Domestic wastewater treatment plants as sources of macrolide-lincosamide-streptogramin B- and penicillin-resistant *Staphylococcus aureus* in Brazil

Silvia Leticia Oliveira Toledo¹, Renata Michelle Silveira Silva¹, Isabella Cristina Rodrigues dos Santos¹, William Gustavo Lima², Leticia Gonçalves Rodrigues Ferreira¹, Magna Cristina Paiva¹*

¹Laboratório de Diagnóstico Laboratorial e Microbiologia Clínica, Universidade Federal de São João del-Rei, Campus Centro-Oeste Dona Lindu, Divinópolis, Minas Gerais, Brasil.

²Laboratório de Microbiologia Médica, Universidade Federal de São João del-Rei, Campus Centro-Oeste Dona Lindu, Divinópolis, Minas Gerais, Brasil.

*Corresponding author E-mail address: magnacpaiva@ufsj.edu.br

Received: 21 October 2019
Revised: 14 April 2020
Accepted: 15 April 2020

**Summary**

*Staphylococcus aureus* is one of the main bacteria that affect human health. Its reduced susceptibility to beta-lactam antibiotics has driven the clinical use of macrolides and lincosamides. However, the presence of macrolide-lincosamide-streptogramin B (MLS₉)-resistant *S. aureus* strains is increasingly common. Wastewater treatment plants (WWTPs) are the main anthropogenic source of resistance determinants. However, few studies have assessed the importance of this environment on the dissemination of MLS₉-resistant *S. aureus* strains. Thus, we aimed to evaluate the impact of a domestic WWTP on the resistance to MLSB and penicillin in *S. aureus* in southeast Brazil. Of the 35 isolates tested, 40.6% were resistant to penicillin. Resistance to erythromycin (8.6%) and quinolones (2.8%) was less common. Despite the low rate of resistance to clindamycin (2.8%), many isolates showed reduced susceptibility to this antibiotic (57.1%). Regarding the resistance phenotypes of staphylococci isolates, inducible MLS₉ resistance (D-test positive) was found in two isolates. In addition, 27 *S. aureus* isolates showed the ability to produce penicillinase. In this article, we report for the first time the importance of WWTPs in the dissemination of MLS₉ resistance among *S. aureus* from southeast Brazil.

**Key words:** Antibiotic resistance, D-test, penicillin zone-edge test, quinolones, *Staphylococcus aureus*, water environment.
Resumen

Plantas de tratamiento de aguas residuales domésticas como fuentes de *Staphylococcus aureus* resistente a macrólidos-lincosamida-estreptogramina B- y penicilina en Brasil

*Staphylococcus aureus* es una de las principales bacterias que afectan la salud humana. Su susceptibilidad reducida a los antibióticos betalactámicos ha impulsado el uso clínico de macrólidos y lincosamidas. Sin embargo, la presencia de cepas resistentes a macrólido-lincosamida-estreptogramina B (MLS\(_B\)) de *S. aureus* es cada vez más común. Las plantas de tratamiento de aguas residuales (PTAR) son la principal fuente antropogénica de determinantes de resistencia. Sin embargo, pocos estudios han evaluado la importancia de este entorno en la diseminación de cepas de *S. aureus* resistentes a MLS\(_B\). Nuestro objetivo fue evaluar el impacto de una PTAR doméstica en MLS\(_B\) y la resistencia a la penicilina en *S. aureus* en el sureste de Brasil. De los 35 aislamientos analizados, el 40,6% eran resistentes a la penicilina. La resistencia a la eritromicina (8,6%) y quinolonas (2,8%) fue menos común. A pesar de la baja tasa de resistencia a la clindamicina (2,8%), muchos aislamientos mostraron sensibilidad reducida a este antibiótico (57,1%). Con respecto a los fenotipos de resistencia de los aislamientos de estafilococos, la resistencia inducible a MLS\(_B\) (prueba D positiva) se encontró en dos aislamientos. Además, 27 aislamientos de *S. aureus* mostraron la capacidad de producir penicilinasa. En este artículo informamos, por primera vez, la importancia de las PTAR en la difusión de la resistencia a MLS\(_B\) entre *S. aureus* del sureste de Brasil.

Palabras clave: Resistencia a antibióticos, prueba D, prueba de borde de zona de penicilina, quinolonas, *Staphylococcus aureus*, ambiente acuático.

