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Evaluation of bacterial contamination in indoor swimming pools

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ABSTRACT

Background: The quality of water is a crucial factor in maintaining public health, as contaminated water can lead to the transmission of various waterborne diseases. One of the primary concerns is the contamination of swimming pools that represent a ubiquitous feature of recreational facilities particularly during the warmer months.
Aim: Investigation the prevalence of bacterial contamination in water and environments of indoor swimming pools.
Materials and methods: Totally, 100 swab samples were collected equally from the changing rooms, pool edges, pool ladders and water of various swimming pools in Wasit province (Iraq) during June and July (2024). Then, the swabs were cultured separately on three media (Blood Agar, Eosin Methylene Blue Agar and MacConkey Agar), purified and to identified based on their morphological characteristics and biochemical test.
Results: Overall 37% of swab samples were contaminated with various species of bacteria; which found in pool edges (56%), pool ladders (44%), changing rooms (36%), and water (12%). Subsequently, the number of identified bacterial isolates was 68 (37.99%), 39 (21.79%), 45 (25.14%) and 27 (15.08%) in water, changing rooms, pool edges, and pool ladders, respectively. In changing rooms, 12 species of bacteria was reported, more significantly was Escherichia coli as well as Klepsellia aerogenes. In pool edges, 11 bacterial species were recorded; in which, significant increases were seen in Klepsellia pneumonia, Escherichia coli, as well as in Aeromonus hydrophila, and Vibrio cholera. Concerning the pool ladders, 10 bacterial species were diagnosed, particularly Vibrio cholera, Escherichia coli and Klepsellia pneumonia. Regarding water, 15 species of bacteria were recorded including Escherichia coli in addition to Vibrio cholera, Aeromonus hydrophila, Pseodomonus argenosa, Proteus mirabrillis, and less significantly in Klepsellia pneumonia, Acintopacter baumannii, Klepsellia aerogenes, Proteus vulgaris, Salmonella epidermidis, Streptococcus faecalis, Anteropacter cloacae, Citropecter frndii, Edwardsiella tarda, and Serratia liquefaciens.
Conclusions: Based on results of the current study, swimming pools represent a source of various bacteria that might be transmitted to swimmer causing different infections in particular in skin. Hence, we advocated to implementation of comprehensive water quality monitoring programs and using of advanced diagnostic techniques to identify and tracking the potential pathogens with applying of effective disinfection strategies to mitigate the risk of bacterial contamination in swimming pools.

INTRODUCTION

Water is an essential resource for sustaining the life, and its contamination poses a significant threat to human health and the environment (Mishra, 2023). The prevalence of water-borne pollutants (physical, chemical and biological) has become a global concern since over one billion people lack access to clean water sources (Manetu and Karanja, 2021). One of the most widespread water quality issues is pathogen loading through the deposition of sewage and untreated wastewater, which leading degradation of aquatic ecosystems, and spreading of infectious diseases, with accumulation of hazardous substances such as heavy metals that can result in chronic poisoning and a variety of health problems (Akpor and Muchie, 2011; Karri et al., 2021; Jan et al., 2022). To address the issue of water contamination, a multifactorial approach is required involving regular monitoring, effective treatment technologies, and the implementation of stricter regulations to control the discharge of pollutants into water bodies (Altenburger et al., 2015; Brack et al., 2017).
Swimming pools are ubiquitous features of many recreational facilities, providing a refreshing and enjoyable environment for people of all ages to engage in physical activity, socialize and beat the heat during the warmer months (Anderson et al., 2014; Pangrazi and Beighle, 2019; Chaúque et al., 2022). However, the very nature of swimming pools with their high bather loads and intermittent water circulation can make them susceptible to bacterial contamination posing potential health risks to swimmers (Barna and Kádár, 2012; Bonadonna and La Rosa, 2019). One of the primary concerns regarding bacterial contamination is the presence of pathogenic microorganisms that can cause a wide-range of infections even death (Okafor, 2011; Stec et al., 2022). Furthermore, the formation of biofilms by bacterial communities within the pool and existence of wild animals such as birds, dogs and cats can act as a source of contamination and enhance their ability to resist disinfection (Guida et al., 2016; Khan et al., 2016; Nguyen et al., 2024). Additionally, investigations have been revealed the presence of multi-drug resistant bacteria on inanimate surfaces within the pool environment highlighting the potential cross-contamination and needing to rigorous cleaning and disinfection protocols (Cantón et al., 2013; Kramer and Assadian, 2014; Koeck et al., 2018; Ekowati, 2019).
In Iraq, the numbers of swimming pools have been increased significantly in last 20 years. Nonetheless, almost national studies have focused on water hygiene or water quality (Khalaf et al., 1992; Aenab and Singh, 2012), and data described the microbiological characteristics of public swimming pools remain very rare of old (Ali et al., 2009). Hence, the current study was conducted to investigate the almost prevalent bacterial contamination in indoor swimming pools in Baghdad and Wasit provinces (Iraq).

