


Original Article

First record of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from the Meia Ponte River and effluent in Brazil: an analysis of 1198 isolates

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ABSTRACT

Objective: This study aimed to detect environmental reservoirs of multidrug-resistant *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in Central Brazil.

Methods: Membrane filtration with growth on Baird-Parker agar base was used to recover *S. aureus* and MRSA from the effluents of a sewage treatment plant (STP) and surface water from an important Cerrado river. Effluent samples were collected at two points in the STP (pre- and post-treatment). Surface water was collected at six points along the Meia Ponte River, from its source to the mouth. Air samples were collected at all these collection points in 2022, totaling four campaigns between the dry and rainy seasons.

Results: *S. aureus* was isolated at all collection points in all campaigns, totaling 87715 isolates. Of the 1198 selected isolates, 83 were ceftioxin (FOX)-resistant, indicating a methicillin-resistance phenotype. The antimicrobial profiles of the 83 FOX-resistant isolates were investigated, and 88 % could be considered multidrug-resistant *S. aureus*. Of the 83 FOX-resistant isolates, more than 70 % were resistant to clindamycin, erythromycin, and rifampicin. The presence of the *mecA* gene was confirmed in 18 isolates collected along the Meia Ponte River and inside the STP, with 15 exhibiting multidrug-resistant phenotypes. The results of the principal component analysis (PCA) revealed an association between *S. aureus* isolates and the urbanized environment/STP, as well as the presence of antimicrobials.

Conclusion: This study is a pioneer in identifying the presence of MRSA in Brazilian water bodies, highlighting the urgent need for monitoring antimicrobial resistance in urban rivers.

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium that commonly colonizes the skin and mucosal membranes of mammals [1]. However, *S. aureus* has the potential to cause acute and chronic diseases via an array of virulence factors, including toxins, such as Pantone-Valentine leukocidin, toxic shock toxin (TSST-1), exotoxins and enterotoxins; enzymes, such as coagulase, hyaluronidase, and staphylokinase; adhesins that facilitate adhesion to cells; and biofilm formation as a protective layer that hinders the action of antibiotics [2]. The infection occurs

through tissue invasion, with evasion of phagocyte killing, internalization, persistence, and distribution of the infection, potentially leading to antimicrobial resistance (AMR), making treatment difficult [3].

S. aureus belongs to the ESKAPE group of nosocomial pathogens (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*). These pathogens can evade antimicrobial treatment through resistance mechanisms [4]. Some *S. aureus* strains have developed resistance to several antimicrobials, including β -lactams, such as methicillin. The primary mechanism of methicillin resistance is the production of PBP2a,

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an altered penicillin-binding protein (PBP) that exhibits decreased affinity for most semi-synthetic penicillins, typically encoded by the *mecA* gene [5].

Several recent investigations have reported the emergence of virulent multidrug-resistant (MDR) pathogens from various sources [2, 6–10]. It represents one of the greatest threats to global public health [11]. This scenario highlights the urgent need to (i) develop new antimicrobial agents and alternative therapies; (ii) establish rational antimicrobial use programs and continuous AMR surveillance [12]; and (iii) invest in research, innovation, and education on the conscious use and discard of antibiotics. Thus, the routine application of antimicrobial susceptibility testing to detect the antibiotic of choice is important, as well as hospital and environmental screening for emerging MDR strains

[10].

In addition to its association with nosocomial infections, methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported as a leading cause of community-associated infections in patients with no prior hospitalization history [13]. MRSA can be spread by colonized persons and animals, as well as through the air and aquatic environments, such as rivers in urban centers that receive both domestic and hospital sewage [1,14]. In this sense, monitoring the aquatic environment is necessary to prevent transmission [15].

However, data about the presence of MRSA in freshwater in tropical countries is scarce. In Brazil, MRSA was identified in drinking water sources in urban parks of São Paulo city, with 27.3 % of the isolates carrying the *mecA* gene [16]. We recently identified MDR *S. aureus* in a

