View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Journal of Preventive Medicine and Hygiene (JPMH)

OPEN ACCESS J PREV MED HYG 2021; 62: E48-E53

RESEARCH ARTICLE

Assessment of hygienic conditions of recreational facility restrooms: an integrated approach

GIUDITTA FIORELLA SCHIAVANO[1], GIULIA BALDELLI[2], VERONICA CEPPETELLI[3], GIORGIO BRANDI[2], GIULIA AMAGLIANI[2]

[1] Department of Humanities, University of Urbino Carlo Bo, Urbino (PU), Italy; [2] Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino (PU), Italy; [3] Diatheva S.r.l., Cartoceto, Fano (PU), Italy

Keywords

Air sampling • Bioaerosol • *Legionella* spp. • *Pseudomonas aeruginosa* • Recreational facility restrooms

Summary

Introduction. *Microbiological quality of recreational environments included restrooms, is generally assessed by water and surface monitoring. In this study, an environmental monitoring, conducted in spring, of swimming pool restrooms of a recreation center located in the Marche region has been carried out. Seven water samples and seven surface swabs were collected. Moreover, six air samples have been included. The aim of this study was to evaluate if air microbiological monitoring, along with molecular detection in real-time PCR, could give additional useful information about the hygienic conditions of the facility.*
Methods. *Heterotrophic Plate Count (HPC) both at 22°C (psychrophilic) and 37°C (mesophilic) was determined by separate cultures in all samples. The presence of Legionella pneumophila and Pseudomonas aeruginosa was evaluated by both culture and real-time PCR.*
Results. *The analysis of shower water recorded a HPC load of mesophilic bacteria (37°C) more than 10-fold higher in men restroom, respect to women's one (> 100 vs < 10 CFU/ml), while in air samples was between < 100 and > 500. Concerning pathogen presence, both species Legionella pneumophila and Pseudomonas aeruginosa were detected only in men restroom, but in different sample types by using different methods (culture and real-time PCR).*
Conclusions. *Air sampling may offer the advantage of giving more representative data about microbial presence in restrooms, including bacterial species transmitted through aerosol, like Legionella. Moreover, the concurrent use of molecular and microbiological detection in an integrated approach could offer the advantage of greater sensitivity.*

Introduction

In recreational facilities, like swimming pools, people can get exposed to pathogens and this may constitute a public health problem; restrooms may represent a potential at-risk environment. The risk of acquiring infections in swimming pools is often associated with microbial contamination of water and inadequate disinfection. Additionally, direct contact with contaminated surfaces and inhalation of air are also potential routes of exposure to pathogens [1].
Legionella pneumophila (*L. pneumophila*) has been recovered from a wide range of artificial water systems including hot water supplies, cooling towers, whirlpools spa but it is also a common inhabitant of natural water [2].
Legionella spp. grows best in warm water, and many legionnaire disease outbreaks are associated with hot water systems [3, 4].
In public swimming pools, especially in the dressing room areas, *L. pneumophila* can spread through shower aerosols produced from contaminated water sources and aerosols can be inhaled [5].
Pseudomonas aeruginosa (*P. aeruginosa*) is also a common environmental bacterium that forms biofilms on wet surfaces [6]. It can also be found in groundwater and drinking water systems [7]. The bacterium is one of the most common causes of opportunistic human infections.
P. aeruginosa is responsible of many episodes of infections associated with attendance at swimming pools. Moreover, *Legionella* spp. and *Pseudomonas* spp. can also resist stressful environmental conditions, like water chlorination, because of their ability to enter into a viable but non-culturable (VBNC) state [8]. VBNC forms typically exhibit a low level of metabolic activity and fail to grow on standard medium, while they retain certain features of viable cells, such as cellular integrity and virulence [9]. Under favorable conditions they can resuscitate, recovering their cultivability and regaining pathogenic potential [10, 11].
Currently, there are several methods for *Legionella* spp. and *Pseudomonas* spp. detection and enumeration including: standard culture methods [12], PCR and real-time PCR [13, 14], also in its quantitative application [15]. Culture methods, using prescribed growth media and specific conditions, recover only culturable organisms [16]. Over the past ten years, molecular methods are used for the detection of airborne and waterborne microorganisms not growing on culture media.

