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Microbiological monitoring of coagulase-negative *Staphylococcus* in public drinking water fountains: Pathogenicity factors, antimicrobial resistance and potential health risks

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ABSTRACT

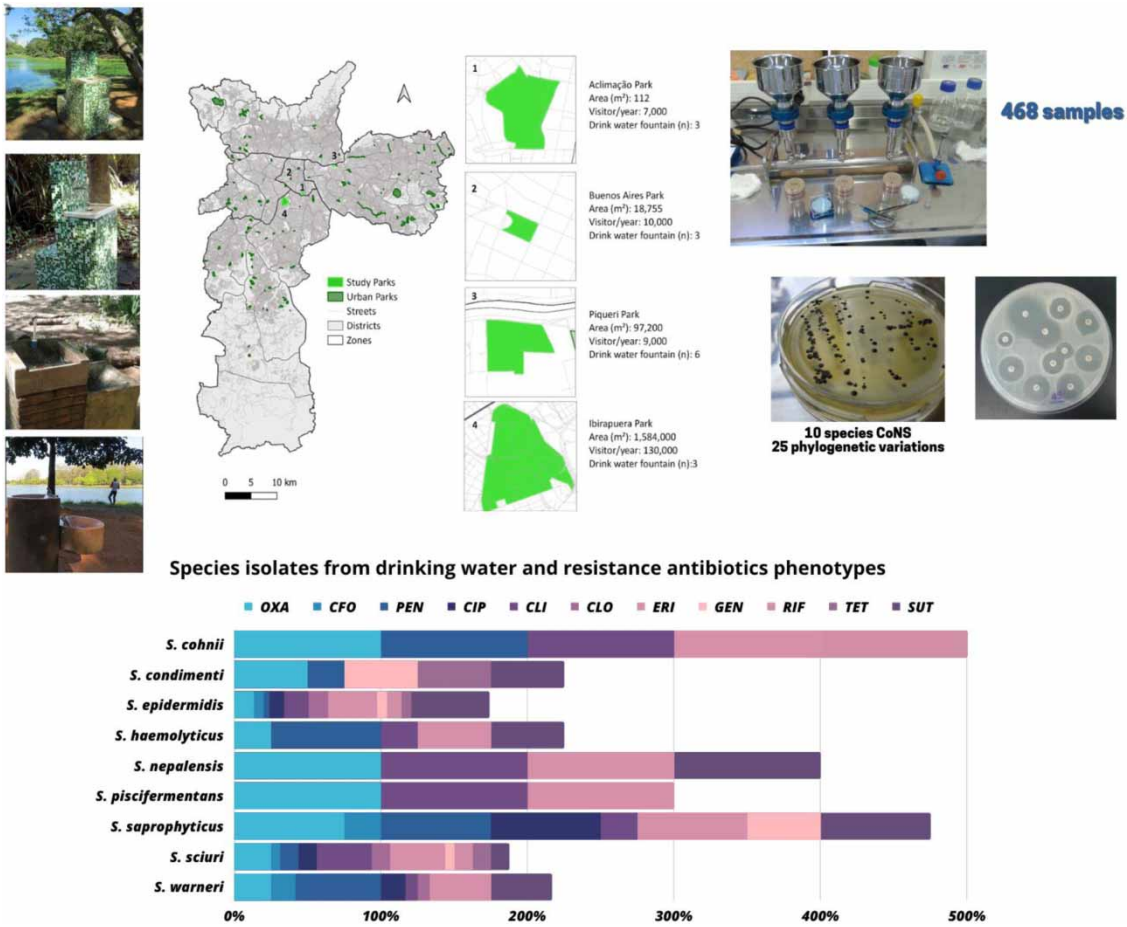
The presence of opportunistic bacteria such as coagulase-negative *Staphylococcus* (CoNS) in drinking water poses public health concerns because of its potential to cause human infection and due to its antimicrobial resistance (AMR) diversity. This study evaluated the occurrence, virulence markers and AMR of CoNS in 468 drinking water samples from 15 public fountains located in four urban parks of São Paulo city (Brazil). Out of 104 samples positive for the presence of *Staphylococcus* genus, we detected CoNS in 75 of them (16%), which did not meet the Brazilian sanitary standards for residual chlorine. All isolates were of concern to public health for being responsible for infection in humans from low to high severity, nine of them are considered the most of concern due to 63.6% being multiresistant to antimicrobials. The results demonstrated that CoNS in drinking water must not be neglected. It is concluded that the presence of resistant staphylococci in drinking water is a potential health risk, which urges feasible and quick control measures to protect human health, especially in crowded public places.