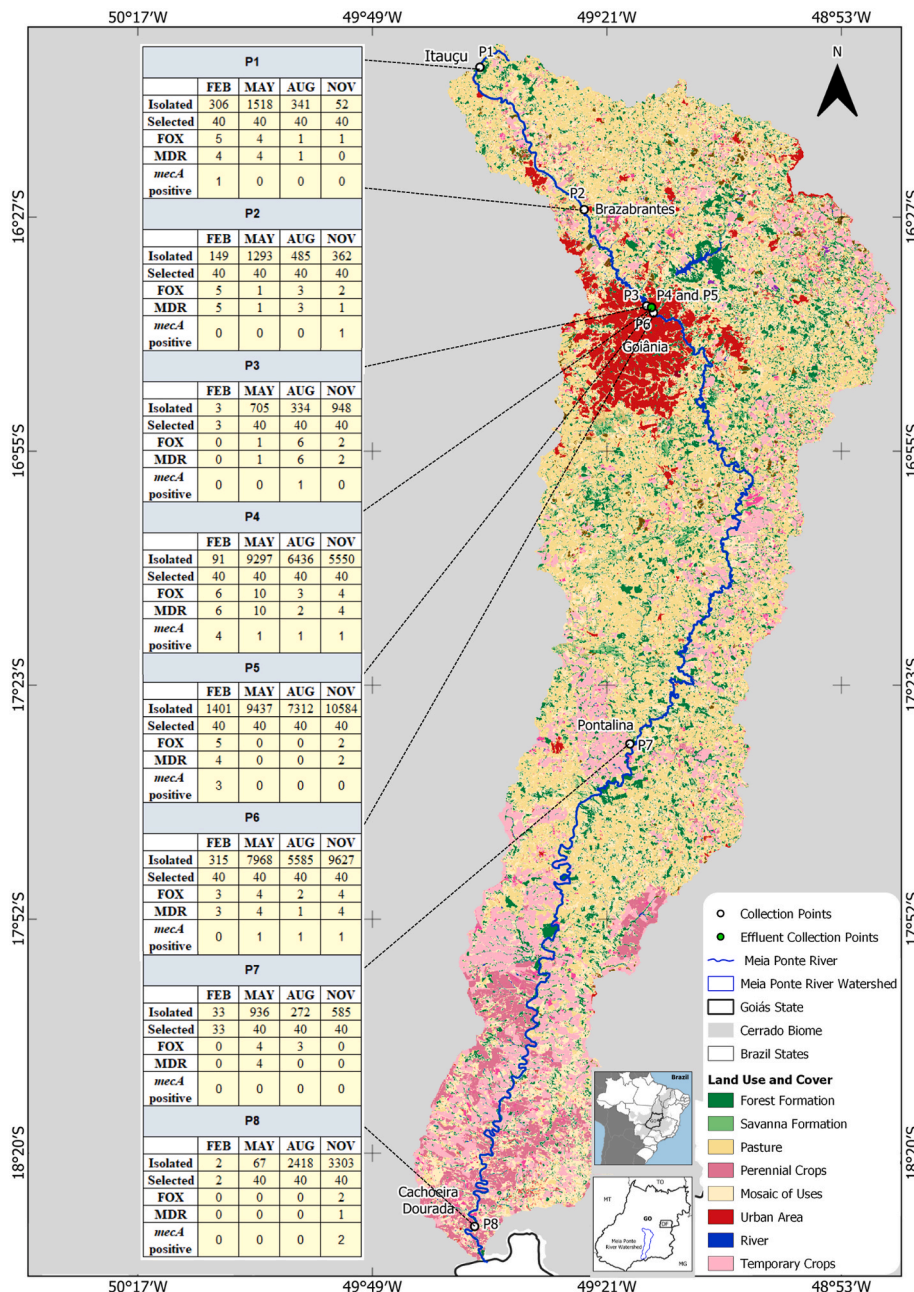


Fig. 1. Collection points for effluents, air, and surface water from the Meia Ponte River collected in 2022, in the State of Goiás, Central Brazil. The "P1 - P8" tables report as Isolated: the total number of *Staphylococcus aureus* isolated in each collection point; Selected: the number of isolates selected for screening on Chromogenic MRSA agar medium; FOX: the number of isolates with a cefoxitin-resistant phenotype; MDR (multidrug resistance): the number of isolates with resistance to at least three different classes of antimicrobials, also including extensively drug-resistant (XDR) and pandrug-resistant (PDR); and *mecA*-positive: the number of isolates positive for the *mecA* gene. Image created in QGIS 3.34.3.

low-order stream, the Extrema River, in Central Brazil [17]. However, we did not identify MRSA. Previous data indicated that the Meia Ponte River in Central Brazil serves as a reservoir of antibiotics (Simão et al., submitted) and antibiotic resistance genes (ARGs) [18]. The Meia Ponte River is one of the most significant rivers in the state of Goiás, as it traverses 39 municipalities and supplies water to nearly 50 % of the population residing in the state capital, Goiânia. Besides, this river receives sewage from the Goiânia sewage treatment plant (STP) and clandestine raw sewage [19].

This study aimed to determine the occurrence of MDR *S. aureus* and MRSA in an urban river environment, including surface water, effluents from an STP, and the air at all collection points. The hypothesis is that the Meia Ponte River could act as an environmental reservoir for these organisms, and urbanization contributes to this.

