



Phenotypic Characterization of Antibiotic Resistance in *Staphylococcus aureus* from Clinical, Hospital Wastewater, and River Sources in Jakarta

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Background: Antimicrobial resistance (AMR) poses a growing threat to public health, particularly in urban settings where effluents from hospital wastewater treatment plants (WWTPs) and river systems may contribute to the spread of resistant bacteria. **Aim:** This study aimed to characterize and compare the phenotypic antibiotic resistance profiles of *Staphylococcus aureus* isolated from clinical samples, hospital wastewater, and the Ciliwung River in Jakarta, to assess potential environmental dissemination and links between clinical and environmental reservoirs of resistance. **Methods:** *S. aureus* isolates were obtained from RSCM clinical samples, four points at the hospital WWTP, and six sites along the Ciliwung River between September and December 2024. Isolates were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry, and antibiotic susceptibility testing was performed via automated systems. **Results:** Two river isolates showed resistance only to penicillin. Among 14 WWTP isolates, resistance to penicillin (43%), tetracycline (19%), and gentamicin (3%) was observed, with evidence of multidrug resistance (MDR). Clinical isolates showed a higher prevalence of resistance, with 32% classified as MDR. All methicillin-resistant *S. aureus* isolates exhibited MDR, with a greater prevalence in clinical (22%) than environmental samples (6%). A vancomycin-resistant *S. aureus* isolate was detected at the WWTP inlet, the first such report in Indonesian wastewater. Half of the environmental isolates were susceptible to all antibiotics tested. **Conclusion:** Similar resistance patterns in clinical and environmental *S. aureus* suggest potential transmission between these reservoirs. However, larger studies are needed to confirm these findings and better assess environmental AMR risks.

Key words: Antimicrobial resistance, Indonesia, methicillin-resistant *Staphylococcus aureus*, wastewater treatment plants

INTRODUCTION

Antimicrobial-resistant bacteria (ARB) are increasingly prevalent due to the overuse of antibiotics for treating infectious diseases in humans, livestock, clinical environments, and the community. Evaluating the risk of antimicrobial resistance (AMR) in aquatic ecosystems is crucial for sustainable human progress,¹ however, Southeast Asian nations have faced criticism for inadequate AMR surveillance.² Methicillin-resistant *Staphylococcus aureus* (MRSA) is a high priority bacterium on the WHO's AMR bacteria list³ and is responsible for both nosocomial and community-acquired infections. Currently, MDR has become more serious in

the water environment, resulting in an increasing number of extensively drug-resistant MRSA strains that are more challenging to control and treat with effective antimicrobial agents, particularly during waterborne outbreaks.^{4,5} *S. aureus* and MRSA have been predominantly identified in water environments in Europe and the USA,^{6,7} and reported as indicator microorganisms in wastewater and river water.^{8,9} They may establish a temporary habitat in hospitals, potentially facilitating their dissemination into the environment through pathways such as hospital wastewater.¹⁰ It has been indicated that these microbes could subsequently infiltrate sewage treatment

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plants (STPs) via the sewerage system, where they may endure treatment and ultimately reenter the environment through STP effluent. Hospital wastewater treatment plants (WWTPs) are recognized as potential sites for the dissemination and reservoirs of AMR.¹¹ Studies have shown that the treatment process often reduces AMR levels in effluent water, and it can also promote the emergence of multidrug-resistant (MDR) bacteria due to selective pressures imposed by microbial treatments in WWTPs. Bacteria can acquire AMR genes through horizontal gene transfer via mobile genetic elements such as transposons and plasmids in environments with elevated levels of these genes, such as wastewater.¹² In Southeast Asian countries, the prevalence and pathogenesis of MRSA have been largely studied in hospitals and the community; however, their persistence in hospital WWTPs and the surrounding environment postrelease remains understudied.

These WWTPs are essential research locations for understanding the dynamics of antibiotic resistance, particularly in *S. aureus*.¹³ River water is highly vulnerable to contamination from wastewater discharge, domestic refuse, and urban runoff, including potential pollutants from various hospital WWTPs. The proximity to densely populated urban regions, where river water is employed for domestic, agricultural, and fishing activities, renders this water supply a potential hazard for human exposure.¹⁴ Furthermore, seasonal flooding exacerbates the risk of disseminating antimicrobial-resistant bacteria into residential zones, increasing the public health threat.

