Staphylococcus aureus and methicillin-resistant *Staphylococcus aureus* (MRSA) in drinking water fountains in urban parks

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ABSTRACT

The presence of *Staphylococcus aureus* in drinking water is a concern because of its potential to cause human infection and also because of its multiple antimicrobial resistance. This study evaluated the water quality of drinking water fountains and mist makers in four municipal parks of São Paulo for 13 months. Although all samples met bacteriological water quality criteria according to Brazilian regulations, the absence of residual chlorine (<0.1 mg/L) was observed. These data were significantly correlated with the frequency of *S. aureus* that was found in 25.2% of the samples. The *mecA* gene was detected in 36.7% of the isolates demonstrating its potential for resistance to several antimicrobials. Furthermore, 27.3% isolates carrying the *mecA* gene had methicillin-resistant Staphylococcus aureus (MRSA) phenotypic potential. The presence of *S. aureus* with characteristics of microbial resistance in water for human consumption is an unprecedented finding. Hence, conducting surveillance for opportunistic bacteria, such as staphylococci in drinking water, is reasonable to take control measures and to protect human health, especially in public places with high attendance.

Key words | antibiotic resistance, drinking water, environmental health, *Staphylococcus aureus*

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HIGHLIGHTS

- *Staphylococcus aureus* species is able to survive in drinking water distributed by public devices.
- Chlorine concentrations below the recommended level favors the growth of the pathogen.
- The isolates presented high frequency of resistance to several antibiotics.
- Some isolates were identified as MRSA.
- The presence of antibiotic-resistant *S. aureus* in drinking water poses a risk to human health.

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GRAPHICAL ABSTRACT

INTRODUCTION

Urban parks are relevant spaces in large cities, which have an important role as a healthy environment to enhance quality of life (Razak *et al.* 2016). These spaces provide several environmental and ecological services for human beings besides their importance for social and psychological benefits (Chiesura 2004). There are around 100 urban parks in São Paulo city (Limnios & Furlan 2013), which must offer safe drinking water access that is provided by drinking water fountains usually supplied by the public water network. Some parks have mist makers used, especially by children, to refresh. In both of these types of urban equipment there is formation of biofilms that can harbor opportunistic pathogenic microorganisms (Sun *et al.* 2014), which can be transferred to the drinking water or to the mist formed by mist makers (Cucarella *et al.* 2001; Otzen & Nielsen 2008). *Staphylococcus aureus* is widely recognized as the major leading community-based bacterial agent in the world. It is worth highlighting its importance as a human pathogen, due to its ability to cause infections as well as its capacity to adapt to diverse environmental conditions and multiple antimicrobial resistance (WHO 2017c). Also, *S. aureus* forms biofilms, which enhances its persistence in water systems (Otto 2006; Høiby *et al.* 2010) and its resistance to antibiotics and disinfectants (Mahapatra *et al.* 2015; Stewart *et al.* 2015). The resistance to antimicrobials by *S. aureus* stands out in its resistance to methicillin (methicillin-resistant *Staphylococcus aureus* (MRSA)), responsible for more than 50% of the isolates associated with infections in health facilities worldwide (Guzmán-Blanco *et al.* 2009). According to the World Health Organization (WHO), it is estimated that people infected with MRSA have 64% more chance of dying than those with a non-resistant form of infection (WHO 2017c). *S. aureus* presence in drinking water is poorly explored and its potential health risks are unknown (WHO 2017a); nevertheless, there are reports of infections associated with several water sources (Armstrong *et al.* 1981; Kessie *et al.* 1998; Harakeh *et al.* 2006; Seyedmonir *et al.* 2015). Considering the importance of these public spaces, the objective of this study was to determine the occurrence of *S. aureus* in drinking water fountains and biofilms from mist makers in urban parks as well as virulence factors and antimicrobial resistance. Currently, various research methods are used to detect and identify pathogens in aquatic environments, such as MALDI-TOF MS (mass spectrometry), which has been highlighted by various studies due to it being a fast, sensitive, and economic method that allows differentiation of subspecies and lineages, besides being applied in epidemiological studies (Singhal *et al.* 2015; Popović *et al.* 2017).