2. Materials and methods

2.1. Water sampling and storage locations

Effluent samples were collected in the capital, Goiânia, at the Dr. Hélio Seixo de Britto Primary Treatment Station (STP) and in surface water along the Meia Ponte River, in the State of Goiás, Brazil (Fig. 1). Eight collection points were defined (P1-P8), as described in Supp. Table S1, with the effluents referring to P4 and P5. At each collection point, three plates with Baird-Parker agar base (Kasvi, Pinhais, PR, Brazil) were opened for 2–3 min in zigzag movements to collect the air samples, totaling 8 points of collection. Four collections were conducted in 2022, spanning the rainy and dry seasons. The first collection was in February (rainy), the second in May (dry), the third in August (dry), and the fourth in November (rainy). For sample collection and storage, the methodologies of Fagundes et al. [20] and Bolívar-Subirats et al. [21] were followed. Briefly, 2 L of surface water and effluents were collected using a stainless-steel bucket with a capacity of 5 L, attached to a rope approximately 8 m long at a depth of about 25–45 cm. The samples were stored in sterile amber glass bottles and transported under refrigerated conditions at 4 °C.

Previous work reported that physical-chemical parameters [temperature, Total Dissolved Solids (TDS in ppm), electrical conductivity ($\mu\text{S cm}^{-1}$), Dissolved Oxygen (DO in ppm), and pH] were also measured at each sampling point using a multiparameter probe [22]. Concomitantly, an analysis of the land use and cover of each sampling point (Pasture, Agriculture, and Urbanized) was performed [22], as well as the quantification (ng L^{-1}) of metronidazole (MTZ) and sulfamethoxazole (SX) (Simão et al., submitted).

2.2. Cellulose nitrate membrane filtration to obtain *Staphylococcus aureus*

Membrane filtration was employed to recover *S. aureus* and MRSA from effluent and surface water samples from the Meia Ponte River, following the methodology of Goldstein et al. [12] with modifications. Briefly, 10 mL of each quadruplicate sample was vacuum-filtered through a cellulose nitrate filter membrane with a pore size of 0.45 μm and a diameter of 47 mm (GVS North America, Sanford, USA). The membranes were placed on Baird-Parker agar base (Kasvi, Pinhais, PR, Brazil) and incubated at 37 °C for 24 h. After this period, the number of colonies that grew on the membranes placed on the Baird-Parker agar base, presumptively identified as *S. aureus*, was counted.

2.3. Methicillin-resistant *Staphylococcus aureus* (MRSA) phenotypic screening

Forty colonies of *S. aureus* were randomly selected from each sampling point in the four campaigns, except those points where fewer than 40 colonies were isolated. The isolates (total N = 1198) were streaked on Chromogenic MRSA agar (Laborclin, Pinhais, PR, Brazil) and incubated

at 37 °C for 24 h [23]. Blue-green colonies were presumptive for MRSA. These colonies were stored in Brain Heart Infusion (BHI) broth (Kasvi, Pinhais, PR, Brazil) with 15 % glycerol at -80 °C in an ultra-freezer (ColdLab®, Piracicaba, SP, Brazil). For quality control and assurance of the isolation process, *S. aureus* ATCC 25923 [American Type Culture Collection, Manassas, VA] was used as a positive control, and phosphate-buffered saline was used as a negative control. Cultures of these isolates were deposited in the Elisa F. L. C. Bailão working collection (Collection of microorganism cultures at the State University of Goiás [CCM-UEG], Central Campus) at the Biotechnology Laboratory (LaBiotec).

2.4. *Staphylococcus aureus* phenotypic confirmation

First, all the selected isolates (total N = 1198) were confirmed to be *S. aureus* using the catalase (1) and coagulase (2) tests, as well as Gram staining (3). To perform the catalase test (1), an emulsion of one section of the colonies was prepared by adding a drop of 35 % hydrogen peroxide (H_2O_2 ; Neon, Suzano, SP, Brazil) to a glass slide, resulting in immediate bubbling, which indicates catalase positivity for *S. aureus* [24]. For the coagulase test (2), the Staphclin latex kit (Laborclin, Pinhais, PR, Brazil) [25] was used, in which a portion of the colonies to be tested containing coagulase or protein A when mixed with latex particles produced agglutination visible to the naked eye within 45 s, being a positive sign for *S. aureus*. For Gram staining (3), a commercial kit (Laborclin, Pinhais, PR, Brazil) was used. The colonies were stained with gentian violet ($\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}$) and then treated with 5 % Lugol's solution (I_3K), forming a dark color between the iodine and the dye, which is not retained by Gram-positive bacteria, such as *S. aureus* [26]. The subsequent treatment with alcohol and 0.25 % Safranin ($\text{C}_{20}\text{H}_{19}\text{N}_4^+\text{Cl}^-$) stains *S. aureus* a purple or violet color, which is organized in clusters, and was visualized under a 40 \times binocular microscope (Primo Star, Zeiss, Oberkochen, Germany).