In this study, *S. aureus* isolates from clinical samples showed a higher proportion of multidrug resistance (8/36) compared to those from hospital wastewater and the Ciliwung River (1/16). While this suggests a potential trend of increased resistance in clinical settings, the limited number of MDR isolates and the overall sample size prevent robust statistical analysis. The similarity in AST profiles between some clinical and environmental isolates suggests possible transmission pathways; however, drawing well-supported conclusions requires a larger, more representative dataset that includes isolates from clinical, environmental, and community sources. The findings remain crucial for understanding the spread of resistant pathogens in environmental and healthcare settings. They will contribute to the development of public health policies and strategies aimed at mitigating AMR in Indonesia and beyond.

MATERIALS AND METHODS

Sample collection

Ethical approval was obtained from the Ethics Committee of Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital with serial number KET-496/UN2.

F1/ETIK/PPM.00.02/2024 on April 1, 2024. RSCM is one of the largest public tertiary care and referral hospitals in Indonesia, offering medical services to a substantial segment of Jakarta's population. The hospital's WWTPs process effluent produced by diverse departments, including clinical, surgical, and diagnostic units. A total of 37 *S. aureus* isolates were obtained from the clinical microbiology laboratory. Thirty-one samples were taken from the WWTPs, Instalasi Pengelolaan Air Limbah (IPAL 1 and IPAL 2), at RSCM between August 2024 and November 2024, eight from raw wastewater (Inlet points), and eight from treated wastewater (Outlet points). An equivalent number of samples was collected from upstream and downstream points of IPAL 1 and IPAL 2, which are in the Ciliwung River. The Ciliwung River flows 120 kilometers from the upstream Bogor districts, which include Mount Gede, Mount Pangrango, and Cisarua, to the downstream areas near Jakarta's northern coastline. The basin encompasses an area of 387 km² and supports a population of approximately 4 million individuals.¹⁵ Six points along the Ciliwung River in south and central Jakarta, shown in Figure 1, were selected for sampling based on their proximity to residential areas and potential contamination exposure. The sampling locations included the downstream of Ciliwung River near RS Bunda Jakarta Hospital, downstream RSCM (within 5 km), Kampung Melayu, South of Tebet (a branch of Ciliwung), and Cikini, Central Jakarta. Water samples were collected using sterile 500 mL Schott glass bottles, labelled with the date, time, and sampling site, and transported to the Clinical Microbiology Laboratory (LMK FKUI) on ice. Samples were placed at 4°C upon arrival and were processed within 12 h.

Isolation of *Staphylococcus aureus* from the environment

Sterile 0.45 µm membrane filters were used to filter 100 mL of water from each site sample. Prepared Brain Heart Infusion Broth (BHI) with 6.5% NaCl, which favors the growth of *Staphylococcus* species, and incubated for 24 h at 37°C. A loopful of the BHI was then spread onto the Mannitol Salt Agar (MSA) plate and incubated for 24 h at 37°C.¹⁶ Three distinct colonies, based on their morphology, were selected and subcultured onto separate MSA plates to ensure purity and incubated at 37°C for 24 h.¹⁷ Subsequently, a colony from each plate was picked and subcultured onto 5% sheep Blood Agar (BA) plates to further assess the hemolytic activity. The clinical isolates were processed using the same procedure, first cultured on MSA, followed by sub-culturing on 5% sheep BA to observe hemolysis.