Resumo

Estações de tratamento de águas residuais domésticas como fontes de *Staphylococcus aureus* resistente a macrólideo-lincosamida-estreptograma e B- e penicilina no Brasil

O *Staphylococcus aureus* é uma das principais bactérias que afetam a saúde humana. Sua reduzida suscetibilidade aos antibióticos beta-lactâmicos tem impulsionado o uso clínico de macrólideos e lincosamidas. No entanto, a presença de cepas de *S. aureus* resistentes a macrólideo-lincosamida-estreptogramina B (MLSB) é cada vez mais comum. As estações de tratamento de esgoto (ETEs) são a principal fonte antropogênica de
determinantes de resistência. No entanto, poucos estudos avaliaram a importância desse ambiente na disseminação de cepas de \textit{S. aureus} resistentes ao MLSB. Assim, nosso objetivo foi avaliar o impacto de uma ETE doméstico na resistência ao MLSB e à penicilina em \textit{S. aureus} no sudeste do Brasil. Dos 35 isolados testados, 40,6% eram resistentes à penicilina. Resistência à eritromicina (8,6%) e quinolonas (2,8%) foi menos comum. Apesar da baixa taxa de resistência à clindamicina (2,8%), muitos isolados apresentaram sensibilidade reduzida a esse antibiótico (57,1%). Em relação aos fenótipos de resistência dos isolados de estafilococos, a resistência induzível ao MLSB (D-teste positivo) foi encontrada em dois isolados. Além disso, 27 isolados de \textit{S. aureus} mostraram a capacidade de produzir penicilinase. Neste artigo relatamos pela primeira vez a importância das ETEs na disseminação da resistência do MLSB entre \textit{S. aureus} do sudeste do Brasil.

\textit{Palavras-chave}: Resistência a antibióticos, D-teste, teste da borda da zona da penicilina, quinolonas, \textit{Staphylococcus aureus}, ambiente aquático.

\section*{Introduction}

\textit{Staphylococcus aureus} is one of the major bacterial pathogens of medical interest [1]. Although it is estimated that approximately 20\% of the general human population are persistently colonized with \textit{S. aureus}, this microorganism can cause a wide variety of clinical complications ranging from self-limited superficial infections to severe bacteraemia or pneumonia [2]. Various classes of antimicrobials are used for the treatment of these infections, such as β-lactams, macrolides, lincosamides and quinolones [3]. In addition, with the emergence of penicillin- and oxacillin-resistant strains since the 1960s, the use of vancomycin (glycopeptide) has also become common. Methicillin-resistant \textit{S. aureus} (MRSA) strains, which are resistant to all β-lactams, were initially only detected in hospital settings [4]. However, since 1990, reports of resistant strains within the community have been described [5]. In the United States, the mortality rate from MRSA-associated infections outnumber those caused by HIV/AIDS and tuberculosis combined [6]. The colonization rate in American hospital settings is quite variable, and in some cases may affect up to 85\% of patients [7]. In Brazil, 31\% of \textit{S. aureus} isolates from hospitalized patients are characterized as MRSA [8].

Macrolides and lincosamides are therapeutic options for the treatment of MRSA infection; however clinical failure of therapy has been reported when the strains harbor the \textit{erm} gene. This gene encodes clindamycin-induced resistance and cross-resistance to erythromycin, conferring the macrolide-lincosamide-streptogramin B (MLS\textsubscript{B}) resistance \textit{phenotype} [9]. In general, exposure to subinhibitory antibiotic concentrations is related to MLS\textsubscript{B} resistance. In this context, wastewater is an important environment
for the development of bacterial resistance as it harbors a complex bacterial community, receives residues of several antimicrobials and is considered a hotspot for gene exchange, including the exchange of genes that confer antimicrobial resistance (e.g., \textit{erm}, \textit{bla\_ampc}, \textit{norA}, \textit{acrABC}, \textit{tetK}, \textit{mecA} and \textit{blaZ}) \cite{10, 11}.