Key words: ATB resistance, coagulase-negative Staphylococcus, drinking water, MRSH

HIGHLIGHTS

- Coagulase-negative *Staphylococcus* (CoNS) was detected in chlorinated drinking water.
- High diversity of CoNs was found in drinking water.
- Ten species of CoNS and 25 phylogenetic variations were identified.
- High frequency of antibiotic-resistant CoNs was observed.
- A strain of *S. haemolyticus* carrying *mecA* gene and resistant to oxacillin and cefoxitin (MRSH) was identified.

GRAPHICAL ABSTRACT



INTRODUCTION

One of the most concerning issues associated with drinking water is the occurrence of microbial contaminants, which impacts human health. Usually, the assessment of drinking water bacteriological quality is based on fecal indicator bacteria (FIB) such as thermotolerant coliforms or *Escherichia coli*. In current Brazilian legislation as well as in several other countries, FIB is used as a bacteriological quality indicator for drinking water in addition to heterotrophic plate count (HPC) and residual chlorine, according to the Ministry of Health of Brazil and the Northern Ireland Environment Agency (NIEA) (Ministério da Saúde Brasil 2011; NIEA 2016). FIB (*E. coli*) acts as an indicator of the potential presence of pathogens and they are effective to identify fragilities in drinking water distribution systems. Nevertheless, these indicators are not always sufficient to indicate the presence of opportunistic bacteria. Furthermore, the usage of indicators alone does not allow a broader view of the drinking water quality regarding the issue of the presence of resistant pathogens to antimicrobials. According to Getahun *et al.* (2020), antibiotic-resistant bacteria are part of a global crisis, requiring urgent action because untreatable drug-resistant infections and diseases pose the threat of a worldwide public health emergency.

The presence of opportunistic pathogens can lead to interspecies and other bacterial lineage interactions. These interactions favor exchanges of genetic elements that are responsible for antimicrobial resistance (AMR) characteristics. Moreover, the response of their metabolites can amplify virulence in other strains, impacting human health (Hartmann *et al.* 2018; Hu *et al.* 2021). Among the diversity of opportunistic bacteria, the group of microorganisms that is increasingly reported in bacterial infections is the coagulase-negative *Staphylococcus* (CoNS). CoNS have received a lot of attention because of the increasing number of cases of resistant infections in inpatients and individuals outside healthcare settings, according to the National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) of Centers for Disease Control

and Prevention (CDC) (NCEZID 2014). In Brazil, according to the Brazilian National Health Surveillance Agency (ANVISA), CoNS infections are the most reported agent in healthcare units and the most resistant agent to antimicrobials used in intensive care units (Ministério da Saúde Brasil 2017).

Although there is ample evidence that opportunistic bacteria exhibit multidrug resistance, their AMR profiles are poorly studied in drinking water (Santos *et al.* 2020; Hu *et al.* 2021). This shortage of data is a threat to human health, especially to individuals with compromised immunity (Sanganyado & Gwenzi 2019).

CoNS comprise 23 coagulase-negative staphylococcal species, all of which show a high frequency of AMR and a natural reservoir of genes associated with a virulence that notably favor characteristics for strains that turn out to be more infectious and more resistant to antibiotic treatments (Becker *et al.* 2014; Argemi *et al.* 2019; Heilmann *et al.* 2019). Considering the importance of CoNS in the potential human health impact from exposure to these opportunistic bacteria, this research evaluated the occurrence of them in drinking water distribution systems in urban parks, since the urban parks are public spaces with a high circulation of people (PMSP 2014). This unprecedented study aimed to detect, identify and characterize CoNS microorganisms in drinking water from public drinking fountains in the city of São Paulo, available in public parks.

