ABSTRACT

As drinking water fountains are the main source of free drinking water in public spaces, microbiological quality standards in such facilities are strongly relevant to public health. The objective of this study was to evaluate the microbiological risk associated with location of drinking water fountains in a public institution, specifically investigating the presence of potentially pathogenic microorganisms. Microbiological analysis was conducted with samples from seven drinking water fountain bubbler faucets located near restroom facilities. We evaluated the number of users of these fountains under two parameters: individuals who only drank water, and individuals who drank water after using the restroom to assess if the number and habits of users could contribute to cross-contamination. Our findings revealed the presence of potentially pathogenic microorganisms, including \textit{Staphylococcus aureus} and \textit{Salmonella} spp. with no correlation between the number of isolated microorganisms and the number of water fountain users. We therefore suggest that the contamination present on the surface of the drinking water fountains is due to the location of the water fountains.

KEYWORDS

RESUMO

Sendo os bebedouros a principal fonte de água potável gratuita nos espaços públicos, os padrões de qualidade microbiológica dessas instalações são bastante relevantes para a saúde pública. O objetivo do presente estudo foi avaliar o risco microbiológico associado à localização de bebedouros em uma instituição pública, especificamente, investigando a presença de microrganismos potencialmente patogênicos. A análise microbiológica foi realizada com amostras de sete válvulas de jato de bebedouros localizados perto de banheiros. Contamos o número de usuários dos bebedouros sob dois parâmetros: indivíduos que somente beberam água, indivíduos que usaram o banheiro e depois beberam água para verificar se o número ou os hábitos dos usuários poderiam contribuir para a contaminação cruzada. Nossos resultados revelaram a presença de microrganismos potencialmente patogênicos, incluindo Staphylococcus aureus e Salmonella spp, sem correlação entre o número de microrganismos isolados e o número de usuários dos bebedouros. Sugerimos, portanto, que a contaminação presente na superfície dos bebedouros se deve à localização dos bebedouros.

PALAVRAS-CHAVE

Microbiologia. Contaminação. Fontes de Água.

RESUMEN

Dado que los bebederos son la principal fuente de agua potable gratuita en los espacios públicos, los estándares de calidad microbiológica de estas instalaciones son muy relevantes para la salud pública. El objetivo del presente estudio fue evaluar el riesgo microbiológico asociado a la ubicación de bebederos en una institución pública, investigando específicamente la presencia de microorganismos potencialmente patogénicos. El análisis microbiológico se realizó con muestras de siete válvulas de bebederos de agua ubicadas cerca de los inodoros. Contamos el número de usuarios de bebederos bajo dos parámetros: individuos que solo bebían agua y personas que usaban el baño y luego bebían agua para ver si el número o los hábitos de los usuarios podían contribuir a la contaminación cruzada. Nuestros resultados revelaron la presencia de microorganismos potencialmente patógenos, incluidos Staphylococcus aureus y Salmonella spp, sin correlación entre el número de microorganismos aislados y el número de usuarios de los bebederos. Por lo tanto, sugerimos que la contaminación presente en la superficie se debe a la ubicación de los bebederos.
1 INTRODUCTION

The ability of microorganisms to grow under high humidity conditions and on inanimate surfaces makes drinking water a suitable environment for microbial survival. Many of these microorganisms are harmless to human health; however, some are pathogenic and can be associated with a spectrum of hazardous effects on their hosts. Under high relative humidity, the adherence and development of most types of microorganisms is favored, which further increases the organism fomite-to-finger transfer efficiency rates (LOPEZ et al., 2013). Pathogenic organisms can survive for hours to months on surfaces or fomites, depending on the number, type of microorganism, and environmental conditions, enabling the formation of biofilms at or near the distal points of use (LOPEZ et al., 2013, KRAMER et al., 2006, DECKER; PALMORE, 2014).

Consequently, water fountains have emerged as an important sanitary concern. It is common for drinking water fountains to be installed next to restroom facilities, sharing a main supply of water; however, due to the eminent cross-contamination risk, the placement of drinking water sources in such potentially high contamination areas is heavily inappropriate. While factors such as hygiene and disinfection procedures are important, the location may also be intrinsically correlated with microbial contamination on fountain surfaces.