https://doi.org/10.15167/2421-4248/jpmh2021.62.1.1455

Bioaerosols are among the less studied particles in the environment, due the lack of standardization in sampling procedures [17, 18]. Moreover, it has been showed that only a small proportion of the total bioaerosol burden is culturable [19].
Recommendations for the control and prevention of legionellosis [20] do not include air sampling. However, this kind of sampling has been used by other authors, in combination with water systems surveillance, for the detection of *L. pneumophila* [21] and *P. aeruginosa* [22]. Airborne *L. pneumophila* may be collected by agar impaction, filtration and liquid impingement. Agar impaction samplers should be used for short sampling times to avoid cell desiccation stress. However, in case of low bacterial concentration, false negative results can occur. To date, numerous liquid-and filter-based samplers have been used for detecting airborne *Legionella* [21]. Liquid-based sampling methods have been described as the best sampling methods to obtain culturable *L. pneumophila* [21, 23], while filtration sampling (gelatine filters) is adequate for capturing total cells. Indeed, collection with liquid impingement relieves the dehydration stress, although a major drawback with conventional impingers is the violent bubbling producing cell damage and reduction of microbial recovery. Some of these problems have been attenuated by swirling aerosol collectors, combining impingement and centrifugal motions to preserve cultivability [24].
The presence of *L. pneumophila* [25] and *P. aeruginosa* [13, 26] in recreational centers has been investigated by several authors by the examination of water through filtration membranes. In our knowledge, other sampling methods for the detection of the above-mentioned pathogens in sport-related environments, included restrooms, are lacking.
Therefore, the aim of this study was to evaluate if air microbiological monitoring, performed through a swirling aerosol collector (Coriolis), could give additional useful information about the hygienic conditions of recreational facility restrooms.
This pilot study aims to propose a new integrated approach, comprising standard microbiological methods and real-time PCR, for the detection of *L. pneumophila* and *P. aeruginosa* and the determination of the Heterotrophic Plate Count (HPC) in recreational facility restrooms, analyzing a complete panel of environmental matrices: water, surfaces and air.
This may offer the advantage of giving more representative data about microbial presence, including also bacterial species known to be transmitted through aerosol. Moreover, the concurrent use of molecular and microbiological detection could give greater sensitivity, especially with VBNC forms possibly originated from disinfection.

Methods

A total of 20 samples were collected from restrooms of one recreational swimming pool, between June and August 2018. This recreational swimming pool is in the Marche Region, Central Italy.

Water samples

Each hot water sample, collected from sink taps and showers in men and women restrooms, was placed in 1 L sterile bottles, containing 10% sodium thiosulfate to neutralize any residual chlorine. Although all samples were collected by turning on the tap hot water, the temperature not exceeded 32°C. Samples were transported to the laboratory in a thermally insulated box and analysed immediately.
Legionella sampling and analysis was performed in accordance with Italian Guidelines [20] and the standard method ISO11731:2017 [27]. Briefly, 1 L of water was filtered through a membrane (0.2 µm, 47 mm diameter; Millipore, Billerica, MA, USA) of cellulose nitrate. Each membrane was put in a tube containing 5 ml of original water sample, shaken and then held at 50°C for 30 min. An aliquot of 0.5 ml was spread on *Legionella* CYE supplemented with *Legionella* BCYE growth supplement (Oxoid Ltd., Hampshire, UK) and incubated for 24 h at 37°C with 2.5% CO_2 for 10 days. Suspected colonies were counted and then confirmed by real-time PCR, as described below.
Isolation and identification of *P. aeruginosa* was performed according to the standardized procedure UNI EN ISO 16266:2008 [12]. Briefly, 100 ml of each water sample was filtered with a cellulose ester membrane (0.45 µm porosity, 47 mm diameter; Millipore), which was then placed onto a *Pseudomonas* Agar with *Pseudomonas* CN Supplement (PACN) (Oxoid) plate. PACN plates were incubated at 35 ± 1°C for 44 ± 4 h before the counting of colonies. Blue/ green pyocyanin-producing colonies were counted as confirmed *P. aeruginosa* according to UNI EN ISO 16266:2008 [12]. Fluorescent non-pyocyanin-producing or reddish-brown colonies were recorded as presumptive *P. aeruginosa* and subjected to confirmation tests according to UNI EN ISO 16266:2008 [12].
The Heterotrophic Plate Count (HPC) at 22°C and 37°C was determined by the pour plate method, using standard Plate Count Agar (PCA, Oxoid) [28]. The plates were incubated at 37°C for 40-48 h and at 22°C for 64-72 h. The results were expressed in colony forming units (CFU)/ml.