METHODS

There are more than 100 municipal parks in the city of São Paulo (Brazil), in which drinking water is provided by public networks and distributed by drinking fountains to visitors according to the São Paulo City Hall (PMSP) (PMSP 2014). According to Brazilian legislation, drinking water must meet the potability quality chemical and bacteriological standards established by the Ministry of Health (Ministério da Saúde, Brasil 2011). The bacteriological standards concern the presence–absence of *E. coli*, HPC with a maximum limit of 500 CFU/mL, to assess the cleanliness and integrity of the water distribution system and reservoirs, and for disinfection standard residual chlorine concentration must be $\geq$0.5 mg/L (Ministério da Saúde, Brasil 2017). Urban parks provide environmental services by improving air quality, reducing noise pollution and stabilizing the microclimate. In addition, the environmental health of these spaces is related to the preservation of fauna, flora and water sources (streams and lakes), which has a positive impact on the visitors' health and on the preservation of green areas in metropolitan areas (Limnios & Furlan 2013). Spaces like these improve people's quality of life and a sense of well-being (Krekel *et al.* 2016; Nguyen *et al.* 2021), promoting outside activities for social interactions and bringing a high flow of visitors (Coley *et al.* 1997). In this study, four public parks were selected based on high visitor frequency (Figure 1), territorial characteristics and location in the city: the Aclimação Park, located in the central region; the Buenos Aires Park, which receives visitors of different ages; the Ibirapuera Park, considered the most important leisure area in the city, attracting people from different parts of the city and the world; and the Piqueri Park, located on the east side of the city.

Samples and sampling

From March 2017 to March 2018, drinking water samples ($n = 468$) from drinking water fountains were collected fortnightly from the four parks. Sample collection, storage and chilling for transportation were carried out in accordance with the recommendations of the Standard Methods for the Examination of Water and Wastewater (2017). Free residual chlorine concentration was performed by a colorimetric method using Free-chlorine Analyzer Policontrol® (São Paulo, BR). *E. coli* quantification was carried out by the membrane filter method according to Standard Methods (2017) as well as the HPC.

Staphylococcus determination and biochemical characterization

A volume of 100 mL of the water samples was concentrated using a filter membrane (0.45 μm, 47 mm, Millipore®, USA) and then transferred on a Petri dish containing Baird-Parker agar (BP) (Difco®, MI, USA) (Standard Methods 2017) followed by incubation for 48 h at 35 $\pm$ 0.5 °C. After the incubation period, typical colonies were observed and then submitted to biochemical characterization for staphylococcal bacteria. The selected colonies were initially screened according to the recommendations of the Brazilian National Health Surveillance Agency (ANVISA 2004): Gram stain, catalase reaction (hydrogen peroxide solution 10 V, Laborclin®, PR – BRA); tube coagulase reaction (Coagu-plasma, Laborclin®, PR – BRA); DNAse agar test (Difco®, MI, USA) and fermentation of Mannitol Salt agar (Difco®, MI, USA).

Identification and genotypic characterization of *Staphylococcus* by PCR

After the screening step, the typical staphylococcal colonies were transferred to a BHI broth and incubated overnight at 35 $\pm$ 0.5 °C. From the bacterial growth, a volume of 1,000 μL was transferred to a microtube and centrifuged at 13,000 rpm for 10 min. The yielded supernatant was discarded, and the pellet was resuspended in 25 μL of lysostaphin enzyme (1 μg/mL)