According to previous studies, the prevalence of \textit{S. aureus} in wastewaters is low compared to clinical environments \cite{12}. However, it should be highlighted that wastewater treatment plants (WWTPs) may be an important reservoir and source of MSL\textsubscript{B}-resistant \textit{S. aureus} \cite{13, 14}. Thus, considering the possibility of bacterial exchange between the clinical and environmental settings, investigation of the presence of resistant \textit{S. aureus} strains in WWTPs is of great relevance as it may contribute to containing the spread of these microorganisms \cite{13}. While many studies have investigated the resistance of enterobacteriaceae present in WWTPs, there is limited information on antibiotic-resistant \textit{S. aureus} in this environment, which is of importance in developing countries \cite{14}. Thus, we aimed to investigate the susceptibility of \textit{S. aureus} isolates recovered from of a community WWTP in Brazil to several clinically important antimicrobials. In addition, the presence of clindamycin-induced resistance and penicillinase production was studied in isolates to determine the potential for this environment to act as a reservoir and source of MSL\textsubscript{B}- and penicillin-resistant \textit{S. aureus}.

\section*{Materials and methods}

\subsection*{Sample collection and recovery of \textit{Staphylococcus aureus}}

The area selected for this study was the city of Divinópolis (Minas Gerais), located in southeast Brazil (232,945 inhabitants). One liter of both raw sewage (RS) and effluent (EF) were collected from the Rio Pará WWTP (geographical coordinates: 20°08’20”S and 44°53’02”W) on June 8, 2015. The WWTP studded adopts the conventional activated sludge treatment system and receive domestic sewage generated by approximately 10% of the population from Divinópolis, been that their effluent is discharged in the Pará River. All samples were stored in sterilized polypropylene bottles and transported on ice to the laboratory within 2 h of collection. The sample collection was authorized by \textit{Companhia de Saneamento de Minas Gerais} (Copasa), a publicly owned company responsible for the collection and treatment of sewage and water supply in the state of Minas Gerais (Brazil).

For the isolation of \textit{S. aureus}, 100 µL of RS and EF were plated directly \textit{onto mannitol salt agar} (Labm, Brazil) \textit{in duplicate} after being serially diluted (10\textsuperscript{-1} to 10\textsuperscript{-5}) in a sterile saline solution 0.85% (NaCl). The plates were incubated at 37 °C for up to 48 h. After this incubation period, plates that had grown 20 to 200 colonies were selected for determination
of the number of colonies forming units (CFU) per milliliter of RS and EF. Mannitol-fermenting colonies, which are yellow in color, were selected, inoculated in brain heart infusion (BHI) broth (Difco, India) and incubated at 37 °C for 24 h. Subsequently, the isolates were repeatedly streaked onto the same agar to check their purity. We also considered tests for catalase, coagulase and DNase, in addition to Gram staining, to confirm the species identification (S. aureus is positive for all these proves) [15]. The colonies isolated and identified as S. aureus were stored in nutrient broth containing 25% glycerol at -80 °C until further use.

**Determination of antibiotic susceptibility profile**

The antimicrobial susceptibility profile was determined by the disc diffusion method according to the recommendations of the Clinical Laboratory Standard Institute [16]. The following antimicrobials (DME Sensidisc, Brazil) were tested: β-lactams (penicillin, PEN), macrolides (erythromycin, ERT), lincosamides (clindamycin, CLN), and quinolones (ciprofloxacin, CIP; ofloxacin, OFX; norfloxacin, NOR). Cefoxitin disk (DME Sensidisc, Brazil) was used to predict the oxacillin susceptibility profile. *Staphylococcus aureus* ATCC 29213 was used as control.

**Inducible clindamycin-resistance assay**

The D-test was performed according to the CLSI (2017) [16] to phenotypically determine resistance to MSL. Briefly, the antimicrobials clindamycin (2 μg) and erythromycin (15 μg) were placed at a distance of 15-26 mm on the surface of Mueller-Hinton agar (Alere, USA) which had been inoculated with each S. aureus isolate. The plates were then incubated at 35 ± 2 °C for 16-18 h. Verification of flattening in the erythromycin inhibition halo resembling the letter “D” indicates inducible resistance to clindamycin (figure 1). *Staphylococcus aureus* ATCC25923 and S. aureus ATCC29213 were used as controls.