2.5. Antimicrobial resistance profile screening

All the selected isolates (total N = 1198) were submitted to the disk diffusion test with cefoxitin (FOX). The 83 FOX-resistant isolates were submitted to the antimicrobial resistance profile screening in triplicate. Antibiotic susceptibility tests were performed using the disk diffusion method, as outlined in the standard protocol published by the Clinical and Laboratory Standards Institute [27] and the methodology described by Tsai et al. [28]. Predicted MRSA isolates were cultured in BHI broth at 37 °C for 24 h. After adjusting to the 0.5 McFarland scale, the diluted medium was spread on Baird Parker agar base using a sterile swab to facilitate the fixation of the antibiotic discs (Laborclin, Pinhais, PR, Brazil). Nine antibiotics were tested (Supp. Table S2). Despite we did not test the *S. aureus* isolates against all of the antimicrobial agents within the antimicrobial categories, multidrug resistance (MDR) was defined by an isolate of *S. aureus* that grew in the presence of at least three different categories of antibiotics; extensively drug-resistant (XDR) was defined as an isolate of *S. aureus* that remain susceptible to only one or two different classes of antibiotics; and pandrug-resistant (PDR) was defined as an isolate of *S. aureus* that was resistant to all classes of tested antibiotics [29]. The mean and standard deviation of the triplicate were calculated for evaluation.

The MAR (Multiple Antibiotic Resistance Index) proposed by Krumperman [30] was used to assess the risk and selective pressure of antibiotics in contaminated environments. The index is calculated by the number of antibiotics to which the isolate is resistant divided by the total number of antibiotics tested. $\text{MAR} > 0.20$ indicates that the isolate may have originated in environments with high pressure of antibiotic use, such as hospitals, farms, industries, or contaminated sewage. $\text{MAR} \leq 0.20$ suggests lower antibiotic exposure, possibly from a more controlled environmental source.

2.6. DNA extraction and polymerase chain reaction (PCR) for *mecA* and *nuc* genes amplification

For the FOX-resistant isolates (total N = 83), DNA extraction was performed using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, California, USA) according to the manufacturer's protocol. The extracted nucleic acid was stored at -80 °C until further analysis. Two gene regions of *S. aureus*, *mecA* [31] and *nuc* [32], were amplified via PCR. The primers (Sigma-Aldrich Brasil Ltda, Barueri, SP, Brazil) used for amplification were as follows: *mecA* forward sequence 5'-AAAATCGATGGTAAAGGTTGGC-3', *mecA* reverse sequence 5'-AGTTCCTGCAGTACCGGATTGC-3', *nuc* forward sequence 5'-GCGATTGATGGTGATACGGTT-3', and *nuc* reverse sequence 5'-AGCCAAGCCTTGACGAATAAGC-3', resulting in amplicons of 533 and 279 base pairs, respectively. The PCR amplification for each primer (singleplex) was performed using a TC9639 thermal cycler (Benchmark Scientific, New Jersey, USA) and GoTaq® Green Master Mix (Promega, California, USA) in a final reaction volume of 10 µL, following the manufacturer's protocol. For both primers (*mecA* and *nuc*), the PCR cycle program was as follows: 1x cycle for an initial denaturation step at 94 °C for 2 min; 40x cycles for denaturation step at 94 °C for 15 s, annealing step at 55 °C for 20 s, and extension step at 72 °C for 20 s 1x cycle for the final extension step at 72 °C for 5 min. The PCR amplicons were visualized by agarose gel electrophoresis. A 1.5 % agarose gel was prepared in 100 mL of TBE 1x buffer (pH 8.3, 90 mM Tris, 90 mM boric acid, 2 mM EDTA). For DNA intercalation, 10 µL of PCR products were mixed with Novel Juice DNA stain (Sigma-Aldrich, Massachusetts, USA) following the manufacturer's protocol. Gels were run in 1x TBE buffer at 100 V for 60 min. The PCR products were visualized and photographed on an LED transilluminator (Kasvi, Pinhais, PR, Brazil). A 100 bp ladder (Invitrogen by ThermoFisher Scientific, Waltham, MA, USA) was added to the gel to compare the fragments amplified.

