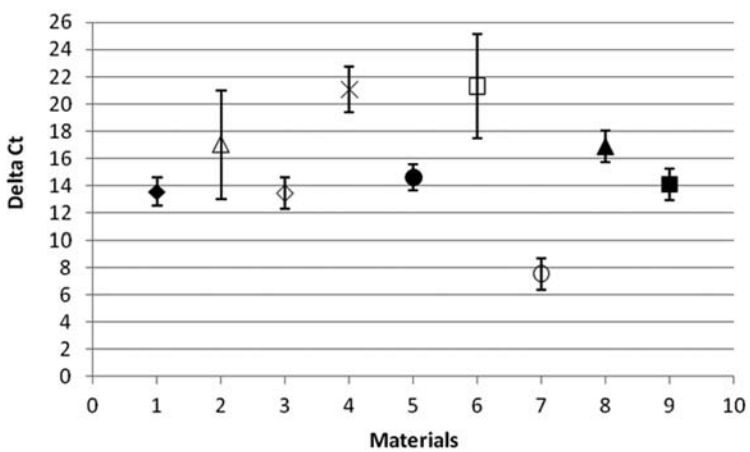

Figure 4 - Persistence of *P. aeruginosa* in several materials after water exposition in the prototype. Delta C_T is the difference between C_T (exposed sample t24) and C_T (control t0).

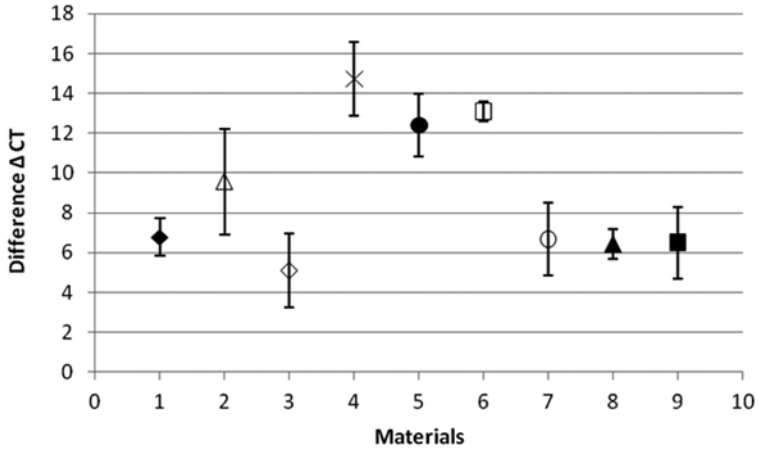

Figure 5 - Persistence of *E. faecalis* in several materials after water exposition in the prototype. Delta C_T is difference between C_T (exposed sample t_{24}) and C_T (control t_0).

the surface of these materials did not show a surface smoothness so elevated to justify a low physical entrapment mechanism, suggesting a specific role related to the properties of the materials in the pool water.

Discussion

Swimming pools represent an extraordinary opportunity to promote health and physical activity for individuals of different ages, even with disabilities (33, 34). Swimming and other water activities, indeed, play a fundamental role in all the phases of prevention: from contrasting sedentary life styles in healthy people, up to support rehabilitation in conditions such as ictus or polytrauma. Sport or adapted physical activity in swimming pools involves a heterogeneous and large population, including childhood, the elderly, pregnant women or subjects at risk like chronic patients or individuals with secondary immunodeficiency (35-37). Even if mandatory duties are already available and regulations are well established for

Table 2 - Biofilm formation on several materials. Raw data at different Chlorine levels. Molecular analysis of residual bacterial DNA measured in Cycle Threshold. Mean value from experiments in triplicate performed with P. aeruginosa. *n.d: below detection limit.

Materials	Chlorine (0.7 ÷ 1.5 mg/l)		Chlorine (<0.7 mg/L)		Chlorine (<0.3 mg/L)	
	Time 0 h (C_T)	Time 24h (C_T)	Time 0 h (C_T)	Time 24 h (C_T)	Time 0 h (C_T)	Time 24h (C_T)
M1	25.12	38.665	23.09	26	23.09	24.20
M2	19.04	38.47	21.23	28.9	21.13	23.03
M3	21.9	35.33	22	21.59	22.2	19.81
M4	20.93	n.d*	23.72	29	23.72	28.5
M5	23.69	38.295	22	23	21.5	23.14
M6	23.69	n.d*	21.03	25	21.03	27.64