Air samples

Air contamination in recreational facility restrooms was assessed by a wet cyclone technology (Coriolis® µ Exonder, Borgo Ticino, NO, Italy). The Coriolis cyclone sampler was adjusted to sample 3,000 L of air (300 L/min for a period of 10 min). Airborne bacteria were collected in Coriolis® µ sterile cones filled with 15 ml phosphate buffered saline (PBS) + 0.005% Tween 80. HPC were conducted on PCA. 100 µl of sample were placed on the plates and incubated at 22 and 37°C. Next, the number of bacterial colonies was counted and recalculated as CFU per m^3 (CFU/m^3). The liquid material was filtered

through 0.2 µm cellulose ester membranes of 47 mm diameter (Millipore). For isolation of *Pseudomonas*, filter was placed on *Pseudomonas* Agar Base with CN (Oxoid) and incubated at 35 ± 1°C for 44 ± 4 h. For isolation of *Legionella*, filter was placed on *Legionella* CYE Agar Base supplemented with *Legionella* BCYE growth supplement (Oxoid) and incubated for 24 h and 10 days, checking the plates at intervals of 2-4 days, at 2.5% CO_2 at 37°C.
A second series of samples was analyzed in parallel by real-time PCR, as described below.

Swab samples

Bacterial microbiota present on the surface of the shower head (inside and out) was collected using sterile non adsorbent cotton swabs rubbed on a 10 x 10 cm^2 area, covering the entire surface by moving the swab back and forth across the surface horizontal and vertical strokes; then resuspended in 5 ml of physiological solution (0.9%, w/v, NaCl). For microbiological analyses, sample volumes of 0.1 mL were spread over the surface of PCA, PACN and *Legionella* CYE Agar Base supplemented with *Legionella* BCYE growth supplement. All plates were incubated for optimal temperature and time as per water analysis and after incubation the number of colonies was counted and was expressed as the number of CFU per cm^2 (CFU/cm^2).

PCR testing

Molecular detection in real-time PCR of *P. aeruginosa* and *L. pneumophila* was performed in water, air and swab samples. Sample analysis was performed according to Schiavano et al. [29] for *P. aeruginosa* and with the DI-Check Legionella pneumophila kit (Diatheva, Fano, Italy) for *L. pneumophila*.

Results

Concerning pathogen presence, both species were detected only in men restroom. *P. aeruginosa* was found in shower water and air samples by microbiological method and in shower swab surfaces by real-time PCR, while *L. pneumophila* was detected at a very low level in the external surface of shower heads with the real-time PCR, namely 4 and 7 genomic units (GU)/PCR, approximately corresponding to 160-280 GU in the sampled surfaces (Tab. I).
The analysis of shower water recorded a HPC load of mesophilic bacteria (37°C) more than 10-fold higher in men restroom, respect to women's one (Tab. II). These values are in accordance with those reported by [30, 31]. Similarly, in air samples the HPC load of psychrophilic flora (22°C) was higher in men restroom respect to women's one (Tab. II).

