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MICROBIOLOGICAL EVALUATION OF SWIMMING POOL WATER AT HOTEL UNITS

Stéphanie Lopes Ferreira
Escola Superior da Saúde – Instituto Politécnico do Porto
Porto
ORCID 0009-0003-6295-8783

Maria Manuela da Silva e Sousa Amorim
Escola Superior da Saúde – Instituto Politécnico do Porto
Porto
ORCID 0000-0002-7328-2163

Teresa Raquel Lemos Moreira
Escola Superior da Saúde – Instituto Politécnico do Porto
Porto
ORCID 0009-0003-7272-2483

António Augusto Araújo Gomes
Escola Superior da Saúde – Instituto Politécnico do Porto
Porto
ORCID 0000-0003-1330-0638

Sandra Marlene da Silva Mota
Escola Superior da Saúde – Instituto Politécnico do Porto
Porto
ORCID 0000-0002-2803-7230

Abstract: The swimming pools are currently operated by public and private entities for the development of sports, recreational and therapeutic activities. Therefore, it is essential to guarantee the quality of the pool water, as they can be the cause of various pathologies.
The objective of this work was to analyze the data from the microbiological evaluation of the water in the indoor and outdoor pools of hotels in mainland Portugal and Madeira in 2016, in order to verify the quality of the water.
A descriptive cross-sectional study was performed using outcome records from a northern laboratory. The microbiological parameters studied to characterize indoor and outdoor pool waters included viable microorganisms at 37ºC/24h, total coliforms, *Escherichia coli*, *Enterococcus spp*., *Pseudomonas aeruginosa*, *Staphylococcus* totals and *Staphylococcus* coagulase producers. The samples were characterized as compliant and non-compliant according to the reference intervals indicated in Normative Circular nº 14/DA of 08/21/2009 of the General Health Directorate.
Of the total number of indoor pools (n=610) analyzed, 24.9% (n=152) were classified as non-compliant, with viable microorganisms at 37°C being the most frequent cause of non-compliances (n=103), followed by total coliforms (n=47) and *Staphylococcus* totals (n=42). For outdoor swimming pools (n=1982), 29.9% (n=592) were also classified as non-compliant, once again microorganisms viable at 37°C being the most frequent cause of non-compliances (n=419), followed by total coliforms (n=154).
Indoor pools have a lower frequency of non-compliance compared to outdoor pools. The ambient temperature and the presence of dirt influence the microbiological quality of the water. These results also suggest that water treatment is not effective, indicating water pollution, with hygienic care being another factor that influences the microbiological quality of water. The determination of these parameters is useful for a constant monitoring of the microbiological quality of the water.
Keywords: Microbiological analysis; microbiological quality; pool water; fecal contamination indicators

INTRODUCTION

Currently, the exploitation of swimming pools by public and private entities for the development of sports, recreational and therapeutic activities represents a major challenge in maintaining the water quality of swimming pools (Chase NM, 2008; Khodaee M, 2016).

Ensuring the chemical and microbiological quality of swimming pool water is essential, as these can be the cause of certain pathologies. There are several risks associated with the contamination of swimming pool water, both from the point of view of microbiological agents that may be present, and from the chemical products used in water treatment and disinfection by-products, with consequences for users and professionals in these public spaces. (Pedroso MJ, 2003; World Health Organization, 2006).

Biological risks are related to the presence of pathogenic microorganisms for public health. They can be bacteria, fungi, protozoa and viruses. In swimming pools, there are biological risks associated with the production of aerosols (presence of bacteria of the genus *Legionella*) and associated with water quality (presence of microorganisms of fecal and non-fecal origin), responsible for the development of various clinical conditions such as dermal and ear infections, ocular, gastrointestinal and even the central nervous system (World Health Organization, 2006).

Regulatory Decree nº 5/97 of March 31st, of the Ministry of Equipment, Planning

and Territorial Administration, Normative Circular nº 14/DA of 08/21/09 of the General Health Directorate (DGS) and the most recent Norm Portuguesa NP 4542-2017 are the only existing references in the country, since in Portugal there is no specific legislation regulating the quality of swimming pool water.