METHODS

Area characterization

The main characteristics of the four public parks located in São Paulo city are shown in Table 1. The water samples were collected monthly from March 2017 to March 2018 from drinking water fountains ($n = 468$) and biofilm from mist makers ($n = 84$), totaling 552 samples. Water samples were collected according to *Standard Methods for the Examination of Water and Wastewater* (AWWA 2012) and examined within a 24 h period. For collecting biofilm samples sterile swabs were used and preserved in Stuart agar gel medium (COPAN, CA, USA) and refrigerated until analysis within a 24 h period.

Fecal indicator bacteria (FIB), heterotrophic plate count (HPC) and residual chlorine

For determining water quality as issued by the Drinking Water Brazilian legislation (Brasil 2017) testing of FIB – *Escherichia coli*, heterotrophic bacteria and residual chlorine were performed. Residual chlorine was measured in samples by a colorimetric method using Free-chlorine Analyzer Policontrol (São Paulo, Brazil). For each test the enumeration and determination of *E. coli* and the heterotrophic plate count (HPC) in drinking water samples were performed according to *Standard Methods for the Examination of Water and Wastewater* (AWWA 2012).

Determination of *S. aureus*

The determination of *S. aureus* was carried out according to *Standard Methods for the Examination of Water and Wastewater* (AWWA 2012). Briefly, a volume of 100 mL was concentrated through 0.45 μm of porosity and 47 mm diameter membrane filter (Millipore). Then, the membranes were transferred to Petri dishes containing Baird Parker (BP) agar (Difco, MI, USA). For biofilms' samples the collected material on swabs was transferred to Petri dishes containing BP agar (Difco). All plates were incubated for 48 h at 35 ± 0.5 °C, and after the incubation period typical colonies (black, shiny, convex, and surrounded by a clear zone) were submitted to confirmatory analysis or tests for phenotypical characterization of the *S. aureus*.

Characterization of the isolates

Typical colonies of *S. aureus* were transferred to Brain Heart Infusion (BHI) agar (Difco) and incubated at 35 ± 0.5 °C overnight. Each typical colony was submitted to Gram stain and tested as follows: catalase reaction (Hydrogen Peroxide Solution 10 V, Laborclin, PR – BRA); tube coagulase reaction (Coagu-plasma, Laborclin); DNase agar test (Difco) and fermentation of Mannitol Salt agar (Difco), which was performed according to the recommendations by the Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância, in Portuguese) (ANVISA 2013).

Table 1 | Main characteristics of the four public parks located in São Paulo city considered for this study

Parks	Characteristics	Attendance (people per weekend)	Drinking fountains (n)	Mist makers (n)
Aclimação (112,000 m^2)	Located in west part of the city. There is an artificial lake, diversity of vegetation and different types of bird species	7,000	6	1
Buenos Aires (25,000 m^2)	Located in central area of the city. There is a diversity of vegetation and different types of bird species	10,000	3	1
Ibirapuera (1,584,000 m^2)	Located in the south area, it is the most important park for leisure	130,000	3	3
Piqueri (97,200 m^2)	Located in the east area of the city, it consists of a forest	9,000	6	0

Source: São Paulo City Council, 2018.

Molecular identification

DNA extraction

DNA was extracted according to the method described by Costa *et al.* (2005) with modifications as follows: (i) isolates were inoculated in 5 mL BHI broth (Difco) and incubated overnight at 35 ± 0.2 °C; (ii) after the incubation period 1 mL of the inoculum was transferred to a microtube and centrifuged at 130,000 rpm for 10 min. The pellet yielded was resuspended in a solution of 25 μL lysostaphin (1 μg/mL) (Sigma, MO, USA) and 25 μL deionized water (Sigma) and heated at 37 °C for 10 min. Then, 50 μL of proteinase K (20 mg/mL) (Roche, CA, USA) and 150 μL Tris buffer (0.1 M, pH 7.5) (USB Corp., OH, USA) were added followed by an incubation at 37 °C for 10 min and then heated at 95 °C for 10 min. After the incubation time a pulse centrifugation at 13,000 rpm for 10 min was carried out (Heraeus Biofuge Pico, Kendro Laboratory Products, Hanau, Germany) and 5 μL of the extracted DNA were used for the polymerase chain reaction (PCR) reaction and another aliquot of 5 μL for the PCR-gel assay.