Discussion

Detection of microbial contamination in environmental matrices in recreational environments is important for safeguarding the state of hygiene and the health of pool users. The HPC at 22 and 37°C is a mandatory criterium to assess water potability, according to law provisions in Italy [32] and can be considered an indicator of hygiene. It is currently used to determine air quality in indoor environments, including recreational facilities [33].
In this study two different incubation temperatures were used to quantify the presence of mesophilic and psychrophilic species in water. According to the microbiological classification of air quality in non-industrial environments provided by the European Collaborative Action [34], a level of psychrophilic flora < 100 CFU/m^3, which was found in women restrooms, corresponds to the category of "low contamination", while an HPC load > 500, as that recorded in men restrooms, can be included in the "high contamination" category. Microbial pollution is a key element of indoor air pollution. It is caused by hundreds of species of bacteria and fungi, filamentous fungi (mold), growing indoors when sufficient moisture is available [35].
HPC are not considered an indicator of health risk. But, the *Legionella* presence in water appears to be in relationship with load and generic water quality parameters, i.e. HPC at 22°C and HPC at 37°C [36]. It is important to consider that the presence of these mesophilic and psychrophilic bacteria could be correlated with the colonization of water system by *Legionella* [37]. In our study, the water samples were collected from the hot water tap because numerous scientific evidences thought the hot water distribution system to be the most frequent source of cases or outbreaks of legionella in hotels, schools, sport facilities, offices and private residences [38].
In recreational facilities, the potential risk of infection with *Legionella* could be associated with the inhalation of aerosols containing the bacteria, in particular the aerosols created in the showers [39].
Although the limited sampling number does not allow a statistical analysis of significance about the association of high levels of HPC at 37°C and pathogen presence, which is out of the scope of this study, results confirmed previously reported data [25]. The lack of positive results for *Legionella* with the standard microbiological method could be due to the presence of VBNC cells [9] or bacteria contained in amoebae [10]. Moreover, PCR positive samples in absence of isolation of living bacteria could be ascribed to the presence of dead microorganisms, i.e. inactivated by disinfection procedures. Nevertheless, in water and air monitoring, filter-concentration before culture could have improved sensitivity, in contrast to real-time PCR, in which only a fraction of the extracted sample was PCR-amplified.
The integrated approach used in the present study takes advantage from the examination of different types of matrices, not limiting to water samples. Indeed, for some pathogens like *L. pneumophila*, aerosol inhalation is the main pathway of exposure, thus air sampling may offer

Tab. I. Results of pathogen detection by microbiological and real-time PCR methods in samples from men restrooms.

Sample type		Microbiological methods		Real-time PCR	
		P. aeruginosa UNI EN ISO 16266:2008	*L. pneumophila* UNI EN ISO 11731:2017	*P. aeruginosa*	*L. pneumophila*
Water	Shower water	+	-	-	-
	Tap water	-	-	-	-
Air	Air	+	-	-	-
Surfaces	Shower head (inside)	-	-	+	-
	Shower head (out)	-	-	+	+*

* Values are: 4 and 7 GU/PCR, in the first and second sample, respectively.

Tab. II. Results of HPC load in men and women restrooms.

Location	Sample type		HPC*	
			22°C	37°C
Men restroom	Water	Shower water	< 10	> 100
		Tap water	< 10	< 10
	Air	Aerosol	> 500	> 300
	Surfaces	Shower head (inside)	n.c.	n.c.
		Shower head (out)	n.c.	n.c.
Women restroom	Water	Shower water	< 10	< 10
		Tap water	< 10	< 10
	Air	Aerosol	< 100	> 300
	Surfaces	Shower head (inside)	n.c.	n.c.
		Shower head (out)	n.c.	n.c.

n.c.: not counted. After incubation many colonies were confluent and this made counting impossible. * Values are reported as CFU/ml for water samples and CFU/m^3 for air samples.

useful information about its presence and give a better knowledge about the microbiological quality of such environments. Moreover, the application of two kinds of methodologies, culture-based and real-time PCR, increased detection sensitivity, especially for surface analysis.

Conclusions

Microbiological monitoring of water, air and surface quality for the presence of important species, like *Legionella* spp. and *Pseudomonas* spp., is useful and crucial in order to determine the potential exposure of swimming pool users. The use of different sampling methods gave integrated information that allowed to highlight the contamination by pathogens on different matrices.