Isolation of *Staphylococcus aureus* from the hospital

Clinical *S. aureus* isolates were retrieved from the microbiology laboratory at RSCM. These isolates were

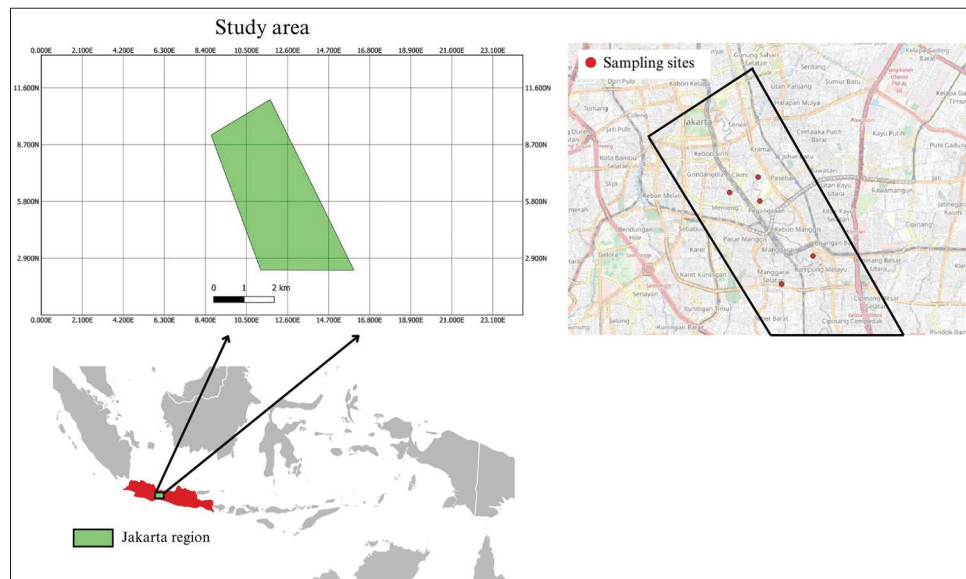


Figure 1: Study area and sampling location along the Ciliwung River in Jakarta, Indonesia. The map illustrates the study region, with the Jakarta province highlighted in red (bottom left). The green polygon (top left) represents the designated study area, while the right-side map provides a detailed view of the sampling locations (red dots) within the Ciliwung River watershed, marked by a black boundary. Base map sourced from OpenStreetMap

derived from clinical samples, including blood, sputum, and swabs, collected from patients in various hospital wards and analyzed microbiologically at the same laboratory from September 2024 to December 2024.

Identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry

Matrix-assisted laser desorption ionization (MALDI) Biotyper-BD Sirius One IVD was used following the manufacturer's guidelines. A single *S. aureus* colony from a BA plate was transferred onto an MBT Biotarget 96 plate using a sterile toothpick. Each spot was covered with 2 μL of MALDI matrix, dried, and loaded into the MALDI-time of flight mass spectrometry (TOF MS) for analyses. The Bruker Bacterial Test Standard was inoculated on each plate for automatic calibration and quality control. Spectral data were then analyzed using the Biotyper software to identify the bacterial species.¹⁸

Antimicrobial susceptibility testing by BD phoenix

Antimicrobial susceptibility testing (AST) for *S. aureus* from WWTPs and river water was carried out using the PMIC/ID-55 panel on the Phoenix Automated Microbiology System. The panel included 21 antibiotics across multiple classes, including β -lactams, macrolides, aminoglycosides, tetracyclines, and glycopeptides, to determine minimum inhibitory concentrations. Pure bacterial colonies were grown on 5% sheep Blood agar and incubated overnight at 37°C. Fresh colonies were added into the

Phoenix ID broth and maintained to 0.5 McFarland standard by using a nephelometer. A 25 μL aliquot of the suspension was mixed with AST broth and an AST indicator, reaching a bacterial concentration of $\sim 5 \times 10^5$ CFU/mL. Samples were loaded into the Phoenix system, incubated at 35°C, and analyzed for species identification and susceptibility profiles.¹⁹

RESULTS

To effectively isolate *S. aureus* from environmental samples while minimizing contamination, a three-step subculturing process was employed. The sample was cultured twice on Mannitol Salt Agar (MSA), followed by a final subculture on Blood Agar (BA), as shown in Figure 2, to obtain pure colonies. The growth on MSA exhibited mixed colony morphologies, ranging from yellow, round, smooth colonies to white, nonsmooth colonies, with some displaying white-centred features. Of the 16 environmental *S. aureus* isolates, 9 exhibited beta-hemolysis as shown in Table 1. While *S. aureus* is typically hemolytic, the presence of nonhemolytic isolates may reflect strain variability or environmental adaptation, warranting further investigation through genotypic methods. All clinical isolates showed beta-hemolysis on BA and displayed the characteristics of the *S. aureus* colony morphology.