Regulatory Decree No. 5/97 of March 31st regulates the technical and safety conditions of water sports venues (Ministry of Equipment, Planning and Land Administration, 1997). Normative Circular nº 14/DA of 08/21/09 gave rise to the Swimming Pool Sanitary Surveillance Program (PVSP), which aimed to standardize the procedures related to the sanitary surveillance of swimming pools and indicate the microbiological and physical-chemical parameters to be analyzed, as well as the respective reference value (Direção Geral da Saúde, 2009). The Portuguese Standard NP 4542 of 2017 establishes the requirements for the quality and treatment of water for use in tanks (Portuguese Quality Institute, 2017).

Swimming pools designed in hotel units are considered as type II (semi-public) swimming pools, intended to provide a complementary service to the main activity of an enterprise and whose use is considered "public" (Direção Geral da Saúde, 2009). The Health Authorities are responsible for requesting the pool management entities to carry out the microbiological and physical-chemical analyzes provided for in the PVSP by accredited laboratories and for sending the results of the analyzes, at least monthly, as a complement and support in the characterization of the installation (Nogueira JMR, 2022).

Table 1 describes the PVSP guidelines regarding the microbiological analysis of water (Direção Geral da Saúde, 2009).

These microbiological parameters can be grouped into fecal indicators and non-fecal indicators. The group of faecal indicators includes total coliforms, Escherichia coli and faecal Streptococci and Enterococci, which make it possible to assess the presence of fecal matter in the water. In turn, the group of non-faecal indicators includes pathogenic microorganisms for humans such as *Pseudomonas aeruginosa*, total Staphylococci and coagulase producers and *Legionella* (Ministry of Equipment, Planning and Land Administration, 1997). Most of these non-fecal bacteria can accumulate in biofilms and represent a challenge in the treatment of swimming pool water.

The count of viable microorganisms at 37ºC allows estimating the total number of microorganisms present in the water. This information is useful in assessing and monitoring water quality, as it naturally contains a certain number of microorganisms from different sources such as soil, vegetation, among others. This way, this parameter allows the evaluation of the microbiological integrity of the pool water and the effectiveness of its treatment.

The importance of determining this parameter lies in its permanent monitoring, so that results above the limit value are indicative of pollution and need to be investigated (World Health Organization, 2006).

Total coliforms and *Escherichia coli* are both indicators of faecal contamination in pool water. The presence of these microorganisms in pool water is a warning sign indicative of contamination or treatment failure.

Escherichia coli belongs to the group of fecal coliforms, existing naturally in the human body. Because it is pathogenic, it cannot be present in swimming pool water (Silva N, 2017; Carroll KC, 2019).

Fecal Enterococci are also indicators of fecal contamination. Considered pathogenic microorganisms, they cannot be present in recreational waters (Silva N, 2017; Carroll KC,

PARAMETERS	EXPRESSION OF RESULTS	ANALYTICAL METHOD	REFERENCE VALUE		PERIODICITY
			VR	VL	
Cultivable microorganisms at 37ºC – 24h	UFC/mL	ISO 6222	≤ 100*	-	Monthly
Total coliforms	UFC/100mL	ISO 9308-1 modificada	0	10	
Escherichia coli	UFC/100mL	ISO 9308-1 modificada	-	0	
Fecal streptococci and enterococci	UFC/100mL	ISO 7899-2	-	0	
Pseudomonas aeruginosa	UFC/100mL	ISO 12780 modificada	-	0	
Coagulase-producing staphylococci	UFC/100mL	NP-4343	-	0**	
Total staphylococci	UFC/100mL	NP-4343	≤20*	-	
Legionella***	Nº/1000mL	ISO 11731:1998	-	100	Quarterly

Caption: **VR** – Recommended value; **LV** - Limit value

*The recommended amount may be exceeded once per public opening season or per calendar year.

**0/100mL in 90% of the samples, it being the responsibility of local health services to carry out the assessment at the end of the season or calendar year.

***In hydromassage tanks or in swimming pools with devices that promote the formation of aerosols

Table 1- Microbiological parameters to be analyzed within the scope of the PVSP

MICROBIOLOGICAL PARAMETER	PROTOCOL COMPLETED
Culturable microorganisms at 37ºC-24h (CFU/mL)	ISO 6222:1999
Coliform bacteria (CFU/100 mL) *Escherichia coli* (UFC/100 mL)	IT-DLM-03/V05
Enterococci (CFU/100 mL)	ISO 7899-2:2000
Pseudomonas aeruginosa (UFC/100 mL)	ISO 16266:2006
Total staphylococci(UFC/100 mL)	NP 4343:1998
Coagulase Positive Staphylococci (CFU/100 mL)	

Table 1 - Protocol carried out for each of the microbiological parameters.