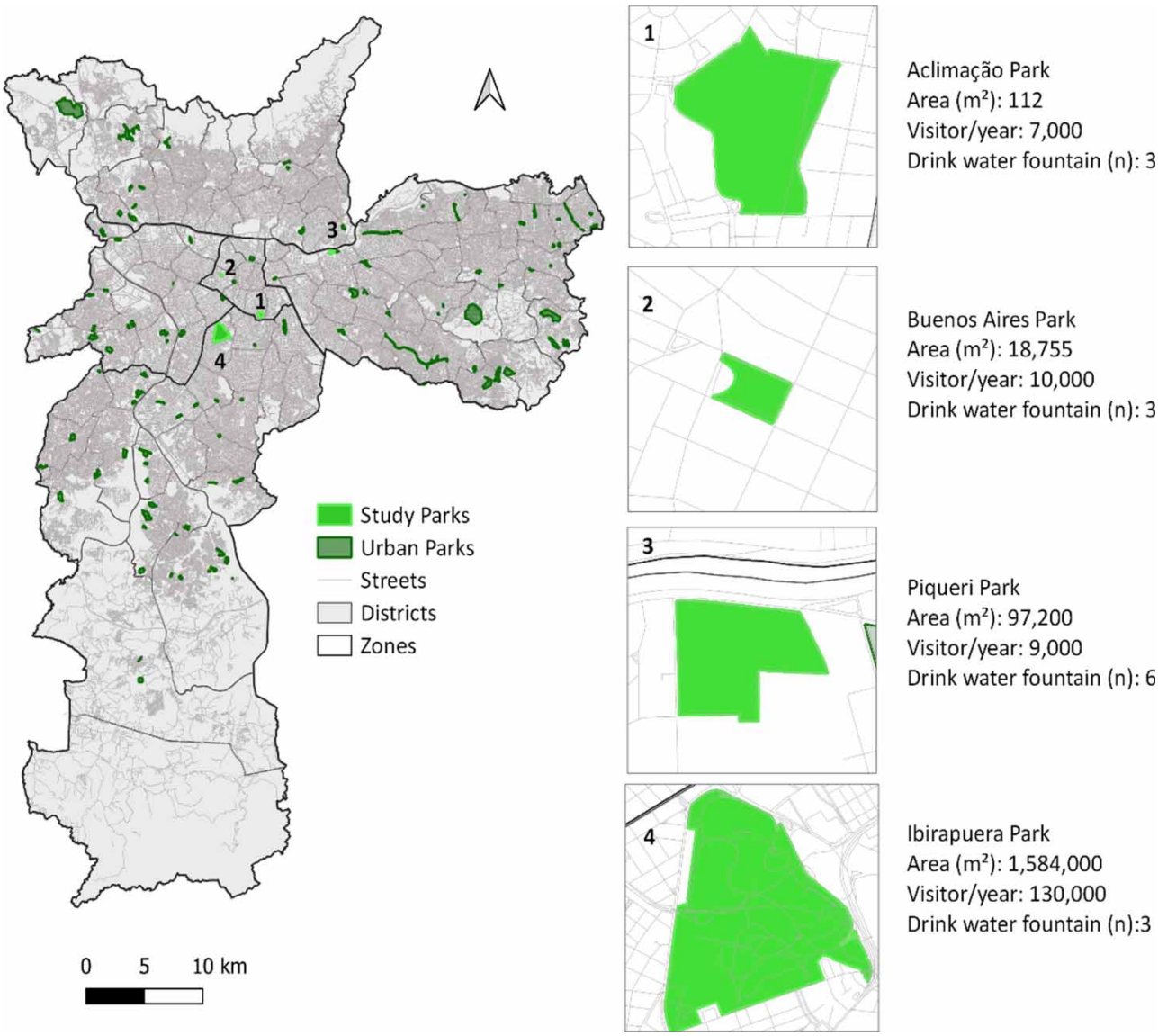


Figure 1 | Location of public parks in Sao Paulo city, selected in the research: (1) Aclimação, (2) Buenos Aires, (3) Piqueri and (4) Ibirapuera. Characteristics such as number of visitors a year, area in m^2 and number of drinking water fountains are analyzed in each park.

(Sigma®, Missouri, USA) and 25 μL of ultrapure water and incubated for 10 min at 37° ± 0.2 °C with agitation. After this step, 50 μL of proteinase K (20 mg/mL in sterile deionized water) (Roche®, California, USA) and 150 μL of Tris buffer (0.1 M, pH 7.5) (USB Corp.®, Ohio, USA) were added and incubated in a shaking bath at 37 °C for 10 min, followed by incubation in a water bath at 95 °C for 10 min. Finally, the solution was centrifuged at 13,000 rpm for 10 min and the supernatant was stored. The primers used to track seven genes are shown in Table 1.

Identification of species of *Staphylococcus* and lineages by MALDI-TOF MS

We used the Bruker MALDI Biotyper for the identification of the isolates. The identification was performed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) (Moreno *et al.* 2018). This technique is useful to analyze peptides and proteins in relatively complex samples, such as samples from environmental matrices. It allows us to differentiate species or subspecies, as well as new species and lineages. Mass spectra were obtained by Microflex™ mass spectrometer (Bruker Daltonik®, Leipzig, Germany). The interpretative criteria were applied as follows: scores ≥2.0 were accepted for species assignment and for gender identification scores ranging from ≥1.7 to ≤2.0 according to the Bruker standard.