2.7. Statistical analysis

In general, data are presented in relative and absolute frequency. A Principal Component Analysis (PCA) between environmental variables (temperature, TDS, electrical conductivity, DO, and pH), land use and land cover (pasture, agriculture, and urbanized), and biological/antimicrobial samples was conducted (Supp. Table S3). A Pearson correlation was performed between phenotypic (growth in the presence of a FOX disk) and genotypic (presence of the *mecA* gene) FOX resistance. The analyses were performed using Statistica 7 and PAST software.

3. Results

3.1. Occurrence of *Staphylococcus aureus* in the Meia Ponte River

S. aureus was isolated at all collection points in all campaigns (Fig. 1), totaling 87715 isolates. The isolates were predominantly collected in P4-P6 (Fig. 1), which correspond to points inside the STP (P4 and P5) and the point where the treated effluent is released into the Meia Ponte River (P6), located in the urban perimeter of a big city (Goiânia, Goiás, Brazil). From this, 1198 isolates were selected to investigate the antimicrobial resistance.

3.2. Phenotypic characteristics of the recovered isolates

Of the 1198 selected isolates, 83 were FOX-resistant, indicating a methicillin-resistance phenotype. Most of these isolates (27.7 %) were from P4 (inside the STP, before the treatment). However, it is important to highlight that ~13 % of the isolates were from P1 (the spring of the Meia Ponte River). In addition, the number of FOX-resistant isolates was ~45 % higher in P6 than in P3, suggesting that the treated effluent released in the Meia River contributes to the increase of the FOX-resistant isolates in the river (Figs. 1 and 3). The antimicrobial profiles

of the 83 FOX-resistant isolates were investigated, and ~88 % (n = 73) could be considered multidrug-resistant *S. aureus* (MDR, XDR, or PDR) (Fig. 2). Of this, 9, 10, 9, 22, 6, 12, 4, and 1 were isolated from P1 – P8, respectively.

Regarding the patterns of resistance, MDR corresponds to 74.70 % (n = 62), XDR to 8.43 % (n = 7), and PDR to 4.82 % (n = 4) of the 83 FOX-resistant isolates. The highest number of MDR occurred in P4, corresponding to 19.28 % (n = 16); XDR was also highest in P4, corresponding to 6.02 % (n = 5); and PDR was highest in P2, corresponding to 2.41 % (n = 2) (Supp. Table S4). Regarding the MAR index, 74 isolates (89.16 %) had a MAR > 0.20, and 9 isolates (10.85 %) had a MAR index < 0.20. The highest number of isolates that may have originated in environments with high antibiotic pressure was in P4 (MAR index average = 0.56), corresponding to 27.71 % (n = 23); and the highest number of isolates that may have originated from a more controlled environmental source was in P7 (MAR index average = 0.11), corresponding to 3.61 % (n = 3) (Supp. Table S4).

It is important to highlight that four isolates were collected from the air (one in P1, one in P3, and two in P4), with three being MDR (Fig. 2 and Supp. Table S4). Of the 83 FOX-resistant isolates, 86.7 %, 85.5 %, and 74.7 % were resistant to CLI, ERY, and RIF, respectively. Four PDR isolates (one from P1, two from P2, and one from P4) were resistant to all the antimicrobials investigated in this work (Fig. 2 and Supp. Table S4).

3.3. Genotypic characteristics of the recovered isolates

In the *nuc* gene screening, all the 83 FOX-resistant isolates were positive, and then *S. aureus* genotypic confirmed isolates (Supp. Fig. S1). The presence of the *mecA* gene was confirmed in 18 isolates (Supp. Fig. S2), corresponding to 21.69 % of the 83 FOX-resistant isolates. The highest occurrence of *mecA*-positive isolates was observed in P4 (n = 7, corresponding to ~ 39 %). The STP treatment was not efficient in eliminating the *mecA*-positive isolates (P5). The Meia Ponte River had three times more MRSA after the STP (P6) than before the STP (P3) (Fig. 1). The antimicrobial profiles of the 18 *mecA*-positive isolates revealed that 13 were MDR (72.22 %), 1 was XDR (5.55 %), and 1 was PDR (5.55 %) (Fig. 2 and Supp. Table S4). Of these, 77.8 % were resistant to CLI, ERY, and/or RIF. One isolate from P4 was resistant to all antimicrobials tested in this work. Conversely, one isolate from P5 and another from P6 were susceptible to all other antimicrobials tested, except FOX (Fig. 2). *mecA*-positive isolates were not observed in P7. In the *mecA* gene screening, 72.2 % of the isolates were from the rainy season (February and November). Finally, a significant correlation (R = 0.74; p = 0.04) was observed between genotypic ($\bar{x} = 2.25 \pm 2.19$) and phenotypic ($\bar{x} = 10.38 \pm 6.12$) resistance.