The multipoint analysis approach used in this preliminary study, with the application of both culture and molecular methods, can increase the probability of a reliable detection. In conclusion, an accurate environmental monitoring of restrooms in recreational pool facilities including air, along with the application of good hygienic practices, can be of main importance to prevent or reduce the exposition of pool users to microbiological risks.

Acknowledgements

Funding sources: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors wish to thank: the swimming pool facility manager, Gabriele Ponti, for the access and assistance; dr. Michela Nalin at Exonder srl for providing Coriolis® µ air sampler used in this study and dr. Enrica Omiccioli at Diatheva Srl for her precious collaboration.

Conflicts of interest statement

The authors declare no conflict of interest.

Authors' contributions

Conceptualization and data curation: SGF and GA; Formal analysis: SGF, GA and GBr; Methodology: SGF, GA and CV; Supervision: SGF, GA and GBr; Validation: SGF, GA, VC and GBr; Writing original draft: SGF and GA; Writing, review & editing: SGF, GA, GBr, GB and CV.

References

[1] Bouillard L, Michel O, Dramaix M, Devleeschouwer M. Bacterial contamination of indoor air, surfaces, and settled dust, and related dust endotoxin concentrations in healthy office buildings. Ann Agric Environ Med 2005;12:187-92.

[2] Schoen ME, Ashbolt NJ. An in-premise model for Legionella exposure during showering events. Water Res 2011;45:5826-36. https://doi.org/10.1016/j.watres.2011.08.031

[3] Barna Z, Kádár M, Kálmán E, Scheirich Szax A, Vargha M. Prevalence of Legionella in premise plumbing in Hun-

gary. Water Res 2016;90:71-8. https://doi.org/10.1016/j.watres.2015.12.004

[4] Serrano-SuárezA, Dellundé J, Salvadó, S. Cervero-Aragó H, Méndez J, Canals O, Blanco S, Arcas A, Araujo R. Microbial and physicochemical parameters associated with Legionella contamination in hot water recirculation systems. Environ Sci Pollut Res 2013;20:5534-44. https://doi.org/10.1007/s11356-013-1557-5

[5] Bouwknegt M, Schijven JF, Schalk JA, de Roda Husman AM. Quantitative risk estimation for a Legionella pneumophila infection due to whirlpool use. Risk Anal 2013;33:1228-36. https://doi.org/10.1111/j.1539-6924.2012.01909.x

[6] Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrock-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, Lim K, Smith R, Spencer D, Wong GK, Wu Z, Paulsen IT, Reizer J, Saier MH, Hancock RE, Lory S, Olson MV. Complete genome sequence of Pseudomonas aeruginosa PA01, an opportunistic pathogen. Nature 2000,406:959-64.

[7] Banning N, Toze S, Mee BJ. Persistence of biofilm-associated Escherichia coli and Pseudomonas aeruginosa in groundwater and treated effluent in a laboratory model system. Microbiology 2003;149:47-55.

[8] Lutz JK, Lee J. Prevalence and antimicrobial-resistance of Pseudomonas aeruginosa in swimming pools and hot tubs. Int J Environ Res Public Health 2011;8:554-64. https://doi.org/10.3390/ijerph8020554

[9] Kirschner AKT. Determination of viable legionellae in engineered water systems: do we find what we are looking for? Water Res 2016;93:276-88. https://doi.org/10.1016/j.watres.2016.02.016

[10] Garcia MT, Jones S, Pelaz C, Millar RD, Abu Kwaik Y. Acanthamoeba polyphaga resuscitates viable non-culturable Legionella pneumophila after disinfection. Environ Microbiol 2007;9:1267-77.

[11] Alleron L, Merlet N, Lacombe C, Frere J. Long-term survival of Legionella pneumophila in the viable but nonculturable state after monochloramine treatment. Curr Microbiol 2008;57:497-502. https://doi.org/10.1007/s00284-008-9275-9

[12] UNI EN ISO 16266:2008 Water quality. Detection and enumeration of Pseudomonas aeruginosa. Method by membrane filtration. International Organization for Standardization, Technical committee ISO/TC 147, Subcommittee SC 4, 2008.