2019).

The *Pseudomonas aeruginosa* is an opportunistic pathogenic microorganism. This microorganism is capable of developing in conditions of low nutrients and can be found in the environment, in the soil, in water, being a good indicator of non-fecal contamination. Bathers are primarily responsible for their presence in swimming pools. Under favorable conditions (deficiency in disinfection and in the hydraulic circuit), this bacterium grows quickly and tends to accumulate in biofilms on filters, tubes and surfaces (Silva N, 2017; Guida M, 2016; Rice SA, 2012).

Staphylococci belong to the *Micrococcaceae* family, and Gram positive cocci are facultative anaerobes. Many of the species belonging to this genus are part of the human body. Of the existing species, *Staphylococcus aureus* is pathogenic for man. For this reason, research on this microorganism is especially important in swimming pool water. Their presence is a good indicator of non-fecal contamination, as the vast majority are part of the normal flora of the skin, ears and nose (Silva N, 2017).

They have a high resistance to the action of disinfectants and accumulate in the superficial film of the water, but they are not, normally, a problem in swimming pools where the operation of disinfection and maintenance is adequate (World Health Organization, 2006).

The presence of any previously mentioned pathogenic microorganism puts the bather's health and safety at risk.

The quality of swimming pool water has to be constantly evaluated and controlled in order to prove the effectiveness of the treatment. In cases where the water is classified as inappropriate, the supervisory body proceeds to close it until it presents the necessary conditions again.

Inadequate water disinfection/treatment, high temperatures, overuse and insufficient water renewal are some of the main factors associated with poor pool water quality (World Health Organization, 2006).

OBJECTIVES

The present work aims to evaluate the microbiological quality of pool water in hotel units in mainland Portugal and Madeira, analyzed by a Portuguese company in 2016, with the aim of gathering relevant information for adequate risk management for the health of users.

The specific objectives of this study are to verify the frequency of nonconformities in outdoor and indoor pools, compare the results obtained and try to establish their possible causes.

MATERIAL AND METHODS

Type of Study: Descriptive cross-sectional study.

SAMPLE

The sample consists of records of the results obtained after microbiological analysis of swimming pool water from hotels in mainland Portugal and Madeira in 2016.

Regarding the exclusion criteria, all inconclusive or invalid sample results were eliminated, namely those that did not present the necessary information for this study (swimming pools not characterized as to the nature of the activity and type of construction).

INSTRUMENTS

The instruments used were the Excel ® database provided by a laboratory accredited by Norm NP EN ISO/IEC 17025 within the scope of water tests and responsible for carrying out the analyses. This database was imported into the IBM® SPSS® Statistics software for organization and statistical treatment of the data.

PROCEDURES

Data were collected by providing pre-existing records of microbiological analyzes carried out in pool water during 2016 by an accredited public health laboratory.

Sample collection and microbiological analysis were performed by a specialized laboratory technician, according to the PE-DSQ10/V18 ISO19458:2006 protocol.

Table 1 lists the normative references for each microbiological parameter.

Statistical analysis of the microbiological results was performed using the software *IBM® SPSS® Statistics*. The data were organized and treated, using descriptive statistics, by analyzing absolute and relative frequencies. Finally, graphs and tables were prepared for the microbiological evaluation of the water in the pools under study, in order to respond to the proposed objectives. For this purpose, the microbiological profile of swimming pool water was analyzed, the frequency of non-conformities was evaluated and the results obtained by microbiological parameter were compared.

ETHICS

The procedures carried out are in accordance with the ethical standards of the responsible entity and the confidentiality of the elements involved in the study is assured.

RESULTS

The sample was entirely composed of 2592 swimming pool records, of which 76.5% (n=1982) referred to outdoor swimming pools and 23.5% (n=610) to indoor swimming pools.

Regarding the assessment of water quality, following the legislation in force, around 71.3% (n=1848) were classified as Compliant and 28.7% (n=744) as Non-Compliant. The relative frequency of Compliant and Non-Compliant samples in the different types of pools follows the same distribution (Figure 1).

In outdoor pools, microorganisms viable at 37ºC showed a higher percentage in waters classified as Non-Compliant. Followed by total coliforms, *Pseudomonas aeruginosa* and total *Staphylococci*.

In indoor pools, the parameters that showed the highest percentage in waters classified as Non-Compliant were viable microorganisms at 37ºC. Fecal coliforms and total Staphylococcus followed with the same value (Table 2).