3.4. Principal component analysis (PCA)

The first two axes of the PCA explained 66.02 % of the total variance (Fig. 3 and Supp. Table S5). The red dots indicate collections during the dry season, while the blue dots indicate the rainy season. The seasons (rainy and dry) did not influence the number of *S. aureus* isolates (Figs. 1 and 3). It is possible to note a positive association between P4, P5, and P6, as well as urbanization, physicochemical parameters, multidrug-resistant *S. aureus* isolates, and the presence of the antimicrobials MTZ and SX. This data indicates that urbanization/STP contributes to the increase in multidrug-resistant *S. aureus* and MRSA isolates in the Meia Ponte River.

4. Discussion

Global deaths associated with AMR reached nearly 5 million in 2019, of which 1.27 million deaths were directly caused by drug-resistant bacteria [33]. AMR is a complex problem that requires a coordinated approach across different sectors, such as human and animal health,

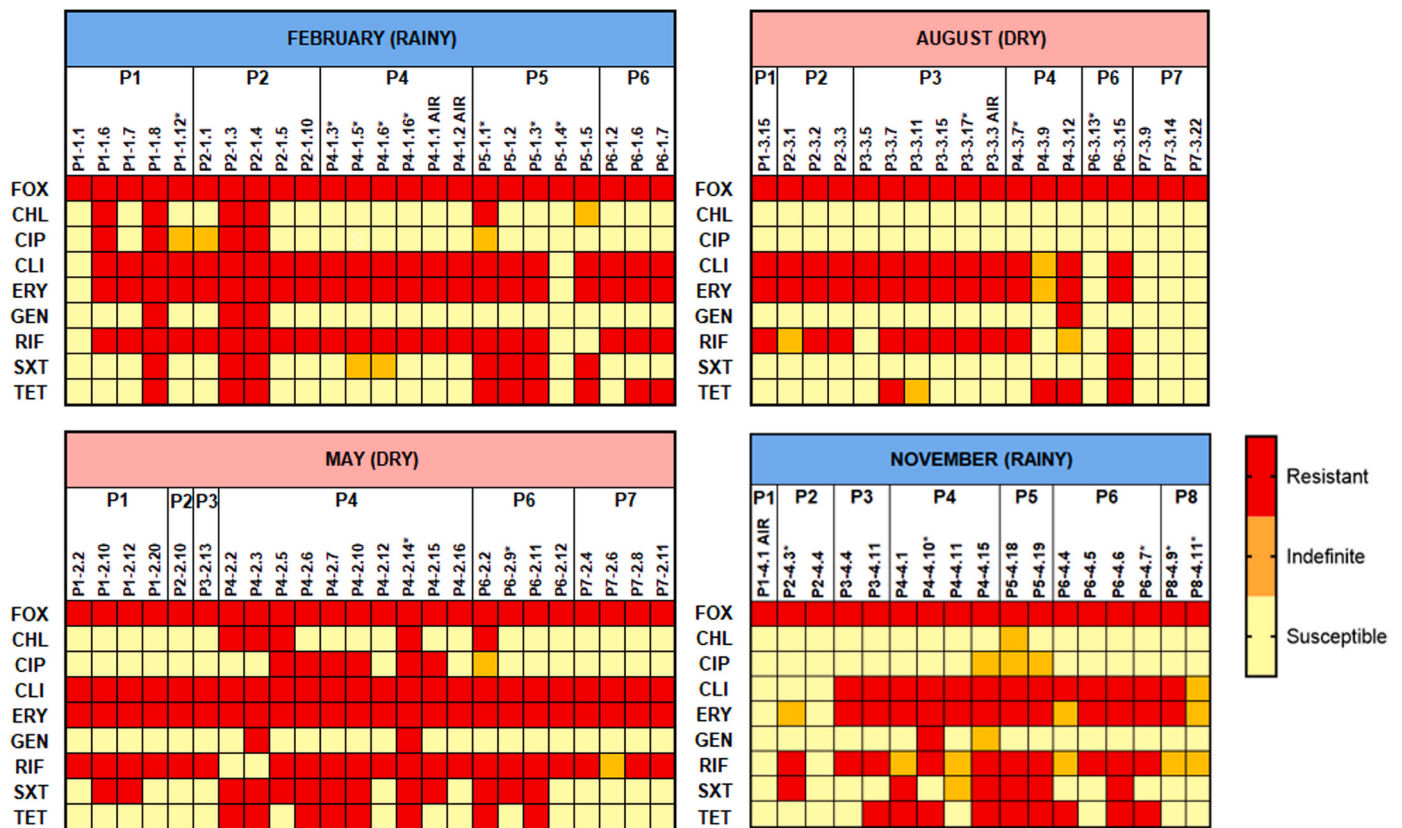


Fig. 2. Antimicrobial profile of the 83 cofoxitin-resistant *Staphylococcus aureus* isolated from effluents, air, and surface water from the Meia Ponte River collected in 2022, in the State of Goiás, Central Brazil. The resistance against cofoxitin (FOX), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), rifampicin (RIF), trimethoprim-sulfamethoxazole (SXT), and tetracycline (TET) was evaluated. The asterisks mark the *mecA*-positive isolates.