Table 1 | Primers used in the study

Gene	Primers sequence	Function	bp
nuc	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	Nuclease encoding specific characteristics found in staphylococci (Barski *et al.* 1996)	278
coa	CGTTACAAGGTGAAATCGTT CCATATTGAGAAGCTTCTGTTG	Difference between positive and negative coagulase isolates (Nagaraj *et al.* 2014)	247
RecN	CAGTTAATCGGTATGAGAGC CTGTAGAGTGACAGTTTGGT	Synthesizes extracellular polysaccharides as a precursor in the biofilm formation (Iorio *et al.* 2011)	219
icaAB	TTATCAATGCCGCAGTTGTC GTTTAACGCGAGTGCGCTAT	Synthesizes enzymes of adhesion intercellular that contribute in biofilm resistance process (Iorio *et al.* 2011)	154
sea	GAAAAAAGTCTGAATTGCAGGGAACA CAAATAAATCGTAATTAACCGAAGGTTC	Genes encoding staphylococcal enterotoxins (Jarraud *et al.* 2002)	560
seg	AATTATGTGAATGCTCAACCCGATC AAACTTATATGGAACAAAAGGTACTAGTTC		642
Luk- PVL	ATCATTAGGTAAAATGTCTGGACATGATCCA GCATCAASTGTATTGGATAGCAAAAGC	Encode Panton-Valentine leucocidin (PVL) production, toxin cytotoxic often associated with staphylococci infection (Jarraud *et al.* 2002)	433
mecA	CTATCCACCCTCAAACAGG	Encode protein that methicillin resistances mediate (Okuma *et al.* 2002)	280
mecA	CGTTGTAACCACCCCAAGA		

Antimicrobial susceptibility profile

The susceptibility to antibiotics was determined by using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI 2020). CLSI has been developing international standards for the past 50 years which are used in more than 50 countries, and benefit safe and transparent laboratory practices (Weinstein & Lewis 2020). Antimicrobials ($n = 11$) tested were: cefoxitin (30 μg); ciprofloxacin (5 μg); clindamycin (2 μg); chloramphenicol (30 μg); erythromycin (15 μg); gentamicin (10 μg); penicillin g (10 μg); rifampicin (5 μg); sulfazotrin (25 μg); tetracycline (30 μg) and oxacillin (1 μg) (DME®, SP, Brazil). Vancomycin susceptibility was also assessed by microdilution broth microplate technique for concentrations ranging from 0.5 to 32 μg/mL of Vancomycin hydrochloride (Sigma®, Missouri, USA).

RESULTS

Out of 468 samples, 393 (83.9%) were in accordance with the standards set by the Brazilian legislation concerning *E. coli* and HPC, and free chlorine (Ministério da Saúde, Brasil 2011), while 75 (16%) of them did not meet the standard value for free chlorine. *Staphylococcus* genus was found in 104 (22.2%) samples within which 75 samples presented a lack of free residual chlorine. CoNS were found in 16% of the isolates in which the residual chlorine did not meet the established standard, as follows: 38.7% (29/75) in Aclimação Park; 22.7% (17/75) in Buenos Aires Park and Piqueri Park; and 16% (12/75) in Ibirapuera Park.

All of the 75 isolates were positive for the catalase test, and within them, 53.3% (40/75) were positive for mannitol fermentation and for the DNAse test. For the coagulase reaction 13.3% (10/75) of the isolates were positive as well as for gene *coa* presence. Genes *sea*, *seg* and *luk*-PVL were not detected; however, their genes *recN* and *icaAB*, commonly associated with CoNS, were detected in 4% (3/75) and in 6.7% (5/75), respectively.

A wide diversity of CoNS was identified in our samples (Table 2): 10 species and 25 phylogenetic variations were identified; 85.3% (64/75) of the isolates were identified at a taxonomic level showing logarithmic scores ≥2.0 while 12.3% with scores ≤2.0 were identified at the genus level. Within the 64 isolates, 40.6% (26/64) were identified as *Staphylococcus epidermidis* while the frequency of *Staphylococcus sciuri* and *Staphylococcus warneri* was 17.2% (11/64); 6.3% (4/64) for *Staphylococcus saprophyticus* and *Staphylococcus condiment*; 4.7% (3/64) for *Staphylococcus haemolyticus*; 3.1% (2/64) for *Staphylococcus nepalensis*; and 1.6% (1/64) for *Staphylococcus cohnii*, *Staphylococcus gallinarum* and *Staphylococcus pscifermentans*. A wide diversity of phylogenetic variation was identified. The greatest variation occurred for the following species: *S. haemolyticus* with 66.7% (2/3), *S. warneri* with 45.5% (5/11), *S. sciuri* with 27.2% (3/11) and *S. epidermidis* with 11.5% (3/26).