**Penicillinase production**

Penicillinase production was investigated in all S. aureus isolates by the penicillin zone-edge method according to the CLSI (2017) [16]. This test is based on the appearance of the inhibition zone edge surrounding the penicillin G disk (DME Sensidisc, Brazil) after the disc-diffusion assay. The result was defined as negative when the appearance of the edge was fuzzy, resembling a “beach”, and as positive when the edge was sharp like a “cliff”. *Staphylococcus aureus* ATCC25923 and S. aureus ATCC29213 were used as controls.
Results and discussion

Methicillin-resistant *S. aureus* is one of the most prevalent multidrug-resistant microorganisms that cause infection in both the community and in health-care settings. Macrolides and lincosamides are therapeutic options for the treatment of MRSA-infections; however, resistance to these antibiotics has increased in recent years [17]. This phenomenon in *S. aureus* has rapidly emerged, mainly due to exposure to subinhibitory antibiotic concentrations combined with the acquisition of antibiotic-resistance genes, such as those of the *erm* family [18, 19]. Wastewater treatment plants combine these two factors, as well as having a nutrient-rich environment that favors microbial proliferation [10]. However, despite the importance of WWTPs in the dissemination of antimicrobial resistance, there is little available information concerning the impact of this environment on MSL$_B$ resistance in *S. aureus*. Furthermore, only few studies have evaluated the influence of domestic WWTPs on antimicrobial resistance in developing countries, and the dynamics of this phenomenon remain to be fully elucidated in these regions [10, 14]. Thus, in this study we aimed to evaluate the resistance profile as well as the phenotypic characteristics related to the clindamycin-induced resistance and penicillinase production in *S. aureus* isolated from a full-scale domestic WWTP in Brazil.

Figure 1. Representation of a positive result in the phenotypic test for inducible MSL$_B$ resistance (D-test) in *S. aureus* isolates. 1 - Erythromycin. 2 - Clindamycin.
Microbiological analyses revealed 260 and 20 CFU/mL of *S. aureus* from RS and EF samples, respectively. In fact, several studies have revealed that, although there is often a high level of microbes present in the initial stages of wastewater treatment, microorganisms are either eliminated or reduced in final stage [12, 20]. Similar to this study, the sewage treatment employed in WWTPs in Spain (88.3%) [21] and Germany (99.9%) [22] also showed high clearance rates for *Staphylococcus*. The drastic reduction in the *S. aureus* population after wastewater treatment can, at least in part, be explained by the retention time of the effluent. According to Li *et al.* (2015) [23], the retention time of effluent has a negative effect on the survival of *S. aureus* because it disrupts important cell surface properties such as the zeta potential, hydrophobicity, and charge density.

A total of 35 different colonies (33 from RS and 2 from EF) were isolated on the manitol agar, and these were included in the antimicrobial susceptibility tests and for phenotypic identification of resistance. As observed in table 1, in general the isolates showed high sensitivity to the antimicrobials tested except penicillin, which showed a considerable resistance rate (40.6%, 13/32) (figure 2). In accordance with our data, a high percentage of penicillin-resistant *S. aureus* in domestic WWTPs has also been found in Tunisia (100%) [24], Portugal (57.1%) [25] and Spain (40.62%) [21].

Despite previous studies having indicated the presence of MRSA in WWTPs [14, 13, 26-28], our study did not identify any isolates with this phenotype (figure 2). Gram-negative bacteria are the predominant infectious agents in Latin America and the Caribbean, while Gram-positive bacteria are more frequent in the USA, Europe, and countries of the Pacific region [26]. Thus, it is expected that the selective pressure driving the spread of MRSA will be less frequent in Latin countries such as Brazil. Corroborating this hypothesis, MRSA isolates are most common in WWTPs in the USA [13, 14], Australia [27], Taiwan [28] and Spain [21].

One of the most interesting findings from the current study was the low level of resistance to quinolones observed among the isolates. These antibiotics are partially metabolized by humans and animals, remaining active in the aquatic environment, and they are not removed by the treatments normally performed in WWTPs [29]. Thus, quinolone-resistant strains can be easily found in this environment. In this study, of the *S. aureus* isolates tested, none showed resistance to levofloxacin and norfloxacin, and only one isolate from EF was resistant to ofloxacin (3.1%, 1/32). Similarly, most *S. aureus* isolates were sensitive to erythromycin (91.4%, 32/35) (figure 2). In the past decade, the clinical use of erythromycin has been limited and is often substituted with other antibiotics due to their better pharmacokinetic proprieties and fewer side effects [30]. Thus, the low rate of erythromycin resistance reported in this study can be explained by reduced selective pressure related to this antibiotic. In turn, although clindamycin-resistant *S. aureus* was uncommon in the WWTP studied, it should be
Table 1. Summary of resistance profile and identification phenotypic of inducible MSL\textsubscript{II} resistance and penicillinase production from \textit{S. aureus} isolated in a full-scale domestic wastewater treatment plants (WWTP).