Identification of *S. aureus* and virulence and resistance genes by PCR

The identification of the isolates of presumptive *S. aureus* species was performed using the pair of genes *coa* described by Nagaraj *et al.* (2014) and the pair of *nuc* described by Barski *et al.* (1996). For virulence factors the pair of genes *sea*, *seg* (enterotoxins) and *luks-PV* (Panton–Valentine leucocidin) described by Jarraud *et al.* (2002) were used.

Identification of MRSA

MRSA was classified according to Goering *et al.* (2019) and CLSI (CLSI 2018), which considering the strains' genotype profile the presence of gene *mecA* and also phenotypic resistance to oxacillin or cefoxitin. Amplification of the *mecA* gene was performed according to Okuma *et al.* (2002).

PCR-gel conditions

The PCR-gel assay was performed with 5 μL DNA extracted from a bacterial culture described previously and added to 20 μL of amplification mix containing 1X reaction buffer, 15.8 μL ultrapure water (LGC Biotecnologia, São Paulo, Brazil), 2.5 μL of 5X colorless GoTaq flexi buffer (Promega, Madison, WI, USA), 1.0 μL of 25 mM $MgCl_2$ (Promega), 1.0 μL of 10 μM each pair of primers, 0.5 μL of 200 μM dNTP (Thermo Fisher Scientific, MA, USA), and 0.2 μL of 5 μ/μL GOTaq® Flexi DNA Polymerase (Promega). The amplified PCR fragments were loaded in 2% agarose gels (Bio-Rad Laboratories, Hercules, CA, USA) in 1× Tris-borate–EDTA buffer (0.05 M Tris-borate, 0.03 M EDTA) containing 2 μL ethidium bromide (10 mg/mL, Pharmacia Biotech, Uppsala, Sweden) run at 70 V for 40 min and visualized using UV illumination.

Identification by mass spectrometry (MALDI-TOF MS)

The DNA isolates were extracted according to the method described by Wolters *et al.* (2011) with modifications as follows: isolates were inoculated in 5 mL BHI broth (Difco) and incubated overnight at 35 ± 0.2 °C and transferred to Petri dishes containing BHI agar (Difco) that were incubated for 48 h at 35 ± 0.5 °C. After incubation, one to three colonies were suspended in 300 μL of sterile water in a microtube and density was adjusted based on McFarland 0.5 scale. The adjusted suspension was mixed with 900 μL of ethanol and then centrifuged at 12,000 rpm for 2 min. After the centrifugation step the supernatant was discarded, ethanol was removed, and pellets were dried at room temperature. The pellet was resuspended in 30 μL of formic acid (70%), 30 μL acetonitrile was added and the mixture was homogenized by vortexing. Again, the mixture was centrifuged at 12,000 rpm for 2 min and 40 μL of the supernatant was collected and stored at −20 °C until use. For MALDI-TOF analysis, 1 μL aliquots of the supernatant were spotted onto the ground steel MALDI target (Bruker Daltonik, Leipzig, Germany) and dried at room temperature. To each sample was added 2 μL of matrix solution (saturated solution of α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile with 2.5% trifluoroacetic acid) (Sigma-Aldrich, Saint Louis, MO, USA)

and then samples were dried at room temperature. The analysis was done as previously described by Moreno *et al.* (2018). Mass spectra were acquired using a Microflex™ mass spectrometer (Bruker Daltonik, Leipzig, Germany); spectra were acquired in linear positive mode at a laser frequency of 20 Hz within a mass range from 2,000 to 20,000 Da. Acceleration voltage was 20 kV, IS2 voltage was maintained at 18.6 kV, and the extraction delay time was 200 ns. For each sample spot, one sum spectrum was accumulated from 300 measurements (6×50 laser shots on different locations). For each of the 23 isolates of the reference set, each extract was analyzed in duplicate with ATTC 23213. The extracts were identified with the manufacturer's software MALDI BioTyper™ 3.0 and standard Bruker interpretative criteria were applied; scores $\geq$2.0 were accepted for species assignment and scores $\geq$1.7 but $\leq$2.0 for genus identification.