[13] Amagliani G, Parlani ML, Brandi G, Sebastianelli G, Stocchi V, Schiavano GF. Molecular detection of Pseudomonas aeruginosa in recreational water. Int J Environ Health Res 2012;22:60-70. https://doi.org/10.1080/09603123.2011.588325

[14] Amagliani G, Schiavano GF, Stocchi V, Bucci G, Brandi G. Application of real-time PCR to Pseudomonas aeruginosa monitoring in a public swimming pool. Microchemical Journal 2013;110:656-9. https://doi.org/10.1016/j.microc.2013.08.001

[15] Omiccioli E, Schiavano GF, Ceppetelli V, Amagliani G, Magnani M, Brandi G. Validation according to ISO/TS 12869:2012 of a molecular method for the isolation and quantification of Legionella spp. in water. Mol Cell Probes 2015;29:86-91. https://doi.org/10.1016/j.mcp.2014.12.004

[16] Dutil S, Meriaux A, de Latremoille MC, Lazure L, Barbeau J, Duchaine C. Measurement of Airborne Bacteria and Endotoxin Generated During Dental Cleaning. J Occup Environ Hyg 2009;6:121-30. https://doi.org/10.1080/15459620802633957

[17] Blais-Lecours P, Perrott P, Duchaine C. Non-culturable bioaerosols in indoor settings: impact on health and molecular approaches for detection. Atmos Environ 2015;110:45-53. https://doi.org/10.1016/j.atmosenv.2015.03.039

[18] Mbareche H, Brisebois E, Veillette M, Duchaine C. Bioaerosol sampling and detection methods based on molecular approaches: no pain no gain. Sci Total Environ 2017;599:2095-104. https://doi.org/10.1016/j.scitotenv.2017.05.076

[19] Peccia J, Hernandez M. Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: a review. Atmos Environ 2006;40:3941-61. https://doi.org/10.1016/j.atmosenv.2006.02.029

[20] Proceedings of Italian Guidelines for the Prevention and Control of Legionellosis 2015, approved in State-Regions Conference on 05.07.2015] 2015 Italian.

[21] Chang CW, Chou FC. Methodologies for quantifying culturable, viable, and total Legionella pneumophila in indoor air. Indoor Air 2011;21:291-9.

[22] Hopman J, Meijer C, Kenters N, Coolen JPM, Ghamati MR, Mehtar S, van Crevel R, Morshuis WJ, Verhagen AFTM, van den Heuvel MM, Voss A, Wertheim HFL. Risk assessment after a severe hospital-acquired infection associated with carbapenemase-producing Pseudomonas aeruginosa. JAMA Netw Open 2019;2:e187665. https://doi.org/10.1001/jamanetworkopen.2018.7665

[23] Montagna MT, De Giglio O, Cristina ML, Napoli C, Pacifico C, Agodi A, Baldovin T, Casini B, Coniglio MA, D'Errico MM, Delia SA, Deriu MG, Guida M, Laganà P, Liguori G, Moro M, Mura I, Pennino F, Privitera G, Spica VM, Sembeni S, Spagnolo AM, Tardivo S, Torre I, Valeriani F, Albertini R, Pasquarella C. Evaluation of Legionella air contamination in healthcare facilities by different sampling methods: an Italian multicenter study. Int J Environ Res Public Health 2017;14:670.

[24] Lin X, Reponen TA, Willeke K, Wang Z, Grinshpun SA, Trunov M. Survival of airborne microorganisms during swirling aerosol collection, Aerosol Sci Technoln 2000;32:184-96.

[25] De Filippis P, Mozzetti C, Amicosante M, D'Alò GL, Messina A, Varrenti D, Giammattei R, Di Giorgio F, Corradi S, D'Auria A, Fraietta R, Gabrieli R. Occurrence of Legionella in showers at recreational facilities. J Water Health 2017;15:402-9. https://doi.org/10.2166/wh.2017.296

[26] Golpayegani A, Douraghi M, Rezaei F, Alimohammadi M, Nabizadeh Nodehin R. Propidium monoazide-quantitative polymerase chain reaction (PMA-qPCR) assay for rapid detection of viable and viable but non-culturable (VBNC) Pseudomonas aeruginosa in swimming pools J Environ Health Sci Eng 2019;17:407-16. https://doi.org/10.1007/s40201-019-00359-w.eCollection 2019 Jun

[27] ISO11731:2017. Water Quality - Enumeration of Legionella. Geneva, Switzerland: International Organization for Standardization 2017.