| \textit{S. aureus} isolated | Antimicrobial resistance profile\textsuperscript{a} | Phenotypic tests\textsuperscript{b} |
|-----------------------------|-----------------------------------------------|----------------------------------|
|                             | ERT  | CLN  | PEN  | LEV  | NOR  | OFX  | OXA  | D-test | Penicillinase |
| Raw sewage                  |      |      |      |      |      |      |      |        |               |
| SA1BEB                      | S    | S    | S    | S    | S    | S    | S    | -      | +             |
| SA2BEB                      | S    | I    | S    | S    | S    | S    | S    | -      | +             |
| SA3BEB                      | S    | I    | R    | S    | S    | S    | S    | -      | +             |
| SA4BEB                      | S    | S    | S    | S    | S    | S    | S    | -      | +             |
| SA5BEB                      | S    | S    | R    | S    | S    | S    | S    | -      | +             |
| SA6BEB                      | S    | S    | S    | S    | S    | S    | S    | -      | +             |
| SA7BEB                      | S    | S    | S    | S    | S    | S    | S    | -      | +             |
| SA8BEB                      | S    | S    | R    | S    | S    | S    | S    | -      | +             |
| SA1CEB                      | S    | S    | R    | S    | S    | S    | S    | -      | +             |
| SA2CEB                      | S    | S    | S    | S    | S    | S    | S    | -      | +             |
| SA3CEB                      | S    | S    | S    | S    | S    | S    | S    | -      | +             |
| SA4CEB                      | S    | R    | R    | S    | S    | S    | S    | -      | +             |
| SA5CEB                      | S    | I    | S    | S    | S    | S    | S    | -      | +             |
| SA6CEB                      | S    | I    | S    | S    | S    | S    | S    | -      | -             |
| SA1DEB                      | S    | I    | R    | S    | S    | S    | S    | -      | +             |
| SA2DEB                      | S    | I    | S    | S    | S    | S    | S    | -      | +             |
| SA5DEB                      | S    | I    | S    | S    | S    | S    | S    | -      | +             |
| SA7DEB                      | S    | I    | S    | S    | S    | S    | S    | -      | +             |
| SA8DEB                      | S    | I    | S    | S    | S    | S    | S    | -      | +             |
| SA1EEB                      | I    | I    | R    | S    | S    | S    | S    | -      | +             |
| SA2EEB                      | S    | I    | R    | S    | S    | S    | S    | -      | +             |
| SA5EEB                      | S    | I    | S    | S    | S    | S    | S    | -      | +             |
| SA1FEB                      | S    | I    | S    | S    | S    | S    | S    | -      | -             |
| SA2FEB                      | S    | S    | S    | S    | S    | S    | S    | -      | +             |
| SA5FEB                      | S    | I    | R    | S    | S    | S    | S    | -      | +             |

(Continued)
Table 1. Summary of resistance profile and identification phenotypic of inducible MSL \(_a\) resistance and penicillinase production from \(S.\) \(aureus\) isolated in a full-scale domestic wastewater treatment plants (WWTP).

| \(S.\) \(aureus\) isolated | Antimicrobial resistance profile\(^a\) | Phenotypic tests\(^b\) |
|-----------------------------|--------------------------------------|-----------------------|
|                            | ERT  | CLN | PEN | LEV | NOR | OFX | OXA | D-test | Penicillinase |
| SA6FEB                     | R    | I   | S   | S   | S   | S   | S   | +      | -               |
| SA2GEB                     | R    | I   | NT  | NT  | NT  | NT  | S   | -      | NT             |
| SA3GEB                     | I    | S   | NT  | NT  | NT  | NT  | S   | -      | NT             |
| SA4GEB                     | S    | I   | R   | S   | S   | S   | S   | -      | +               |
| SA7GEB                     | S    | S   | S   | S   | S   | S   | S   | -      | -               |
| SA8GEB                     | I    | S   | R   | S   | S   | S   | S   | -      | -               |
| SA2HEB                     | R    | I   | NT  | NT  | NT  | NT  | S   | +      | NT             |
| SA3HEB                     | S    | I   | S   | S   | S   | S   | S   | -      | +               |

| Effluent                   | S    | I   | R   | S   | S   | S   | S   | -      | +               |
|                            | S    | S   | R   | S   | S   | R   | S   | -      | +               |