Antimicrobial susceptibility

The isolates were tested for antimicrobial susceptibility according to Clinical and Laboratory Standards Institute (CLSI 2017), as quality control of the antibiogram *S. aureus* ATCC 2832 was used. The Kirby–Bauer disk diffusion method was used to determine the antimicrobial susceptibility for the following antibiotics (μg): cefoxitin (30) (CFO); ciprofloxacin (5) (CIP); clindamycin (2) (CLI); chloramphenicol (30) (CLO); erythromycin (15) (ERI); gentamicin (10) (GEN); penicillin g (10) (PEN); rifampicin (5) (RIF); sulfazotrin (25) (SUT); tetracycline (30) (TET); and oxacillin (1) (OXA) (DME, SP Brazil). The isolates were grown in BHI broth (Difco) at 37 ± 2 °C for 24 h. The density was adjusted based on McFarland 0.5 scale (1.5×10^8 CFU/mL) by direct suspension of the colonies in Mueller Hinton (MH) broth (Difco). The inoculum was transferred to MH agar (Difco) and incubated at 35 ± 2 °C for 24 h. The antimicrobial susceptibility for vancomycin was determined by microdilution method based on CLSI (2017). The strains were cultured in BHI broth at the same conditions mentioned before and inoculum density was adjusted for the McFarland 0.5 scale (5×10^5 CFU/mL) by direct colony suspension in MH broth (Difco). Resistance to Vancomycin was tested at concentrations (μg/mL) as follows: 0.15, 0.25, 0.5, 1, 2, 4, 8, 16, and 32, and the minimal inhibitory concentration (MIC) was read manually after 24 h of incubation.

RESULTS

All water samples ($n = 468$) met the Brazilian bacteriological standards of quality, *E. coli* was not detected and the HPC was below the maximum value of 500 CFU/mL as established by the Brazilian legislation (Brasil 2017). The concentration of residual chlorine ($\geq$0.5 mg/L) was in accordance with the legislation standard in 86.3% (404/468) of the samples. Out of 404 samples, 18.3% (74/404) were from Ibirapuera park, 34.9% (141/404) from Piqueri park, 16.1% (65/404) from Buenos Aires park, and 30.7% (124/404) from Aclimação park. Taking into account all 552 samples analyzed (468 water samples plus 84 mist maker samples) 30 (5.4%), 27 from drinking water fountains samples and three from mist maker biofilm were contaminated with *S. aureus* according to biochemical profile (described in method section). From the contaminated samples, all 119 isolates of *S. aureus* were submitted to confirmation that was performed by polymerase chain reaction (PCR), 104 from drinking water fountain samples and 15 from biofilm (mist makers). Out of 119 isolates, 30 (25.2%) were confirmed as *S. aureus*. Furthermore, analysis for identification of all 119 isolates carried out using mass spectrometry (MALDI-TOF MS) confirmed the results obtained by molecular assays (Table 2 and Table S1, Supplementary material). All samples contaminated with *S. aureus* were not in accordance with the residual chlorine values established by the current legislation.

Detection of virulence genes (*sea*, *seg* and *luk*-PVL), antimicrobial susceptibility and methicillin resistance gene (*mecA*)

The virulence marker genes, *sea*, *seg* and *luk*-PVL, were not detected. All confirmed isolates of *S. aureus* ($n = 30$) were tested with an antimicrobial susceptibility test. All of them were susceptible to vancomycin and to at least one out of the other 11 antibiotics tested. The results concerning antimicrobial resistance are presented in Table 3.