Materials and methods

Ethical approval

Morphological and biochemical characterization

Initially, all bacterial isolates were used to preparation the Gram stained slides that tested by light microscope (Zeiss, Germany) at a magnification of 1000X to identify their morphological characteristics. Finally, biochemical testing was done to the species of study isolates (Jasim et al., 2024).

***Diagnosis* of bacterial species via VITEK2 technology**

The species of bacterial isolates were identified in current study using the VITEK2 Compact System (bioMérieux, France). Briefly, bacterial suspensions were employed in 45% sterile NaCl as similar to McFarland Standards (0.5×10^8 CFU/ml).

Statistical analysis

The *t*-test and One Way-Analysis of Variance (ANOVA) in the GraphPad Prism Software (*version 8.0.2*) was applied to detect significant differences between study values (mean ± standard error) at p<0.05 (Gharban, 2024).

Results

Our findings revealed that overall 37% (37/100) of swab samples were contaminated with various species of bacteria (Figure 1). Significantly ($p<0.0283$, CI = 7.430 to 66.57, r^2 = 0.8409), pool edges were showed the higher rate of contamination [56% (14/25)] while water having the lower one [12% (3/25)] when compared to values of changing rooms [36% (9/25)] and pool ladders [44% (11/25)], (Figure 2).

This study licensed by the Scientific Committee of the Department of Biology, College of Education for Pure Sciences (University of Wasit).

Preparation of culture media and transport broth

In this study, three culture media (Blood Agar, Eosin Methylene Blue Agar and MacConkey Agar) and one transport broth, Brain-Heart Infusion (BHI), in addition to seven biochemical tests (catalase, coagulase, gas and indol production, sugar fermentation and urease activity), and Gram stain were prepared according to their manufacturers' instructions (HiMedia, India).

Samples

A total of 100 swab samples were collected from the changing rooms (total no=25), pool edges (total no=25), pool ladders (total no=25) and water (total no=25) of various indoor swimming pools found in Wasit province (Iraq) during June and July (2024). The swabs were kept in tubes containing the BHI broth and transported to the laboratory under cooled condition to be cultured as soon as possible.

Bacteriological examination

Under aseptic conditions, the swabs were streaked separately on the prepared three media, and incubated at 37°C for 24 hours, and the grown colonies were re-cultured additionally for purification (Ibraheim et al., 2023).

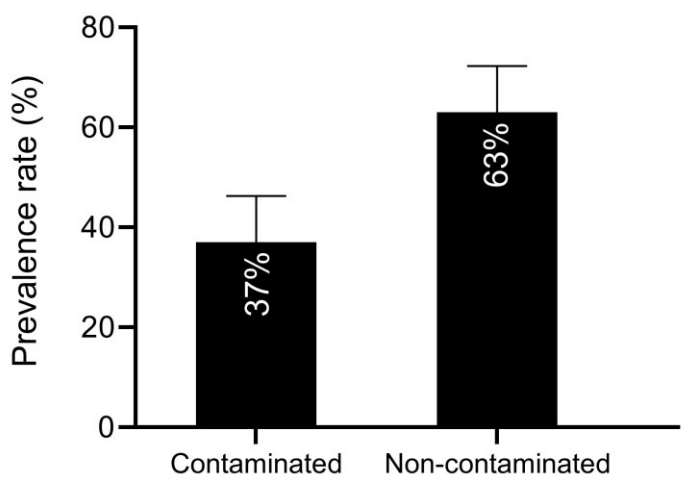

Figure (1): Total results of testing the environments and water of swimming pools (total No=100)