\(^a\)S: susceptible; R: resistant; I: intermediate. \(^b\)positive test (+); negative test (-). NT: not tested; ERT: erythromycin; CLN: clindamycin; PEN: penicillin; CIP: ciprofloxacin; OFX: ofloxacin; NOR: norfloxacin; OXA: oxacillin.

Figure 2. Susceptibility profile of \(Staphylococcus aureus\) isolated from a full-scale domestic wastewater treatment plants (WWTP). ERT: erythromycin; CLN: clindamycin; PEN: penicillin; OFX: ofloxacin; LEV: levofloxacin; NOR: norfloxacin; MET: methicillin.
noted that 20 isolates (57.1%) showed an intermediate level of susceptibility to this lincosamide, which highlights the possibility of the spread of resistance within this environment (figure 2). Goldstein et al. [13] reported that sewage represents an important route of dissemination of MLS<sub>B</sub>-resistant <i>S. aureus</i>, and the most common resistance gene related to this phenotype was identified to be <i>ermC</i>. The family of erythromycin ribosomal methylase (<i>erm</i>) genes encodes an adenine-specific N-methyltransferase that methylates the 23S region of rRNA, conferring resistance to all macrolides, lincosamides, and streptogramin B [19].

Erythromycin-induced MLS<sub>B</sub> resistance was investigated by D-zone test. Two of 35 isolates tested, both derived from RS, were found to be D-test-positive (5.7%). This finding corroborates a study by Hess & Gallert [22], which reported that inducible MLS<sub>B</sub> resistance (D-test-positive) in <i>S. aureus</i> from sewage (14-19%) occurs at a lower frequency than constitutive MLS<sub>B</sub> resistance (62.2-75.5%). Penicillinase production was also investigated in 32 <i>S. aureus</i> isolates by the penicillin zone-edge method. A total of 27 isolates were found to produce penicillinase, although several of these (55.5%) were susceptible to penicillin. According to Kaase et al. [31] the penicillin zone-edge test is the most sensitive phenotypic method for penicillinase detection, but some species that not showed genetic determinants to this beta-lactamase, might have positive result in test. Thus, the inconsistencies between the findings in this study highlight the need to confirm, by molecular methods, whether <i>blaZ</i> gene is present in the positive isolates.

In summary, MRSA isolates were absent, and we found a reduced rate of resistance to erythromycin in full-scale domestic WWTPs studded. The high frequency of resistance to penicillin, in turn, suggests the indiscriminate use of this antibiotic in the region of station. In addition, the high rate of intermediate sensitivity to clindamycin among the isolates suggests that domestic sewage can contribute to the advancement of MLS<sub>B</sub>-resistant <i>S. aureus</i>. However, future studies should be performed to better understand the dynamics of this phenomenon, especially in WWTPs from other regions of Brazil.

**Acknowledgements**

The authors would like to thank the University of São João del-Rei for its support during the research. W.G.L. is grateful to Coordenação de Aperfeiçoamento de Pessoal do Nível Superior (CAPES) for a Ph.D. fellowship.

**Disclosure statement**

No potential conflict of interest was reported by the authors.
Wastewater treatment plants as sources of antibiotic-resistant *Staphylococcus aureus*

References

1. S.Y.C. Tong, J.S. Davis, E. Eichenberger, T.L. Holland, V.G. Fowler, Jr., *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management, *Clin. Microbiol. Rev.*, 28, 603-661 (2015).

2. J.A. Lindsay, M.T. Holden, *Staphylococcus aureus*: superbug, super genome?, *Trends Microbiol.*, 12, 378-385 (2004).

3. P. Moreillon, New and emerging treatment of *Staphylococcus aureus* infections in the hospital setting, *Clin. Microbiol. Infect.*, 14, 32-41 (2008).