Table 2 | Phenotypical and genotypical profile of *S. aureus* isolated from water samples and biofilms from municipal parks

				Phenotypical characteristics					Gene presence						
Park	Collection date	Sample	RC (mg/L)	Coag 4 h	Coag 12 h	Cat	DNase	Mannitol	*mecA*	*nuc*	*coa*	*sea*	*seg*	*Luk*-PVL	MALDI-TOF score
Aclimação	04/24/2017	Water	<0.1	+	+	+	+	+	−	−	−	−	−	—-	2.447
Aclimação	04/24/2017	Water	<0.1	+	+	+	+	+	−	−	+	−	−	−	2.4
Aclimação	04/24/2017	Water	<0.1	+	+	+	+	+	−	+	+	−	−	−	2.471
Aclimação	06/05/2017	Water	<0.1	+	+	+	+	−	−	+	+	−	−	−	2.188
Aclimação	06/05/2017	Water	<0.1	+	+	+	+	+	−	+	+	−	−	−	2.506
Aclimação	12/04/2017	Water	<0.1	+	−	+	+	+	−	−	+	−	−	−	2.511
Aclimação	01/29/2018	Water	<0.1	+	−	+	+	−	−	−	−	−	−	−	2.402
Aclimação	03/05/2018	Water	<0.1	+	+	+	+	+	−	−	+	−	−	−	2.541
Aclimação	03/05/2018	Water	<0.1	+	-	+	−	+	−	+	+	−	−	−	2.464
Buenos Aires	04/03/2017	Water	<0.1	−	−	+	+	+	+	−	−	−	−	−	2.483
Buenos Aires	04/03/2017	Water	<0.1	+	+	+	+	−	+	−	−	−	−	−	2.437
Buenos Aires	04/24/2017	Water	<0.1	+	+	+	+	+	-	−	+	−	−	−	2.448
Buenos Aires	05/08/2017	Water	<0.1	−	−	+	+	+	+	+	+	−	−	−	2.501
Buenos Aires	05/08/2017	Water	<0.1	+	+	+	+	−	+	+	−	−	−	−	2.391
Buenos Aires	06/05/2017	Water	<0.1	+	+	+	+	+	+	+	+	−	−	−	2.065
Buenos Aires	09/25/2017	Water	<0.1	+	−	+	+	+	−	−	−	−	−	−	2.527
Buenos Aires	09/25/2017	Water	<0.1	+	−	+	+	−	+	−	−	−	−	−	2.371
Buenos Aires	09/25/2017	Water	<0.1	+	−	+	+	+	−	−	−	−	−	−	2.49
Ibirapuera	13/03/2017	Biofilm	N/P	+	−	+	+	−	−	−	−	−	−	−	2.243
Ibirapuera	22/05/2017	Water	<0.1	+	+	+	+	+	−	+	+	−	−	−	2.459
Ibirapuera	26/06/2017	Biofilm	N/P	−	−	+	+	+	−	+	−	−	-	−	2.423
Ibirapuera	26/06/2017	Water	<0.1	−	−	+	+	+	+	+	+	−	−	−	2.432
Ibirapuera	16/10/2017	Water	<0.1	+	+	+	+	−	−	−	−	−	−	−	2.481
Ibirapuera	16/10/2017	Biofilm	N/P	+	+	+	+	−	−	+	−	−	−	−	2.449
Ibirapuera	04/12/2017	Water	<0.1	+	−	+	+	−	−	−	−	−	−	−	2.51
Piqueri	03/27/2017	Water	<0.1	+	+	+	+	+	+	−	−	−	−	—-	2.215
Piqueri	04/24/2017	Water	<0.1	+	+	+	+	+	+	+	+	-	-	-	2.511
Piqueri	06/26/2017	Water	<0.1	−	−	+	+	+	−	−	−	−	−	−	2.523
Piqueri	10/23/2017	Water	<0.1	−	+	+	+	+	+	−	−	−	−	−	2.51
Piqueri	12/04/2017	Water	<0.1	+	−	+	+	−	+	+	+	−	−	−	2.44

RC: residual chlorine; Cat: catalase test; Coag: tube coagulase test; N/P: not performed.

The frequency of resistance was 43.3% (13/30) to penicillin, 30.3% (10/30) to sulfazotrin, 30% (9/30) to erythromycin, 26.6% (8/30) to oxacillin, 16.6% (5/30) to clindamycin and to tetracycline, 10% (3/30) to rifampicin and cefoxitin, 6.6% (2/30) to ciprofloxacin, and 3.3% (1/30) to gentamicin. It is worth emphasizing that 23 isolates (76.6%) were susceptible for at least two antibiotics and some of them were resistant to nine antibiotics.