Various studies have reported restroom surfaces as hotspots of microbial contamination, indicating that routine use of restrooms results in the dispersal of urine- and fecal-associated bacteria throughout the surrounding environment (FLORES et al., 2011, MATINI et al., 2020, MKRTCHYAN et al., 2013, BARKER; BLOOMFIELD, 2000). As surfaces contaminated with pathogens can be sources of indirect transmission, our hands, after using public restrooms, can act as potential vehicles for the transmission of human pathogens between restrooms and adjacent water fountains, representing an important route of contamination of pathogens to users (FLORES et al., 2011, LOPEZ et al., 2013).

As drinking water fountains are the main source of free drinking water in public spaces, the microbiological quality standards in such facilities are strongly relevant to public health. This issue is relatively under-researched, and studies have reported an increase in contamination of outcoming water, compared to supply water, and an elevated microbial count on water device surfaces, evidencing the importance of quality monitoring and adequate disinfection (WALTERS; CRAM, 2002, BRIGHT et al., 2010, SACCHETTI et al., 2014).

The consumption of microbiologically unsafe drinking water can lead to outbreaks of waterborne diseases (LECLERC et al., 2002). To ensure safety, the reduction of microbiological risk associated with water intake to acceptable levels is an important goal for water supply, involving quality pro-
cedures from the treatment plant to the point of consumption (OMAR et al., 2017; VIEIRA, 2007). Post-provision contamination resulting from inadequate handling or storage of dispensed water, poor sanitation, and hygiene issues are all responsible for decline in water quality at the point of consumption, resulting in serious health consequences for users (ERCUMEN et al., 2015, GRILC et al., 2015).

In this study, we sought to investigate the sanitary risk associated with location of drinking water fountains at the Faculty of Integrated Sciences of Pontal, Ituiutaba, Brazil, through the identification of pathogenic microorganisms associated with its surface. We evaluated if the number and habits of users could be risks factors for cross-contamination.

## 2 MATERIAL AND METHODS

The study was conducted at the Federal University of Uberlândia, Pontal Campus, in the city of Ituiutaba, Minas Gerais, Brazil. We evaluated the number of individuals who used seven of the 13 water fountains of the faculty, all of which were located in the three most crowded buildings at the campus. All of the drinking water fountains at the campus Pontal are located next to the restroom facilities. Counting was performed for 3 h in May 2017 to determine the number of individuals who used only the drinking water fountain and those who used the drinking water fountain after using the restroom. All observations were made simultaneously, on a single day, in a period with a constant flux of persons. The drinking water fountains were all located next to the restroom facilities.

After the observation period, the biological samples were collected from the the seven drinking water fountains bubbler faucets. The fountains were chosen randomly at three buildings of the faculty (blocks A, B, and D). Samples were collected using sterile swabs and placed into test tubes containing nutrient broth under aseptic conditions. Following a 24-h incubation at 37 °C to allow microbial growth, samples were diluted $10^{-2}$ or $10^{-3}$. A 100μl aliquot of each sample was inoculated into MacConkey (Merck KGaA), Mannitol-salt (Merck KGaA), *Salmonella-Shigella* (Alere), Sabouraud dextrose (Acumedia), or blood (Kasvi) agar plates in triplicate. Plates were incubated at 37 °C for 24 h, and colonies were subsequently counted, replated, and identified. At each fountain, the water pressure coming out of the fountain was considered adequate for consumption.

## 3 RESULTS

Fungal growth was not observed in any of the samples. The number of bacterial colonies grown in bacterial-specific culture media was high, with the majority of microorganisms growing on blood agar media (FIGURE 1), an enriched bacterial medium used for a wide range of organisms, including pathogens.
**Figure 1**  – Number of colonies in each culture medium tested and identified at each collection point. Colonies were counted manually. Data shown represents the means of three biological replicates. The error bar indicates the standard deviation (SD). Culture media identified as BL (Blood agar); MT (Mannitol-salt agar); MC (MacConkey agar); and SS (Salmonella-Shigella agar)

Source: Research Data

Analysis of the colonies on the differential selective agar plates revealed an elevated number of *Staphylococcus aureus* and *Salmonella* spp. colonies. *Escherichia coli* isolates were not detected in any of the samples. The isolates were not confirmed using complementary tests. *S. aureus* and *Salmonella* spp. are health-relevant pathogens that are capable of forming biofilms on different surfaces, including stainless steel, and are related to the occurrence of poisoning by the consumption of contaminated food or water (JUNGFER et al., 2013, GRILC et al., 2015). Biofilm formation also promotes other unfavorable conditions at drinking water fountains, including reduced flow through blocked tubes and corrosion of stainless steel (PARKAR et al., 2004).