Out of 37 clinical isolates, 8 (21.6%) were identified as MRSA. AST revealed the highest resistance to penicillin (51%), while no isolates exhibited resistance to vancomycin. The second highest resistance was identified against tetracycline (27%), followed by erythromycin (19%). Multidrug resistance (MDR) was

Table 1: β -hemolysis and matrix-assisted laser desorption ionization-time of flight mass spectrometry identification scores of water isolates and their corresponding sampling sites

Isolate ID	Date of collection	Type of specimen	Site of specimen	β -hemolysis on BA	Score value	Species name
JKT0001	August 27, 2024	Raw wastewater	IPAL 2, RSCM, Jakarta	No	2.13	<i>S. aureus</i>
JKT0002	November 4, 2024	Raw wastewater	IPAL 2, RSCM, Jakarta	Yes	2.38	<i>S. aureus</i>
JKT0003	November 4, 2024	Raw wastewater	IPAL 1, RSCM, Jakarta	Yes	2.29	<i>S. aureus</i>
JKT0004	November 4, 2024	Raw wastewater	IPAL 1, RSCM, Jakarta	Yes	2.31	<i>S. aureus</i>
JKT0005	November 4, 2024	Downstream	IPAL2, RSCM, Jakarta	Yes	2.23	<i>S. aureus</i>
JKT0006	November 4, 2024	Downstream	IPAL2, RSCM, Jakarta	Yes	2.18	<i>S. aureus</i>
JKT0007	November 25, 2024	Downstream	IPAL2, RSCM, Jakarta	Yes	2.34	<i>S. aureus</i>
JKT0008	November 12, 2024	Treated water	IPAL 1, RSCM, Jakarta	No	2.18	<i>S. aureus</i>
JKT0009	November 12, 2024	Upstream	IPAL 1, RSCM, Jakarta	No	2.33	<i>S. aureus</i>
JKT0010	November 12, 2024	Upstream	IPAL 1, RSCM, Jakarta	No	2.31	<i>S. aureus</i>
JKT0011	November 12, 2024	Upstream	IPAL2, RSCM, Jakarta	Yes	2.33	<i>S. aureus</i>
JKT0012	November 12, 2024	Downstream	IPAL2, RSCM, Jakarta	No	2.37	<i>S. aureus</i>
JKT0013	November 12, 2024	Raw wastewater	IPAL2, RSCM, Jakarta	No	2.24	<i>S. aureus</i>
JKT0014	November 12, 2024	Downstream	IPAL 1, RSCM, Jakarta	No	2.45	<i>S. aureus</i>
JKT0015	August 27, 2024	River water	Cikini, Central Jakarta	Yes	2.26	<i>S. aureus</i>
JKT0016	October 28, 2024	River water	Kampung Melayu, Central Jakarta	Yes	2.22	<i>S. aureus</i>

S. aureus=*Staphylococcus aureus*; IPAL=Instalasi pengelolaan air limbah; BA=Blood agar

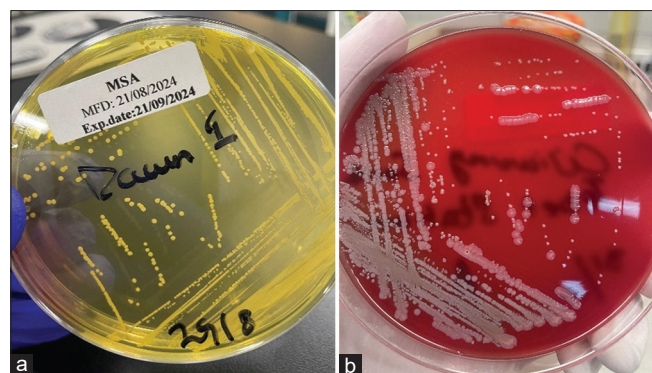


Figure 2: Growth of environmental *Staphylococcus aureus* on Mannitol Salt Agar (a) and Blood Agar (b)

identified in 12 patients (32.4%), indicating nonsusceptibility to at least three different antibiotic classes. Notably, all MRSA isolates were classified as MDR, highlighting their significant resistance profile. The characteristics of the patients are in Table 2. One observable trend is that 19% of the isolates exhibited intermediate susceptibility to levofloxacin, while only one isolate showed intermediate susceptibility to moxifloxacin, as shown in Figure 3.