Out of 27 isolates from water samples, 20 (74%) were resistant to at least two antibiotics tested. From biofilm samples, all the three isolates happened to be resistant at least to one of the antibiotics tested and one of the isolates

Table 3 | Antimicrobial resistance profile of *S. aureus* isolated from water and biofilm samples collected from urban parks of São Paulo city

	mecA −						*mecA* +					
	Susceptible		Intermediate		Resistant		Susceptible		Intermediate		Resistant	
	N	%	N	%	N	%	N	%	N	%	N	%
OXA 01	14	73.7	0	0.0	5	26.3	8	72.7	0	0.0	3	27.3
CFO 30	18	94.7	0	0.0	1	5.3	9	81.8	0	0.0	2	18.2
PEN 10	8	42.1	3	15.8	8	42.1	5	45.5	1	9.1	5	45.5
CIP 05	15	78.9	2	10.5	2	10.5	10	90.9	1	9.1	0	0.0
CLI 02	14	73.7	2	10.5	3	15.8	5	45.5	4	36.4	2	18.2
CLO 30	18	94.7	1	5.3	0	0.0	11	100.0	0	0.0	0	0.0
ERI 15	9	47.4	6	31.6	4	21.1	3	27.3	3	27.3	5	45.5
GEN 10	18	94.7	0	0.0	1	5.3	11	100.0	0	0.0	0	0.0
RIF 05	17	89.5	0	0.0	2	10.5	10	90.9	0	0.0	1	9.1
TET 30	13	68.4	3	15.8	3	15.8	9	81.8	0	0.0	2	18.2
SUT 25	12	63.2	0	0.0	7	36.8	8	72.7	0	0.0	3	27.3

was resistant to five antibiotics (oxacillin, penicillin, clindamycin, erythromycin, and sulfazotrin).

The gene *mecA* was only detected in samples from drinking water fountains. Out of 30 isolates, 11 carried the *mecA* gene (36.7%) as follows: 16.7% (5/30) in Aclimação park, followed by 11.1% (3/27) in Buenos Aires park, 7.40% (2/27) in Ibirapuera park, and 3.70% (1/27) in Piqueri park. Regarding the 11 carrying the gene *mecA* isolates, only one was susceptible for all antibiotics tested and another one resistant to nine of them, with two being resistant to cefoxitin and three to oxacillin, and two were resistant to both of these antibiotics (Table 3), configuring characteristics to be considered as a MRSA strain.

The number of *mecA* non-carrying resistant isolates ranged from one to seven and the number of *mecA* carrying resistant isolates ranged from one to five. Notably, frequency for both isolates' groups (*mecA*$^-$ and *mecA*+) for penicillin resistance ranged from 42.1% to 45.5%, for sulfazotrin resistance ranged from 36.8% to 27.3%, for oxacillin resistance ranged from 26.3% to 27.3%, for erythromycin resistance ranged from 21.1% to 45.5%, clindamycin and tetracycline resistance ranged equally from 15.8% to 18.2%, for rifampicin resistance ranged from 10.5% to 9.1%, and cefoxitin resistance ranged from 5.3% to 18.2%.

DISCUSSION

The results revealed that all drinking water samples were in accordance with bacteriological standards set by the Brazilian legislation (Brasil 2017). Concerning the concentration of residual chlorine (≥0.5 mg/L), 13.7% (64/468) of the samples did not meet the legislation values. It is important to point out that all samples with an absence of chlorine were contaminated with *Staphylococcus* genus bacteria. Wang *et al.* (2014) reports that bacterial communities in drinking water networks have been detected worldwide, whether with residual chlorine absence or not. But it is known that residual disinfectant absence intensifies biofilms' formation, which can play a role as reservoirs for opportunistic pathogens (Berry *et al.* 2006; Douterelo *et al.* 2018) favoring their proliferation, enhancing their resistance to adverse environmental conditions and capacity of dissemination (Høiby *et al.* 2010; Henriques *et al.* 2013; Falkinham *et al.* 2015). Other factors that influence bacterial proliferation are structural, the kind of access and maintenance of the water distribution device (Briscoe *et al.* 2006). Thus, our study emphasizes that the bacteriological standard is not sufficient to indicate the presence of opportunistic pathogens such as *S. aureus*.