Table 2 | Species isolates from drinking water and resistance antibiotics phenotypes

Species	N. of isolates	Species group			OXA		CFO		PEN		CIP		CLI		CLO		ERI		GEN		RIF		TET		SUT		Total
			N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	%
S. cohnii	1	*S. cohnii*	1	1.33	1	100	0	0	1	100	0	0	1	100	0	0	1	100	0	0	1	100	0	0	0	0	45.45
S. condimenti	4	*S. condimenti*	4	5.33	2	50	0	0	1	25	0	0	0	0	0	0	0	0	2	50	0	0	2	50	2	50	45.45
S. epidermidis	30	*S. epidermidis*	30	40	4	13.33	2	6.67	12	40	3	10	5	16.67	4	13.33	10	33.33	2	6.67	3	10	2	6.67	16	53.33	100
S. gallinarum	1	*S. gallinarum*	1	1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. haemolyticus	4	*S. haemolyticus*	4	5.33	1	25	0	0	3	75	0	0	1	25	0	0	2	50	0	0	0	0	0	0	2	50	45.45
S. nepalensis	2	*S. nepalensis*	2	2.7	2	100	0	0	0	0	0	0	2	100	0	0	2	100	0	0	0	0	0	0	2	100	36.36
S. piscifermentans	1	*S. piscifermentans*	1	1.3	1	100	0	0	0	0	0	0	1	100	0	0	1	100	0	0	0	0	0	0	0	0	27.27
S. saprophyticus	4	*S. saprophyticus*	4	5.3	3	75	1	25	3	75	3	75	1	25	0	0	3	75	2	50	0	0	2	0	3	75.00	81.82
S. sciuri	1	*S. sciuri*	16	21.3	4	25	1	6.25	2	12.50	2	12.50	6	37.50	2	12.50	6	37.50	1	6.3	2	12.50	2	12.50	2	12.50	100
S. warneri	12	*S. warneri*	12	16.0	3	25	2	16.67	7	58.33	2	16.67	1	8.33	1	8.33	5	41.67	0	0	0	0	0	0	5	41.67	72.73

OXA, oxacillin; CFO, Cetoxitin; PEN, Penicillin; CIP, ciprofloxacin; CLI, clindamycin; CLO, chloramphenicol; ERI, erythromycin; GEN, gentamicin; RIF, rifampicin; TET, tetracycline; SUT, sulfazotrin.

Concerning the antimicrobial susceptibility of the isolates, all of them were susceptible to vancomycin in the broth microdilution test. It was observed that 3.1% (2/64) of isolates were resistant to 63.6% (7/11) of the antibiotics tested (clindamycin, erythromycin, oxacillin, penicillin, tetracycline, cetoxitin and sulfazotrin). Table 2 shows the results obtained in this study, CoNS of isolates examined, organized by taxonomic group and the respective percentages of antibiotic resistance (or intermediary) phenotypes. The resistance to oxacillin was verified in 34.8% (22/64) of the isolates and resistance to cefoxitin in 9.3% (6/64) and for both was 9.3% (6/64). Regarding the detection of *mecA* gene, 15.6% (10/64) of the isolates carried this gene, which were: *S. epidermidis* (1/10), *S. condimenti* (1/10), *S. haemolyticus* (1/10), *S. warneri* (2/10) and *S. sciuri* (5/10). Within them, only *S. haemolyticus* expressed resistance against oxacillin.