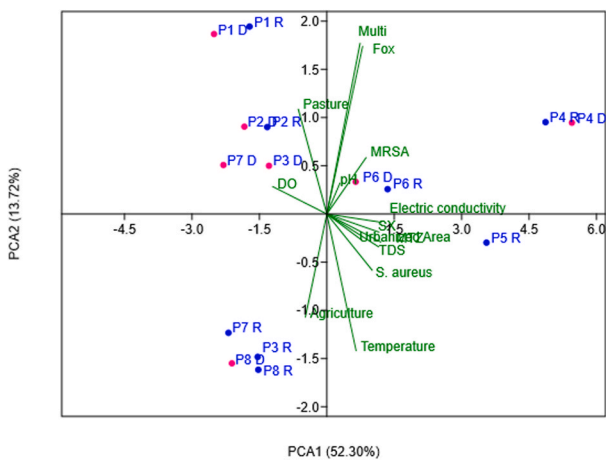


Fig. 3. Principal Component Analysis (PCA) for environmental variables, antimicrobials, and *S. aureus* isolates in a river in Central Brazil. Total Dissolved Solids (TDS), Dissolved Oxygen (DO), Metronidazole (MTZ), Sulfamethoxazole (SX), Methicillin (MRSA), Cefoxitin (FOX). The red dots indicate collections during the dry season, while the blue dots indicate the rainy season.

food production, and environmental. The active surveillance of MRSA enables the early detection of cases, monitoring of the pathogen's spread, and the implementation of control measures, such as isolating infected or colonized patients, maintaining rigorous hand and surface hygiene, and rational antibiotic use in the hospital environment [34]. In response to the growing AMR challenge, 178 countries have developed national action plans to prevent the rise and spread of AMR [35].

However, Brazil does not currently have regular monitoring of resistant bacteria in water quality, similar to more than 80 % of the countries participating in this treaty [33].

Given this scenario, some individual efforts to monitor the occurrence of resistant bacteria in urban rivers and STPs have been carried out. In this study, multidrug-resistant *S. aureus* and MRSA were detected in the Meia Ponte River, being the first record of MRSA in Brazilian rivers. The presence of MDR *S. aureus* and MRSA in the Meia Ponte River indicates that this river could be a source of development, transmission, and spread of AMR to the local population, which uses the river for recreational and water supply purposes. When bacteria enter the water bodies, resistance genes are transferred between microorganisms and pathogens by horizontal gene transfer. Additionally, MRSA can survive for up to 14 days in water [36,37]. The occurrence of MRSA in other rivers around the world, such as in Japan [38], Spain [39], and the USA [40], reinforces that AMR is a global One Health problem.

Multi-drug-resistant *S. aureus* and MRSA were also observed in the Goiânia STP, both before and after sewage treatment, indicating that the treatment is not effective in removing these potential pathogens. Moreover, the Meia Ponte River had three times more MRSA after the STP (P6) than before the STP (P3), suggesting that the sewage amplifies the AMR in the river. Water bodies that receive pollution are more likely to harbor resistant microorganisms and can serve as a source of AMR within the environment [41]. This is the case of Meia Ponte River, which receives treated sewage from the Goiânia STP and raw sewage [19]. This observation was corroborated by the PCA, which showed an association between the points inside the STP (P4 and P5) and in the Meia Ponte River after the STP (P6), urbanization, multidrug-resistant *S. aureus* isolates, and the presence of the antimicrobials MTZ and SX, as well as environmental variables (TDS, temperature, and electrical

conductivity). The PCA approach revealed no significant differences between the dry and rainy seasons for the studied parameters, consistent with previous observations [42].

These data reinforce that polluted water bodies serve as a pathway or reservoir for bacterial resistance [43], in line with environmental parameters. It is also known that efficient sewage treatment is crucial in limiting the environmental dissemination of antibiotic-resistant bacteria [44]. In this sense, the management of STPs needs to be more concerned with water resources. MRSA has been identified in STPs in other countries, such as Sweden [45], the United States [12,46], and Austria [47]. It is also important to highlight that within the Goiânia STP (P4), multidrug-resistant *S. aureus* was isolated from the air, indicating a health risk to the STP employees and the potential for environmental spread, as previously reported in Poland [48]. Several factors influence the concentrations of bacteria in the air of STP, including temperature, humidity, UV radiation intensity, and wind speed [49], with seasonality having a significant impact.