Even though hands are the main route of transmission of this pathogen (WHO 2017a) and there is a lack of

association with the infection's development by drinking water consumption (WHO 2011, 2017a), the presence of *S. aureus* in devices of water system distribution is a concerning scenario. In relation to the investigation of the virulence genes studied – *sea*, *seg* and *luk-PVL* – they were not detected. However, it has to be pointed out that virulence determinants are abundant in opportunistic pathogens, so the absence of these studied genes does not rule out the presence of other genetic elements, including those that are regulated by genetic mobile elements (Novick *et al.* 2010).

Antimicrobial resistance by *S. aureus* and MRSA are recognized as a problem by the WHO (2017b). Our results revealed high frequency of antibiotic resistance by *S. aureus* isolated from drinking water and mist makers.

According to Woolhouse *et al.* (2015), bacteria can alternate between resistant forms and sensitive ones in response to environmental pressure, whose mechanisms remain unclear. But what is known is that the pressure by exposure to antimicrobials promotes resistant strains besides increased capacity to cause infection (Nogueira *et al.* 2019).

In relation to the susceptibility to vancomycin, in this study all isolates were susceptible to this antibiotic. This result was not surprising, as according to Howden *et al.* (2010), the dissemination of vancomycin-resistant *Staphylococcus aureus* (VRSA) is reduced. Unfortunately, our results cannot be compared with others in the literature due to the scarcity of data with this type of sample.

Regards carrying *mecA* isolates, 45.4% (5/11) were classified as MRSA according to criteria stated by Goering *et al.* (2019) and CLSI (2018). These results are worrying because of their widespread dissemination throughout the environment. According to the Centers for Disease Control and Prevention of the United States (US CDC 2013), MRSA kills more Americans each year than AIDS, Parkinson's disease, emphysema, and homicide combined.

MRSA has emerged as a major cause of infections in the general population, including cases with neither health care exposure nor known classical risk factors, but also there were outbreaks reported that affected healthy populations (Levin-Edens *et al.* 2011). According to Goering *et al.* (2019), OS-MRSA (oxacillin-susceptible methicillin-resistant *Staphylococcus aureus*) has been reported in clinical isolates, which can be observed in isolates from livestock and from the environment (Mistry *et al.* 2016).

In 2013, the US CDC stated that resistant bacteria are a substantial threat to public health and national security (CDC 2013). Given the emergence of antimicrobial resistance and its huge impact on human, animal, and environmental health, the WHO has recognized it as one of the top ten threats to global health (Ventola 2015; WHO 2019). The WHO has classified *S. aureus* as a high priority within other resistant bacteria because of soaring resistant strains to the available antibiotics (WHO 2017b). The environment is a hotspot for the dissemination and mobilization of antimicrobial resistance due to the presence of circulating people and animals and a variety of bacteria from several sources.

Not only does this study provide unprecedented results about the presence of opportunistic pathogens in drinking water supplied in public places with high attendance, it also indicates the potential risk of infection for those that are exposed to these sources of water, including sensitive populations such as children, the elderly, and those immunocompromised (Collier *et al.* 2012; CDC 2013; Pandey *et al.* 2014; Wang *et al.* 2014; Beer *et al.* 2015; Van Der Wielen & Lut 2016; Sharaby *et al.* 2019).

CONCLUSIONS

Urban environments, such as parks, are a hotspot for the dissemination and mobilization of antimicrobial resistance due to the presence of circulating people and animals, and then a reservoir for a variety of bacteria from several sources. This study showed that even when drinking water samples were in accordance with bacteriological standards set by the Brazilian legislation, opportunistic bacteria *S. aureus* and *S. aureus* carrying the *mecA* gene were detected in high frequency in the samples analyzed. Thus, our study has provided important results where there were no previous data. It also makes clear the challenge to promote the commitment of all stakeholders, including park goers, in order to have a healthy drinking water source.

For this purpose, it is necessary to act in order to invest in preventive measures to ensure adequate drinking water quality and to take care of the maintenance of water distribution devices. Moreover, it is crucial to keep people

aware of the health risks when they are exposed to a neglected environment.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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