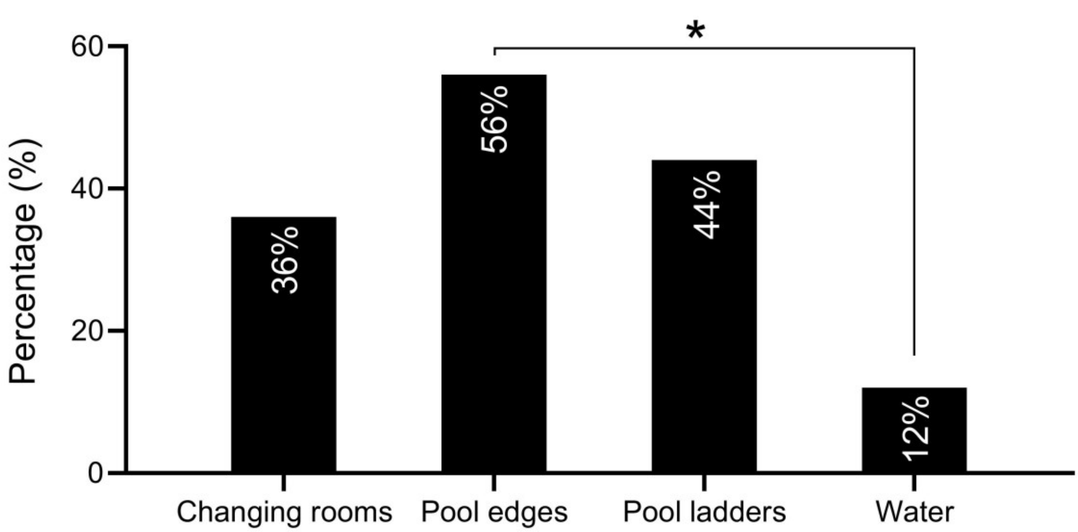

Figure (2): Distribution of contaminated samples according to type of swab sample

Subsequently, significant increase [p<0.0138, CI = 9.697 to 40.30, r^2 = 0.9001] in number of bacterial isolates was identified in water [68 (37.99%)] whereas the lowest was observed in pool ladders [27 (15.08%)] in comparison to changing rooms [39 (21.79%)] and pool edges [45 (25.14%)], (Figure 3).

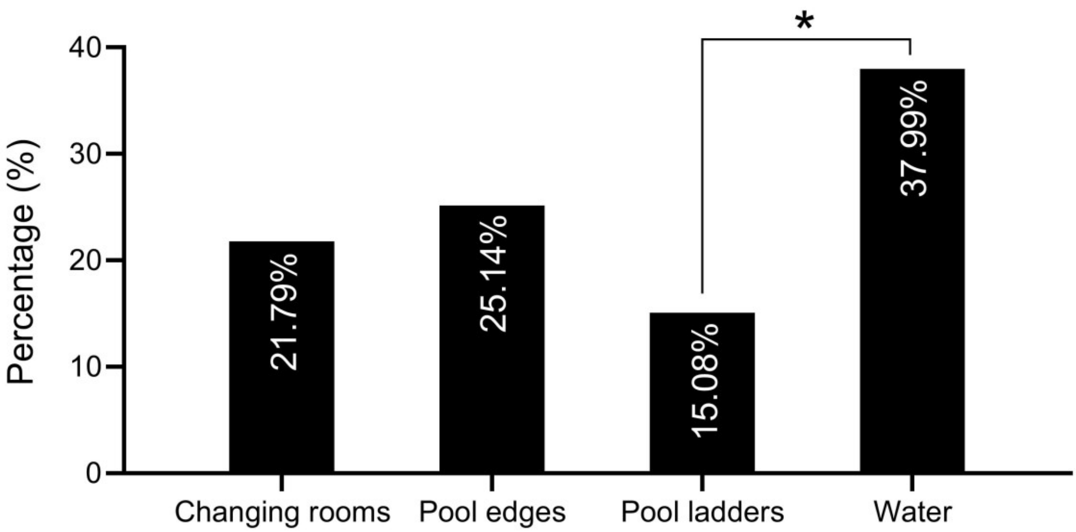

Figure (3): Number of bacterial isolates obtained from the environments and water of swimming pools

In changing rooms, a total of 12 species of bacteria was reported; in which, significant prevalence was seen in *Escherichia coli* (28.21%) as well as in *Klepsellia aerogenes* (12.82%) when compared to other bacterial species including *Pseodomonus argenosa* (10.26%), *Staphylococcus aureus* (10.26%), *Vibrio cholera* (10.26%), *Proteus mirabrillis* (7.69%), *Aeromonus hydrophila* (5.13%), *Klepsellia pneumonia* (5.13%), *Acintopacter baumannii* (2.56%), *Proteus vulgaris* (2.56%), *Salmonella epidermidis* (2.56%), and *Streptococcus faecalis* (2.56%), (Table 1).