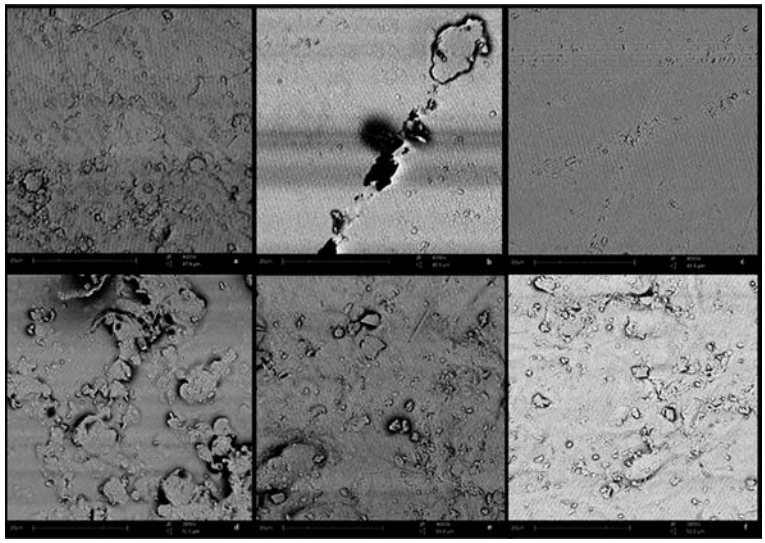

Figure 6 - The spatial structure of deposits on the surface of selected materials: austenitic stainless steel AISI grade 316 2B finish (a), Glossy glazed porcelain tile (b), acrylonitrile butadiene styrene (c), plasticized poly-vinyl chloride (PVC-P) membrane (d), hard PVC Myrtha-technology hot rolled on stainless steel in two different coatings (e, f).

assuring hygiene in recreational waters, the diffusion of swimming pools and their potentialities for health promotion impose highest hygiene levels to assure benefits at the lowest risk. The current revision of the Italian public health regulation on swimming pool construction and management intends to improve effectiveness and appropriateness of hygiene measures, also considering the lack of a European directive in the field (38). Moreover, swimming pool safety is influenced by additional social factors related to architectural and aesthetic aspects, novel engineering solutions, technical innovation in equipment, materials or water treatments (1-3). In the last decades, the growing number of users and the wide diffusion of swimming pools imposed an advancement in safety management as well as an update in monitoring and surveillance. The engineering of waters for recreational uses is not a primary need for a society and the history of pools runs alongside the main civilization processes (4). For this additional reason, the benefits cannot be reduced by avoidable accidents or preventable health risks and safety has to be enforced by hygiene regulations based on scientific evidences. WHO and international networks such as ICSPS provide a continuous support to the development of guidelines for swimming pools and recreational waters (2, 7). However, technological progress in the field is very fast so that public health authorities, researchers, producers as well as all the involved stakeholders need a continuous updating and tools or verifying effectiveness of innovative solutions.

Here, we report the development and application of a laboratory model to test materials or water treatments in a polluted or microbiologically contaminated water environment (Figure 1). The prototype model CavyPool was designed as a scaled system to perform “in vivo” experiments. Fluid-dynamics was studied, confirming constant and stable conditions (Figure 2). We stressed the system to evaluate its capability to reach and maintain a steady state, considering all the basic parameters and controls used