DISCUSSION

The presence of opportunistic bacteria in various aquatic environments has been increasingly reported (Vikramjeet *et al.* 2019), including *Staphylococcus aureus* which has been detected in drinking water samples with the absence of free residual chlorine or below 0.1 mg/L as reported by Santos *et al.* (2020). The colorimetric method is one of the most used for field evaluation of free residual chlorine concentrations in drinking water (Badalyan *et al.* 2009; Standard Methods 2017). In the present study, it was shown that *Staphylococcus* that do not belong to the *aureus* species, including a variety of CoNS, are capable of surviving in drinking water even in the presence of chlorine. *Staphylococcus* infections cause several health concerns related to secondary bacterial infections in clinical outcomes and mortality in humans (Argemi *et al.* 2019). Therefore, evidence that CoNS are present and viable in drinking water is a significant worry for people who are exposed to those drinking water sources (Otzen & Nielsen 2008; Sanganyado & Gwenzi 2019). Taking into account the study carried out by Hu *et al.* (2021) which reported that there are several threatening environmental contaminants such as opportunistic pathogens compromising the safety of drinking water, raises the need to protect drinking water sources and protect human health.

Therefore, we investigated the occurrence of CoNS in drinking water supplied in public areas. Our study is unprecedented in focusing on the occurrence, taxonomy identification, and pathogenic potentials of negative staphylococci (CoNS) in drinking water distributed by public devices. The results reinforce the presence of these opportunistic bacteria in drinking water due to the absence of residual chlorine or its improper concentration. This is evidence for the poor sanitary conditions, which favor the survival of opportunistic pathogens and their proliferation in these environments.

Čuvalová *et al.* (2015), in Slovak Republic, reported that out of 10 coagulase-negative staphylococci species isolated 4 were from drinking water samples. The study carried out by Faria *et al.* (2009) reported the occurrence of coagulase-negative staphylococci in several sources of water, including drinking water from a distribution network. They found the prevalence of *Staphylococcus pasteuri* and *S. epidermidis* in these samples. In our study, 10 species were identified and 25 phylogenetic variations of CoNS, within which the most prevalent species were *S. epidermidis*, *S. warneri* and *S. sciuri* (Table 2).

CoNS has been increasingly reported in nosocomial infections worldwide (Table 3), but our study also shows that the presence of CoNS in drinking water is a risk factor for human health. *S. warneri*, detected in our samples, is considered an emerging pathogen whose pathogenesis and epidemiology have been little explored, but according to Espadinha *et al.* (2019), this species is frequent in infections of immunocompromised individuals and in sepsis of neonates. Another concerning species isolated from our samples is *S. sciuri*, which, according to Nemeghaire *et al.* (2014), is associated with polymicrobial infections and is considered a reservoir of virulence factors, playing a role in the horizontal transfer of genes to other staphylococcal species.

Although there are reports of the presence of genes associated with virulence factors in isolates of CoNS (Heilmann *et al.* 2019), we did not detect the genes *sea*, *seg* and *luk-PVL*. Only the virulence genes *recN* and *icaAB* were detected, which does not exclude that the isolates have other virulence characteristics. However, the identification of isolates using primers for specific *recN* and *icaAB* genes could be used to forecast virulent strains that possess the ability to initiate a lethal infection (Raheema *et al.* 2020).

Regarding antimicrobial susceptibility, the isolates of this study were resistant to penicillin, erythromycin, oxacillin, clindamycin, sulfazotrin and tetracycline, very similar to the resistance showed by clinical isolates (penicillin, erythromycin, oxacillin and gentamicin) (Duran *et al.* 2012; Čuvalová *et al.* 2015). Similar results were reported by Čuvalová *et al.* (2015) for AMR of coagulase-negative staphylococci isolated from drinking water and reported by Faria *et al.* (2009) as well.

We found the *mecA* gene in *S. haemolyticus*, *S. epidermidis*, *S. warneri*, *Staphylococcus caprae* and *Staphylococcus capitis* ssp. *urealyticus*, and the same results were found by Čuvalová *et al.* (2015). Among 10 CoNS isolates carrying *mecA* gene, only *S. haemolyticus* was simultaneously resistant to oxacillin and cefoxitin.