Table (1): Bacterial species identified changing rooms of swimming pools

Species	No.	%	
Acintopacter baumannii	1	2.56	p<0.0038 CI = 2.189 to 9.576 r^2 = 0.4160
Aeromonus hydrophila	2	5.13	
Escherichia coli	11	28.21 **	
Klepsellia aerogenes	5	12.82 *	
Klepsellia pneumonia	2	5.13	
Proteus mirabrillis	3	7.69	
Proteus vulgaris	1	2.56	
Pseodomonus argenosa	4	10.26	
Salmonella epidermidis	1	2.56	
Staphylococcus aureus	4	10.26	
Streptococcus faecalis	1	2.56	
Vibrio cholera	4	10.26	

In pool edges, 11 bacterial species were recorded; in which, significant increases were seen in *Klepsellia pneumonia* (20%), *Escherichia coli* (17.78%), as well as in *Aeromonus hydrophila* (13.33%), *Vibrio cholera* (13.33%), *Proteus mirabrillis* (11.11%) in comparison to other bacterial species; *Acintopacter baumannii* (6.67%), *Klepsellia aerogenes* (4.44%), *Proteus vulgaris* (4.44%), *Salmonella epidermidis* (4.44%), *Serratia liquefaciens* (2.22%), and *Staphylococcus aureus* (2.22%), (Table 2).

Table (2): Bacterial species identified pool edges of swimming pools

Species	No.	%	
Acintopacter baumannii	3	6.67	
Aeromonus hydrophila	6	13.33 *	
Escherichia coli	8	17.78	
Klepsellia aerogenes	2	4.44	**p<0.0023**
Klepsellia pneumonia	9	20 **	**CI = 2.431 to 9.331**
Proteus mirabrillis	5	11.11	**r^2 = 0.4494**
Proteus vulgaris	2	4.44	
Salmonella epidermidis	2	4.44	
Serratia liquefaciens	1	2.22	
Staphylococcus aureus	1	2.22	
Vibrio cholera	6	13.33 *	

Concerning the pool ladders, 10 bacterial species were diagnosed (Table 3). Significant prevalence of *Vibrio cholera* (29.63%), as well as *Escherichia coli* (18.52%) and *Klepsellia pneumonia* (14.81%) was higher than *Salmonella epidermidis* (11.11%), *Proteus mirabrillis* (7.41%), *Aeromonus hydrophila* (3.7%), *Proteus vulgaris* (3.7%), *Pseodomonus argenosa* (3.7%), *Roatinolia terrigena* (3.7%), and *Staphylococcus aureus* (3.7%).

Table (3): Bacterial species identified pool ladders of swimming pools

Species	No.	%	
Aeromonus hydrophila	1	3.7	
Escherichia coli	5	18.52 *	
Klepsellia pneumonia	4	14.81	
Proteus mirabrillis	2	7.41	**p<0.0099**
Proteus vulgaris	1	3.7	**CI = 1.620 to 10.14**
Pseodomonus argenosa	1	3.7	**r^2 = 0.3485**
Roatinolia terrigena	1	3.7	
Salmonella epidermidis	3	11.11	
Staphylococcus aureus	1	3.7	
Vibrio cholera	8	29.63 **	

Regarding water, 15 species of bacteria were diagnosed; most significantly including *Escherichia coli* (19.12%) in addition to *Vibrio cholera* (13.24%), *Aeromonus hydrophila* (10.29%), *Pseodomonus argenosa* (10.29%), *Proteus mirabrillis* (8.82%), and less significantly in *Klepsellia pneumonia* (7.35%), *Acintopacter baumannii* (5.88%), *Klepsellia aerogenes* (5.88%), *Proteus vulgaris* (4.41%), *Salmonella epidermidis* (4.41%), *Streptococcus faecalis* (4.41%), *Anteropacter cloacae* (1.47%), *Citropecter frndii* (1.47%), *Edwardsiella tarda* (1.47%), and *Serratia liquefaciens* (1.47%), (Table 4).

Table (4): Bacterial species identified water of swimming pools

Species	No.	%	
Acintopacter baumannii	4	5.88	
Aeromonus hydrophila	7	10.29	

Anteropacter cloacae	1	1.47	**p<0.0003** **CI = 3.208 to 8.555** **r^2 = 0.5761**
Citropecter frndii	1	1.47	
Edwardsiella tarda	1	1.47	
Escherichia coli	13	19.12 ***	
Klepsellia aerogenes	4	5.88	
Klepsellia pneumonia	5	7.35 *	
Proteus mirabrillis	6	8.82	
Proteus vulgaris	3	4.41	
Pseodomonus argenosa	7	10.29	
Salmonella epidermidis	3	4.41	
Serratia liquefaciens	1	1.47	
Streptococcus faecalis	3	4.41	
Vibrio cholera	9	13.24 **	