By analyzing the distribution of Non-compliant results over the months of 2016, a higher percentage was found in outdoor pools in the summer and early autumn months (July, August, September and October). In indoor swimming pools, the results with the highest percentage fall to the months of early summer (May and June) and late autumn (November and December).

DISCUSSION

The results of this study showed that 71.3% (n=1848) of the pools analyzed were classified as conforming, that is, suitable for bathers.

According to the results obtained, the microbiological parameters mainly responsible for the occurrence of non-conformities are microorganisms viable at 37ºC and total coliforms.

The presence of these parameters with values above the limit value is indicative of fecal contamination and that the water treatment in these pools may not have been effective. However, the pathogenic indicators, Pseudomonas aeruginosa and total Staphylococcus, which are more resistant to disinfection, due to the fact that they form biofilms and resist high temperatures, were not the parameters with the highest percentage of non-conformities. In view of the above, the treatment of water in swimming pools classified as Non-Compliant may have been

Microbiological Parameters	Outdoor pools (n=1982)		
	According to	Nonconforming	
		> VR	> VL
Viable microorganisms at 37ºC/24h[1]	1563 (78,9%)	419 (21,1%)	-
Total coliforms	1828 (92,2%)	68 (3,4%)	86 (4,3%)
Escherichia coli	1941 (97,9%)	-	41 (2,1%)
Enterococcus spp.	1956 (98,7%)	-	26 (1,3%)
Pseudomonas aeruginosa	1863 (94,0%)	-	119 (6,0%)
Coagulase positive Staphylococcus	1969 (99,3%)	13 (0,7%)	-
total Staphylococcus[1]	1866 (94,1%)	116 (5,9%)	-
Total	1390 (70,1%)	592 (29,9%)	

VR – Recommended Value; **VL** – Limit Value

1. The recommended value may be exceeded once per public opening season

Table 3- Absolute frequency and relative frequency of each microbiological parameter determined in outdoor swimming pools

Microbiological Parameters	Indoor pools (n=610)		
	According to	Nonconforming	
		> VR	> VL
Viable microorganisms at 37ºC/24h[1]	507 (83,1%)	103 (16,9%)	-
Total coliforms	563 (92,3%)	15 (2,5%)	32 (5,2%)
Escherichia coli	593 (97,2%)	-	17 (2,8%)
Enterococcus spp.	607 (99,5%)	-	3 (0,5%)
Pseudomonas aeruginosa	581 (95,2%)	-	29 (4,8%)
***Staphylococcus* coagulase positiva**	606 (99,3%)	4 (0,7%)	-
***Staphylococcus* totais**[1]	568 (93,1%)	42 (6,9%)	-
Total	(5,1%)	152 (4,9%)	

VR – Recommended Value; **VL** – Limit Value

1. The recommended value may be exceeded once per public opening season

Table 2- Absolute frequency and relative frequency of each microbiological parameter determined in indoor pools

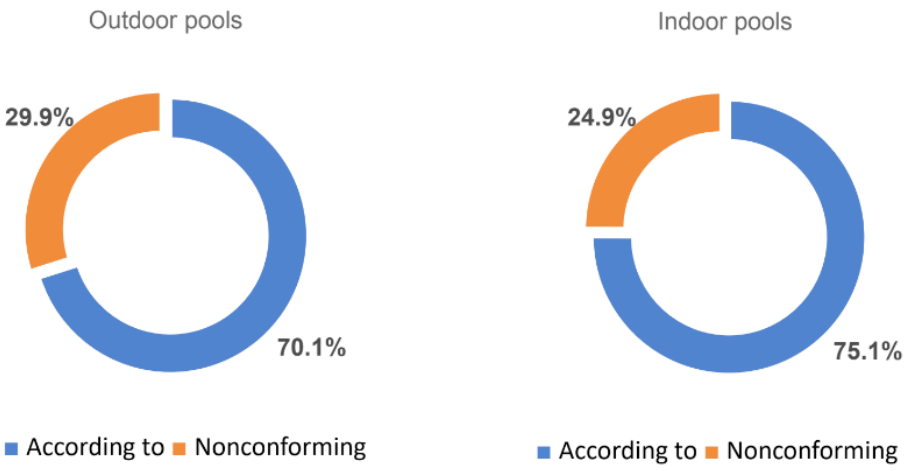

Figure 1 – Microbiological classification of swimming pool water.