4. M. Abbas, M. Paul, A. Huttner, New and improved? A review of novel antibiotics for Gram-positive bacteria, *Clin. Microbiol. Infect.*, 23, 697-703 (2017).

5. M. Bassetti, E.M. Trencarichi, A. Mesini, T. Spanu, D.R. Giacobbe, M. Rossi, E. Shenone, G.D. Pascale, M.P. Molinari, R. Cauda, C. Viscoli, M. Tumbarello, Risk factors and mortality of healthcare-associated and community-acquired *Staphylococcus aureus* bacteraemia, *Clin. Microbiol. Infect.*, 18, 862-869 (2012).

6. H.W. Boucher, G.R. Corey, Epidemiology of methicillin-resistant *Staphylococcus aureus*, *Clin. Infect. Dis.*, 46, 344-349 (2008).

7. H. Grundmann, M. Aires-de-Sousa, J. Boyce, E. Tiemersma, Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat, *Lancet*, 368, 874-885 (2006).

8. A.C. Gales, H.S. Sader, J. Ribeiro, C. Zoccoli, A. Barth, A.C. Pignatari, Antimicrobial susceptibility of gram-positive bacteria isolated in Brazilian hospitals participating in the SENTRY Program (2005-2008), *Braz. J. Infect. Dis.*, 13, 90-98 (2009).

9. D.M.R. Amorim, O.C. Person, P.J. Amaral, I.I. Tanaka, Inducible resistance to clindamycin among *Staphylococcus aureus* isolates, *O Mundo da Saúde, São Paulo*, 33, 401-405 (2009).

10. H. Chen, M. Zhang, Occurrence and removal of antibiotic resistance genes in municipal wastewater and rural domestic sewage treatment systems in eastern China, *Environ. Int.*, 55, 9-14 (2013).

11. C.A. Michael, D. Dominey-Howes, M. Labbate, The antimicrobial resistance crisis: Causes, consequences, and management, *Front. Public. Health*, 145, 1-8 (2014).
12. K.E. Shannon, D.Y. Lee, J.T. Trevors, L.A. Beaudette, Application of real-time quantitative PCR for the detection of selected bacterial pathogens during municipal wastewater treatment, *Sci. Total Environ.*, 382, 121-9 (2007).

13. R.E.R. Goldstein, S.A. Micallef, S.G. Gibbs, J.A. Davis, X. He, A. George, L.M. Kleinfelter, N.A. Schreiber, S. Mukherjee, A. Sapkota, S.W. Joseph, A.R. Sapkota, Methicillin-resistant *Staphylococcus aureus* (MRSA) detected at four U.S. wastewater treatment plants, *Environ. Health Perspect.*, 120, 1551-1558 (2012).

14. A. Naquin, J. Clement, M. Sauce, R. Grabert, M. Sherpa, R. Boopathy, Presence of antibiotic resistant *Staphylococcus aureus* in sewage treatment plant, *J. Water Sustainabil.*, 4, 227-236 (2014).

15. E.W. Koneman, W.C. Winn-Jr, S.D. Allen, W.M. Janda, G.W. Procop, P.C. Schreckenberger, G.L. Woods, “Koneman, diagnóstico microbiológico: texto e atlas colorido”, 6th ed., Guanabara Koogan, Rio de Janeiro, 2008.

16. Clinical and Laboratory Standards Institute (CLSI), “Performance Standards for Antimicrobial Disk Susceptibility Testing”, 28th ed., M02-A2, Wayne, PA, 2017.

17. M. Dadashi, M.J. Nasiri, F. Fallah, P. Owlia, B. Hajikhani, M. Emaneini, M. Mirpour, Methicillin-resistant *Staphylococcus aureus* (MRSA) in Iran: A systematic review and meta-analysis, *J. Glob. Antimicrob. Resist.*, 12, 96-103 (2017).

18. S. Teeraputon, P. Santanirand, T. Wongchai, W. Songjiang, N. Lapsomthob, D. Jaikrasun, S. Toonkaew, P. Tophon, Prevalence of methicillin resistance and macrolide-lincosamide-streptogramin B resistance in *Staphylococcus haemolyticus* among clinical strains at a tertiary-care hospital in Thailand, *New Microbes New Infect.*, 19, 28-33 (2017).