The MAR index indicated that 89.16 % of the isolates may have originated in environments with high pressure of antibiotic use, such as hospitals, farms, industries, or contaminated sewage. Conversely, just 10.85 % of the isolates have lower exposure to antibiotics, possibly from a more controlled environmental source [30]. In this sense, monitoring urban rivers is essential, considering various variables, including both ecological and anthropogenic factors.

Although a positive correlation was observed, a high discrepancy in results was noted between the disk diffusion method using FOX and the presence of the *mecA* gene. FOX resistance in *S. aureus* isolates lacking a *mecA* gene is infrequently reported [50]. However, Volkmann et al. [51] demonstrated that this gene was not commonly found in the municipal wastewater samples. Known mechanisms of non-*mecA*-mediated β -lactam resistance include overexpression or overproduction of penicillinases, β -lactamases, or methicillinases, overexpression or modification of normal constitutive PBP genes, or the expression of *mecA* homologues [52].

To address the AMR challenge, it is crucial to implement a range of strategies. These strategies may include (i) reducing the indiscriminate and excessive use of β -lactam antibiotics; (ii) implementing stringent hygiene practices in healthcare settings; (iii) investing in research to develop new antimicrobial treatments and alternative therapies [12]; (iv) financing research into the impact of the presence of antimicrobials in water; (v) and disseminating measures to control the spread of resistant strains in the environment, such as monitor inadequate disposal of antibiotics, monitor the evolution of the environmental reservoirs of resistant strains, and public awareness of the importance of correct disposal of antibiotics.

In this context, the One Health approach aligns best with national strategy plans to combat AMR. One Health contributes to integrated and coordinated actions among different professionals in a multisectoral manner to monitor, prevent, and control the spread of AMR. AMR is not limited to the hospital environment; it also spreads through environmental sources [53]. For example, animals treated with antibiotics can harbor resistant bacteria, which can be transmitted to humans through direct contact, consumption of contaminated meat, or exposure to environmental matrices such as water, air, and soil. Likewise, improper disposal of medicines and hospital waste contributes to the presence of resistance genes in the environment [54]. Therefore, One Health is essential for comprehensively and sustainably addressing the global challenge of AMR, thereby protecting collective human, animal, and environmental health [55].

5. Conclusion

In summary, this work accepted the hypothesis that the Meia Ponte River can act as an environmental reservoir for MDR *S. aureus* and MRSA, and that urbanization contributes to this. This work is a pioneer in identifying the presence of MRSA in Brazilian water bodies. The Meia

Ponte River, including its spring, harbors MDR *S. aureus* and MRSA, which makes the river a potential source of AMR within the environment. These potential pathogens were also isolated in the Goiânia STP, both in the air and sewage (before and after the treatment). The number of FOX-resistant isolates was ~45 % higher in the river after the STP than before, suggesting that the treated effluent released into the Meia River contributes to the amplification of AMR in the river. Of the 83 FOX-resistant isolates investigated, ~88 % were MDR, XDR, or PDR *S. aureus*. Most of these isolates were resistant to CLI, ERY, and RIF. The presence of the *mecA* gene was confirmed in 18 isolates, which were ~83 % MDR, XDR, or PDR. To reduce the spread of MDR *S. aureus* and MRSA in the environment, it is essential to implement surveillance systems that carefully monitor the release of effluents into the Meia Ponte River, as well as the presence of resistant bacteria and/or ARGs along the river. The present study contributes to understanding where the highest frequency of MDR *S. aureus* occurs along the Meia Ponte River. The DNA sequencing and phylogenetic analysis of these isolates are in progress to suggest the origins of these MRSA isolates.

CRedit authorship contribution statement

Igor Romeiro dos Santos: Writing – original draft, Formal analysis, Conceptualization. **Leandro Fonseca de Lima:** Writing – original draft, Formal analysis, Conceptualization. **Marcelino Benvindo de Souza:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Isabela Náthaly Machado da Silva:** Formal analysis. **Adriano Roberto Vieira de Sousa:** Supervision, Formal analysis. **Alexandre Melo Bailão:** Funding acquisition, Methodology. **Cátia Lira do Amaral:** Writing – review & editing, Funding acquisition, Data curation. **Elisa Flávia Luiz Cardoso Bailão:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

Data availability

Data and materials not included in the manuscript are available under request.

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Declaration of competing interest

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Appendix A. Supplementary data

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