for a classical swimming pool. It revealed successful and easily usable also within a laboratory hood where to perform specific contaminations, assuring safe conditions for operators and work environment. The system appeared easy to use and stable for months, reaching an acceptable equilibrium after a few hours (Figure 3). It can be applied to test different parameters or processes, but here we report its application to the evaluation of some swimming pool materials for their resistance to bacterial adherence. The exposure method revealed effective due to the availability of a specific device to hold in parallel blocks, under identical conditions (Figure 1A). For the detection method, we adopted a molecular biology approach based on real time PCR, using the C_T values as a parameter associable to the number of genomic units, as previously described (30-32). This approach allowed us to measure the adherent cells with high sensitivity appearing fast and highly reproducible. A different behavior in response to bacterial adherence was consistently observed for all the tested materials, showing a good performance for innovative products based on PVC (Figure 4 and 5). Following this approach, the residual microbial DNA represented an effective candidate marker to detect biofilm traces, including dead cells as well as those viable but not cultivable (6, 40). In these studies, indeed, the most relevant parameter is the quantity of microbial material that can adhere to the surface more than microbial cultivability in a medium. So, we considered a model to measure bacterial adherence under known conditions for fluid-dynamics, temperature, redox values. Observed results were in agreement with data from real swimming pools and there was not the need to overextend the experiments on a longer period. We repeated the experiment under different disinfection conditions, further confirming the observed data (Table 2) and showing a best response for innovative products such as M4 and M6. Even if it was not possible to acquire information on the electrostatic, hydrophobic or other physical/chemical properties of the studied materials, we could exclude a role of surface roughness by adding an evaluation through Electron Microscopy (Figure 6). The whole of these results suggests that the observed differences may be due to the intrinsic properties of the materials in contact with the water, rather than surface discontinuities entrapping the bacteria. The same approach can be easily applied to other materials under different disinfection conditions using other bacteria or even a mixture of microorganisms mimicking a specific biofilm. Presently, we are testing environmental microflora from swimming pools by adapting the described exposure/detection method to an mfDNA analysis by next generation sequencing. Preliminary data are promising and further support the extension of this application in this and other fields Several other perspectives are open and feasible through the scaled prototype. Interestingly, also education. It was possible to mimic in the laboratory the management of a standard pool, applying the internal and external controls reported in the Italian law (38). It revealed useful for training students from different courses and operators in the field. By periodic sampling of both the pool-thank water and the recirculating-inlet water, we reproduced in the laboratory the procedures for monitoring chlorine, pH, microbial indicators and the automatized monitoring systems. This approach revealed very effective also for education of personnel involved in pool surveillance. Beside the classical issues related to a chlorinated swimming pool, a further potential application of the CavyPool is for those pools filled with different waters such as sea water or thermal waters, allowing a personalized approach to the management of a specific pool plant.

In conclusion, a device and method was developed for evaluating swimming pool hygiene in a public health laboratory. It can

allow pre-testing on a small scale, providing useful technical information to health authorities, producers or consumers. It can be applied to pre-test water treatments, simulate contaminations or stressing conditions, evaluate procedures, tools or materials. Moreover, it can support education in the field and training of pool operators in surveillance. The CavyPool prototype opened new perspectives in personalizing the hygiene management of a not-chloride pool as those filled with natural thermal waters. The whole of the observed results underlines further different applications. This approach suggests promising potentials to support technical decisions or policy makers in acquiring evidences for comparison or validation of innovative solutions for swimming pool hygiene.

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Riassunto

Modello in scala "CavyPool" per la valutazione igienico-sanitaria dei trattamenti e dei materiali per piscine

Razionale. I requisiti igienico-sanitari e la sorveglianza delle piscine sono stabiliti dalle linee guida dell'Organizzazione Mondiale della Sanità e da leggi nazionali. Il progresso nella gestione delle acque e nella costruzione delle piscine sta rivoluzionando il contesto, introducendo nuovi materiali, sistemi, procedure di disinfezione o indicatori per la sorveglianza. L'innovazione nel settore offre nuove opportunità per l'adeguamento e l'applicazione di linee guida.

Disegno dello studio. Al fine di fornire uno strumento per le prove di laboratorio, un prototipo è stato realizzato e applicato allo studio di materiali per piscina e trattamenti igienico-sanitari.

Metodi. Un modello di piscina in scala è stato progettato e valutato mediante un algoritmo di fluidodinamica computazionale. Un monitoraggio in tempo reale automatizzato ha assicurato il controllo dei parametri e il conseguimento di un equilibrio stazionario. I punti critici di controllo lungo il circuito dell'acqua sono stati identificati e resi accessibili per consentire l'immissione di diversi biocidi o il campionamento dell'acqua. Le simulazioni sono state eseguite in condizioni di sicurezza all'interno di una cappa standard. Alcuni materiali per le superfici della piscina o le tubazioni sono stati esaminati per studiare la formazione di biofilm in diverse condizioni di disinfezione. I microrganismi aderenti sono stati analizzati mediante analisi molecolare di mfDNA utilizzando real time PCR.

Risultati. Il prototipo ha raggiunto lo stato stazionario entro 5-25 ore in diverse condizioni, mostrando stabilità chimica, fisica e fluidodinamica. Un metodo è stato ottimizzato per analizzare i materiali, evidenziando la loro diversa risposta all'induzione di biofilm. Diversi campioni innovativi in PVC presentavano la massima resistenza all'adesione batterica.

Conclusioni. Lo strumento sviluppato permette di valutare sia materiali che trattamenti utilizzabili per l'igiene e la manutenzione di piscine, incluso il controllo della formazione del biofilm. Tale strumento può essere applicato per simulare contaminazioni in presenza di diverse strategie di trattamento o di disinfezione dell'acqua. Inoltre, lo stesso può essere di ausilio all'autorità sanitaria e decisori per acquisire evidenze utili al confronto o alla convalida di soluzioni innovative.

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