19. G. Maravić, Macrolide resistance based on the Erm-mediated rRNA methylation, *Curr. Drug Targets Infect. Disord.*, 4, 193-202 (2004).

20. M.C. Paiva, M.P. Ávila, M.P. Reis, P.S Costa, R.M. Nardi, A.M. Nascimento, The microbiota and abundance of the Class 1 Integron-Integrase gene in tropical sewage treatment plant influent and activated sludge, *PLoS One*, 10, 1-12 (2015).

21. P. Gómez, C. Lozano, D. Benito, V. Estepa, C. Tenorio, M. Zarazaga, C. Torres, Characterization of staphylococci in urban wastewater treatment plants in Spain, with detection of methicillin resistant *Staphylococcus aureus* ST398, *Environ. Pollut.*, 212, 71-76 (2016).

22. S. Hess, C. Gallert, Demonstration of staphylococci with inducible macrolide-lincosamide-streptogramin B (MLSb) resistance in sewage and river water and of the capacity of anhydroerythromycin to induce MLSb, *FEMS Microbiol. Ecol.*, 88, 48-59 (2014).
Wastewater treatment plants as sources of antibiotic-resistant *Staphylococcus aureus*

23. J. Li, X. Zhao, X. Tian, J. Li, J. Sjollema, A. Wang, Retention in treated wastewater affects survival and deposition of *Staphylococcus aureus* and *Escherichia coli* in sand columns, *Appl. Environ. Microbiol.*, 81, 2199-2205 (2015).

24. S.M. Ben, M.S. Abbassi, P. Gómez, L. Ruiz-Ripa, S. Sghaier, C. Ibrahim, C. Torres, A. Hassen, *Staphylococcus aureus* isolated from wastewater treatment plants in Tunisia: occurrence of human and animal associated lineages, *J. Water Health*, 15, 638-643 (2017).

25. C. Faria, I. Vaz-Moreira, E. Serapicos, O.C. Nunes, C.M. Manaia, Antibiotic resistance in coagulase negative staphylococci isolated from wastewater and drinking water, *Sci. Total Environ.*, 407, 3876-3882 (2009).

26. C.M. Luna, E. Rodríguez-Noriega, L. Bavestrello, M. Guzmán-Blanco, Gram-negative infections in adult intensive care units of Latin America and the Caribbean, *Crit. Care Res. Pract.*, 2014, 480463 (2014).

27. J.M. Thompson, A. Gündoğdu, H.M. Stratton, M. Katouli, Antibiotic resistant *Staphylococcus aureus* in hospital wastewaters and sewage treatment plants with special reference to methicillin-resistant *Staphylococcus aureus* (MRSA), *J. Appl. Microbiol.*, 114, 44-54 (2013).

28. M.T. Wan, C.C. Chou, Class 1 integrons and the antiseptic resistance gene (qacEΔ1) in municipal and swine slaughterhouse wastewater treatment plants and wastewater-associated methicillin-resistant *Staphylococcus aureus*, *Int. J. Environ. Res. Public Health*, 12, 6249-6260 (2015).

29. N. Dorival-García, A. Zafra-Gómez, F.J. Camino-Sánchez, A. Navalón, J.L. Vílchez, Analysis of quinolone antibiotic derivatives in sewage sludge samples by liquid chromatography-tandem mass spectrometry: comparison of the efficiency of three extraction techniques, *Talanta*, 106, 104-118 (2013).

30. D. Jelić, R. Antolović, From erythromycin to azithromycin and new potential ribosome-binding antimicrobials, *Antibiotics (Basel)*, 5, 1-29 (2016).

31. M. Kaase, S. Lenga, S. Friedrich, F. Szabados, T. Sakinc, B. Kleine, S.G. Gatermann, Comparison of phenotypic methods for penicillinase detection in *Staphylococcus aureus*, *Clin. Microbiol. Infect.*, 14, 614-616 (2008).

How to cite this article

S.L. Oliveira-Toledo, R.M. Silveira-Silva, I.C. Rodrigues dos Santos, W.G. Lima, L. Gonçalves-Rodrigues-Ferreira, M.C. Paiva, Domestic wastewater treatment plants as sources of macrolide-lincosamide-streptogramin B- and penicillin-resistant *Staphylococcus aureus* in Brazil, *Rev. Colomb. Cienc. Quím. Farm.*, 49(2), 267-279 (2020).