Table 3 | Pathogens identified in drinking water and potential health risks and antimicrobial resistance according to the literature

Species	Diseases	Antimicrobial resistance	References
S. cohnii	Implications in nosocomial infections, including meningitis, primary septic arthritis, septicemia, brain abscess and catheter invasion	Linezolid, penicillin, oxacillin, cefoxitin, clindamycin, erythromycin, azithromycin, levofloxacin, ciprofloxacin and gentamicin	Lavecchia *et al.* (2021) and Song *et al.* (2017)
S. condimenti	Until 2013 was considered to have a medium or low pathogenic capacity. Currently, it has been identified in meningococcal infection hypoxic–normocapnic respiratory failure and dilated cardiomyopathy	Erythromycin and rifampicin	Misawa *et al.* (2015), Gabrielsen *et al.* (2017) and Zecca *et al.* (2019)
S. epidermidis	Healthcare-associated infection and medical devices: prosthetic valve endocarditis, prosthetic joint infections, infections of central catheter	They usually tend to be multidrug-resistant, the resistance to methicillin ranges from 75 to 90% of the cases. It also presents very high resistance to other antimicrobial agents, such as trimethoprim/sulfamethoxazole, clindamycin, fusidic acid and fluoroquinolone	ECDC (2018) and Kozajda *et al.* (2019)
S. gallinarum	Sepsis	Ampicillin, amoxicillin, tetracycline	Nhung *et al.* (2017)
S. haemolyticus	Foreign body-related infections and infections in preterm newborns	Oxacillin, cefoxitin, ampicillin, levofloxacin, gentamicin, clindamycin, erythromycin, tetracycline and fosfomycin	Frickmann *et al.* (2018) and Westberg *et al.* (2022)
S. nepalensis	It was not identified as a human pathogen until 2019, recently identified in human bacteremia	Novobiocin	Hosoya *et al.* (2020)
S. piscifermentans	So far reported only in the production process in the food industry	There is currently no research discussing its antibiotic resistance ability	Zell *et al.* (2008)
S. saprophyticus	Frequently colonizes humans and animals, it is related to urinary tract infections, acute pyelonephritis, nephrolithiasis and patients in UTI with endocarditis	Nalidixic acid and novobiocin	Lawal *et al.* (2021) and Watanabe *et al.* (2022)
S. sciuri	Resistance to novobiocin, β-lactams, tetracyclines, aminoglycosides and aminocyclitols, trimethoprim and fusidic acid	Endocarditis, peritonitis, septic shock, urinary tract infection, endophthalmitis and pelvic inflammatory disease	Nemeghaire *et al.* (2014) and Al-Hayawi (2022)
S. warneri	Rare cases of human disease, but reported in infections of prostheses and endovascular catheters and sepsis	Penicillin G, oxacillin, vancomycin and kanamycin	Gelman *et al.* (2022)

According to Shi *et al.* (2013), disinfection using chlorine can provide a favorable environment for antimicrobial-resistant bacteria (ARB), antimicrobial-resistant genes (ARGs) and mobile genetic elements (MGEs) dissemination. Moreover, infections caused by resistant CoNS are directly associated with worse clinical outcomes, with longer hospital stays, with an increment of mortality, and with an increasing burden and costs on the healthcare infrastructure (Gajdács *et al.* 2021; Kalantar-Neyestanaki *et al.* 2022). Currently, there are no data to estimate the risk due to exposure to AMR in the environment for establishing a safety level of risk (Wuijts *et al.* 2017); however, we have experienced that CoNS may colonize different types of water, including drinking water that fulfills bacteriological quality standards, which poses a risk for human health.

CONCLUSION

CoNS species and phylogenetic variations identified in drinking water samples from public fountains presented antibiotic-resistant profile, posing serious concerns for human health in spaces with a large circulation of people, especially children, newborns, immunocompromised people and elderly people, whose attendance at parks is high.

This verification is reinforced by the fact of detection of a strain of *S. haemolyticus* carrying *mecA* gene, and, moreover, to be resistant to oxacillin and cefoxitin (methicillin-resistant *Staphylococcus haemolyticus*, MRSH). These results bring us to the crux of the matter, the complexity of keeping drinking water safe, even meeting the established standards. Contamination of drinking water by opportunistic and antimicrobial-resistant pathogens is beyond monitoring classic microbiological indicators of drinking water quality, and, despite the increase of relevant studies related to this issue, it is still an ongoing discussion.

A better knowledge of pathogenic organisms toward survivability, antibiotic resistance profile and chlorine resistance in drinking water systems is needed to assess risks, and to better design sanitary barriers and contamination prevention measures in order to supply safe drinking water.

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DATA AVAILABILITY STATEMENT

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

CONFLICT OF INTEREST

The authors declare there is no conflict.

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