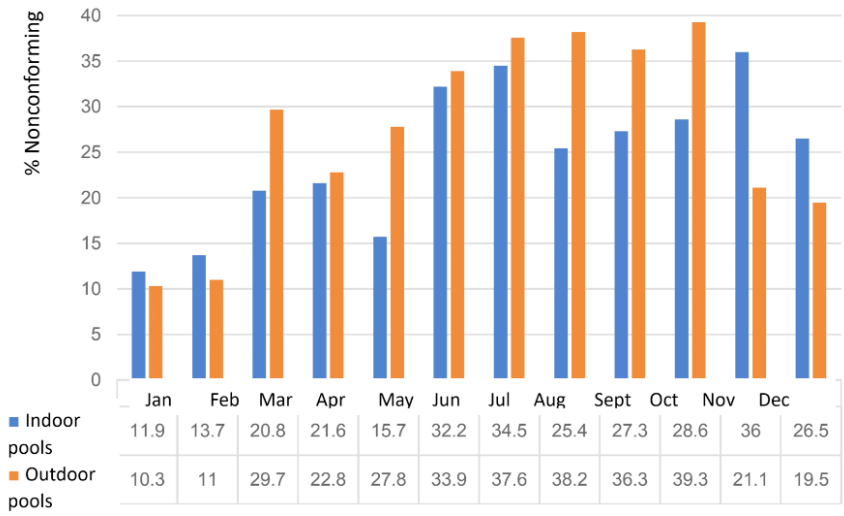

Figure 2 – Distribution of non-compliant results for indoor and outdoor pools throughout 2016

adequate and effective. Compromising the quality of the water in these pools, at the time of collection, may be due to other conditions, namely: the number of bathers, the use of showers immediately before entering the pool, the bathers' own hygiene and accidental excretion (for example: vomit, feces, urine) (World Health Organization, 2006; Pedroso MJ, 2003; Pedroso MJ, 2009).

It is important to mention that the determination of these parameters is useful when microbiological monitoring is carried out continuously, in order to verify possible trends and, if given values above the limit values, the corrective measures were effective.

In general, indoor pools have a lower frequency of non-conformities. However, the number of indoor pools analyzed is significantly smaller compared to the number of outdoor pools.

Bearing in mind that outdoor pools are more exposed to the elements and the surrounding environment that contains microorganisms, it would be expected that the relative frequency of non-conformities would be higher.

By analyzing the distribution of Non-Compliant results over the months of 2016, it can be observed that in outdoor swimming pools higher percentages of Non-Compliant results are observed in the summer months, between June and October, with percentages greater than 30%, which can be justified by the fact of being months associated with the holiday period and greater occupancy of the outdoor swimming pools. However, it would be important to verify this hypothesis by combining these results with the occupancy rate of the swimming pools. In the case of indoor pools, higher percentages of Non-Compliant were observed in the months of June, July and November.

The present work analyzed the data referring to each pool from a national perspective, mainland Portugal and Madeira, without taking into account the meteorological conditions associated with each region at the time of harvest, which is a limiting factor of the study.

Another limitation is due to the fact that the study is cross-sectional, making it impossible to know if the water samples are from different pools or if they are several samples from the same pool, collected throughout the year 2016.

The lack of other works related to this topic for comparison of results, as well as the lack of access to results from previous years, are also other limitations. Access to this information would make it possible to carry out a more detailed analysis of previous non-conformities and also identify critical points in the process that could be improved. It must be noted that the lack of longitudinal information does not allow inferences about the process of treatment and monitoring of the water quality of the pools studied.

CONCLUSION

This study made it possible to evaluate the microbiological quality of swimming pool water in mainland Portugal and Madeira, during the year 2016, following the rules imposed by Regulatory Decree nº5/97 of March 31st.

In short, most pools meet the established requirements and the results obtained showed that there is still a percentage of pools classified as Non-Compliant from the analyzed water samples, which must be taken into account and properly treated for these waters.

It is also concluded that outdoor pools have a higher frequency of non-compliance compared to indoor pools, although the difference is not significant.

For future work, it is suggested to evaluate, in parallel with the microbiological parameters analyzed, other microbiological parameters

such as *Legionella spp* and physical-chemical parameters, namely temperature, pH and residual chlorine, in order to obtain a global assessment of the quality of the water in the swimming pools and identify possible causes for the non-compliant values found.

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