DISCUSSION

Water pollution is one of the most common environmental problems in the world, which constitute a universal call to improve the lives and prospects of people (Muhammed and Abubakar, 2022). Public indoor swimming pools are one of the recreation centers which many people use every day, so, they can be contaminated by infectious agents, such as bacteria that include a large variety of unicellular microorganisms (Papadopoulou et al., 2008; Gerba and Pepper, 2019). Bacteria are ubiquitous in the environment and are able to rapidly colonize, divide and/or found in nutrient, air, soil, and water in addition to its ability to transmission directly or indirectly (Dugan, 2022). In this study, 37% of swab samples were contaminated with various species of bacteria; which found in pool edges (56%), pool ladders (44%), changing rooms (36%), and water (12%). Subsequently, the number of identified bacterial isolates was 68 (37.99%), 39 (21.79%), 45 (25.14%) and 27 (15.08%) in water, changing rooms, pool edges, and pool ladders, respectively. In changing rooms, 12 species of bacteria was reported, more significantly was *Escherichia coli* as well as *Klepsellia aerogenes*. In pool edges, 11 bacterial species were recorded; in which, significant increases were seen in *Klepsellia pneumonia*, *Escherichia coli*, as well as in *Aeromonus hydrophila*, and *Vibrio cholera*. Concerning the pool ladders, 10 bacterial species were diagnosed, particularly *Vibrio cholera*, *Escherichia coli* and *Klepsellia pneumonia*. Regarding water, 15 species of bacteria were recorded including *Escherichia coli* in addition to *Vibrio cholera*, *Aeromonus hydrophila*, *Pseodomonus argenosa*, *Proteus mirabrillis*, and less significantly in *Klepsellia pneumonia*, *Acintopacter baumannii*, *Klepsellia aerogenes*, *Proteus vulgaris*, *Salmonella epidermidis*, *Streptococcus faecalis*, *Anteropacter cloacae*, *Citropecter frndii*, *Edwardsiella tarda*, and *Serratia liquefaciens*. Based on our data, *Escherichia coli*, *Klepsellia* spp. (*K. aerogenes* and *K. pneumonia*) and *Vibrio cholera* were the almost bacterial species identified in environments and water of swimming pools. *Escherichia coli* consider an enteric pathogen that transmitted from human or animal feces to susceptible hosts through interactions with food and environmental compartments (water, surfaces, soil, hands, and flies), (Van Elsas et al., 2011; Loayza et al., 2020). This bacterium is used as indicator to study the sources and fate of fecal contamination in the environment since the presence of *E. coli* in water is associated usually with increased risk of both enteric pathogens and diarrheal diseases (Hunter, 2003; Gomes et al., 2016; Ercumen et al., 2017).

Friedman et al. (1999) detected a cluster of gastrointestinal illnesses, including one case of hemolytic-uremic syndrome and one culture-confirmed *E. coli* infection followed a trailer park pool party. In the United Kingdom, report describes the investigation of an outbreak associated with a local leisure center pool and makes recommendations about safe management of such facilities because it documented that a number of outbreaks are associated with swimming pools due to relative susceptibility of *E. coli* to adequate levels of free chlorine (Verma et al., 2007).

Rasti et al. (2012) mentioned that some bacteria are resistant to sodium hypochlorite that is used for disinfecting pools and considered to be an opportunist microorganism that is involved in different infections. Despite the fact that these bacteria are not considered major threats and play no direct role in producing infections in humans, they should not be ignored when checking the water quality of swimming pools (Purohit et al., 2020; Nowicki et al., 2021).

Ghasemi et al. (2019) reported that swimming pools might be contaminated with microorganisms associated with swimmers like fecal contamination of the water, accidental fecal release or residual fecal material on bodies, and non-fecal shedding like vomit, mucous, saliva, skin, mouth, and upper respiratory tract contamination. The previous studies referred that some bacteria may cause a variety of respiratory, dermal, or central nervous system infections or diseases (Nichols, 2006; Papadopoulou et al., 2008; WHO, 2009). Another ones have been investigated the quality of pool waters during the working day, and some researchers have believed the highest number of swimmers during holidays and weekend are young children, therefore, the water sampling would be better evaluated in these times (Fantuzzi et al., 2010; Lévesque et al., 2015; Carter et al., 2019).

CONCLUSION

Based on results of the current study, swimming pools represent a source of various bacteria that might be transmitted to swimmer causing different infections in particular in skin. Hence, we advocated to implementation of comprehensive water quality monitoring programs and using of advanced diagnostic techniques to identify and tracking the potential pathogens with applying of effective disinfection strategies to mitigate the risk of bacterial contamination in swimming pools.

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