[28] Ente Nazionale Italiano di Unificazione. UNI EN ISO 6222. Water quality - Quantitative assessment of viable microorganisms - Counting of colonies by inoculation on agarized medium. 2001.

[29] Schiavano GF, Carloni E, Andreoni F, Magi S, Chironna M, Brandi G, Amagliani G. Prevalence and antibiotic resistance of Pseudomonas aeruginosa in water samples in central Italy and molecular characterization of oprD in imipenem resistant isolates. Plos One 2017;12(12). https://doi.org/10.1371/journal.pone.0189172

[30] Lee BU, Hong IG, Lee DH, Chong E-S, Jung JH, Lee JH, Kim HJ, Lee IS. Bacterial bioaerosol concentrations in public restroom environments. Aerosol Air Qual Res 2012;12:251-5. https://doi.org/10.4209/aaqr.2011.08.0134

[31] Heo KJ, Lim CE, Kim HB, Lee BU. Effects of human activities on concentrations of culturable bioaerosols in indoor air environments. J Aerosol Sci 2017;104:58-65. https://doi.org/10.1016/j.jaerosci.2016.11.008

[32] Bonadonna L, Ottaviani M. Metodi analitici di riferimento per le acque destinate al consumo umano ai sensi del DL.vo 31/2001. 2007. Metodi microbiologici. [Reference analytical methods for water intended for human consumption accord-

ing to the Italian Legislative Decree 31/2001. Microbiological methods]. https://doi:10.13140/RG.2.1.1419.6088

[33] Grisoli P, Albertoni M, Rodolfi M. Application of airborne microorganism indexes in offices, gyms, and libraries. Appl Sciences 2019;9(6). https://doi.org/10.3390/app9061101

[34] Commission of European Communities. Indoor air quality & its impact on man. Report n. 12. Luxembourg: Commission of European Communities 1993.

[35] WHO. Guidelines for indoor air quality: dampness and mould. 2009.

[36] Mouchtouri V, Velonakis E, Tsakalof A, Kapoula C, Goutziana G, Vatopoulos A, Kremastinou J, Hadjichristodoulou C. Risk factors for contamination of hotel water distribution systems by Legionella species. Appl Environ Microbiol 2007;73:1489-92.

[37] Cunha BA, Burillo A, Bouza E. Legionnaires' disease. Lancet 2016;387:376-85.

[38] Fields, BS, Benson RF, Besser RE. Legionella and Legionnaires' disease: 25 years of investigation. Clin Microbiol Rev 2002;15:506-26. https://doi.org/10.1128/CMR.15.3.506-526.2002

[39] Leoni E, Legnani PP, Bucci Sabatini MA, Righi F. Prevalence of Legionella spp. in swimming pool environment. Water Research 2001;35:3749-53.

Received on December 12, 2019. Accepted on January 15, 2021.

Correspondence: Giuditta Fiorella Schiavano, University of Urbino Carlo Bo, Department of Humanities, via Bramante 17, 61029 Urbino (PU), Italy - Tel.: +39 0722 303546 - E-mail: giuditta.schiavano@uniurb.it

How to cite this article: Schiavano GF, Baldelli G, Ceppetelli V, Brandi G, Amagliani G. Assessment of hygienic conditions of recreational facility restrooms: an integrated approach. J Prev Med Hyg 2021;62:E48-E53. https://doi.org/10.15167/2421-4248/jpmh2021.62.1.1455

© Copyright by Pacini Editore Srl, Pisa, Italy

This is an open access article distributed in accordance with the CC-BY-NC-ND (Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International) license. The article can be used by giving appropriate credit and mentioning the license, but only for non-commercial purposes and only in the original version. For further information: https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en