The number of drinking water fountain users was determined to evaluate a possible relationship between restroom use and microbiological contamination at drinking water fountains. We divided users into two groups: (1) individuals who used the restroom before using the drinking water fountain and (2) individuals who only used the drinking water fountains (Figure 2).

**Figure 2**  – Frequency of use of drinking water fountains. Persons who only used the water fountain (identified as water) and persons who used the water fountain after using the restroom (identified as restroom + water)

Source: Research Data
4 DISCUSSION

The safety of drinking water has always been a health concern worldwide, especially in developing countries. In general, water dispensed from drinking water dispensers, as water cooler devices, was more contaminated than the water supplied to them (SACCHETTI et al., 2014, ZANETTI et al., 2009). The contamination may be associated with a lack of proper cleaning measures at the point of use, deteriorating the quality of dispensed water and with location, next to restroom facilities (DIDUCH et al., 2013, SILVA et al., 2016, WALTERS; CRAM, 2002).

Our study assessed the number of bacterial colonies cultured from the samples collected from drinking water fountains at the campus Pontal. All the drinking water fountains at the campus are located closer to restrooms facilities. We evaluated if the number and habits of users could represent risks factors for cross-contamination.

The water fountain on the ground floor block D presented the highest count of bacterial colonies, and the most elevated diversity of potentially pathogenic species, including *S. aureus* and *Salmonella*. The number of colonies counted from the drinking water fountain on the third floor of block A was considerably high, followed by the fountain on the second floor B. In both block A and B, potentially pathogenic species were identified.

The location with the highest number of users did not correspond to the sample with the highest number of colonies and greater diversity. The drinking water fountain on the first floor, block D, presented the highest number of users and the lowest number of bacterial colonies. The number of bacterial colonies on the second floor, block D, fountain with the highest number of users who used the restroom prior to drinking water, was low compared to the fountains on the ground floor, block D, and the third floor at block A. A high fomite-to-finger transfer efficiency of enteropathogens and pathogen found in the skin (e.g., *S. aureus*) was observed under high relative humidity (FLORES et al., 2011). In our study, a significant number of people had used the restroom prior to drinking water in block D on the first and second floors; however, this factor did not contribute to the diversity or the number of colonies identified in the water fountains.

Although no correlation was observed between the number of users and the number of bacterial colonies identified in the samples from the drinking water fountains, the microbiological data show a high number of potentially pathogenic bacteria. These results may be associated with the location of the drinking fountains regardless of the number of users or the use of the restrooms before getting to drinking fountains.

In a study assessing drinking water fountains installed in restroom areas (WALTERS; CRAM, 2002), the microbiological results from swab samples collected at drinking water fountains presented high colony counts, above the level that would be expected or desired in any drinking water facility. These results indicate that the proximity of restrooms carries an inherent risk of cross-contamination, one reason to avoid such areas for fountain installation.

Another study isolated different bacteria, including *S. aureus* and *Staphylococcus saprophyticus*, from the nozzle of water dispensers positioned closer to restrooms, highlighting an imminent health risk for the users of the drinking water fountains (SILVA et al., 2016).
At the Pontal campus of the Federal University of Uberlândia, fountains were visually free from dirt and impurities such as mold or mud. However, bacteria can remain on surfaces even with the use of chemical cleaning agents (DEQUEIROZ; DAY, 2007).

Our findings suggest that the location of the water fountain near the restrooms, which is a strategy to share an already available major water supply facility, may contribute to the observed elevated microbial count, irrespective of the number of individuals using each fountain. The proximity to restrooms could also contribute to microbial adherence and resistance at fountains, making these locations reservoirs of potentially pathogenic microorganisms.

5 CONCLUSIONS

Microbial contamination of drinking water is a risk to human health because water can act as a vector for the transmission of infectious diseases caused by pathogenic microorganisms, particularly from fecal contamination. A global approach assessing all aspects potentially involved in microbiological contamination, such as transport and distribution, operational monitoring, and sanitary surveillance, may contribute to the promotion of drinking water quality (BOAKYE-ANSAH et al., 2016).

A significant risk of contamination in water fountains is associated with the location of the water fountains near restrooms (SILVA et al., 2016, WALTERS; CRAM, 2002). The repositioning of drinking water fountains may reduce the risk of contamination.

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