Environmental samples yielded 16 *S. aureus* isolates. Fourteen isolates were obtained from WWTPs, with 35% from inlet points, 35% from downstream, 21% from upstream, and only 7% from outlet points. Five were methicillin-sensitive *S. aureus*, exhibited resistance to penicillin only, and one was

MDR vancomycin-resistant *S. aureus* (VRSA). Two isolates from the Ciliwung River showed resistance only to penicillin. Among all 16 isolates, penicillin resistance was observed in 43%. Ampicillin, fusidic acid, clindamycin, ciprofloxacin, erythromycin, gentamicin, linezolid, and rifampicin were detected only in multidrug-resistant (MDR) *S. aureus*, as shown in Figure 4.

A significant finding was an MDR isolate from Inlet IPAL 2, resistant to multiple antibiotics, including vancomycin, as shown in Table 3, classifying it as a VRSA strain. Penicillin G and tetracycline resistance were also detected at Downstream IPAL 2 and Inlet IPAL 2, while gentamicin resistance appeared at Downstream IPAL 2. Isolates from Outlet IPAL 1 and Upstream IPAL 1 exhibited complete susceptibility.

Comparison of clinical and environmental isolates

The majority of isolates from clinical and environmental sources were susceptible to all tested antibiotics. Penicillin resistance was the most prevalent in both groups, showing a similar trend. Tetracycline resistance followed the same pattern, whereas erythromycin resistance was significantly lower in environmental isolates compared to clinical isolates. An isolate showing resistance to penicillin and tetracycline corresponded with the AST profiles of two *S. aureus* wastewater isolates, JKT0002 and JKT0012. The clinical isolate was reported in December, while the water isolates were collected in early and mid-November. These wastewater

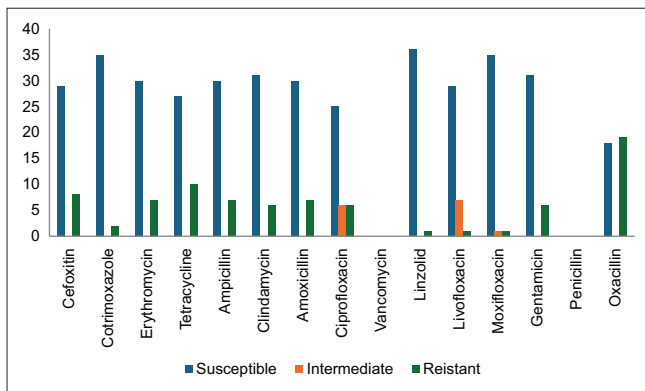


Figure 3: Antibiotic susceptibility pattern of *Staphylococcus aureus* in clinical isolates

Table 2: Characteristics of the patients and percentage of methicillin-resistant *Staphylococcus aureus* among the clinical isolates

Characteristics	<i>Staphylococcus aureus</i> (n)	MRSA, n (%)
Total	37	8 (22)
Age (years)		
>50	10	3 (8)
<5	8	2 (5)
10–50	19	3 (8)
Sex		
Male	25	5 (13)
Female	12	3 (8)
Source		
Blood	32	6 (75)
Sputum	5	1 (13)
Wound	11	1 (13)

MRSA=Methicillin-resistant *Staphylococcus aureus*

isolates originated from both the inlet and downstream points of the WWTP IPAL 2.

DISCUSSION

This study is the first in Indonesia to assess the diversity of AMR in *S. aureus* in river water and WWTPs of a tertiary care hospital and to compare them with the clinical *S. aureus* strains simultaneously. Antibiotic resistance in *S. aureus* can be acquired through enzymatic inactivation of the antibiotic (such as penicillinase and aminoglycoside-modifying enzymes), modification of the target resulting in reduced affinity for the antibiotic (penicillin-binding protein 2a in MRSA and D-Ala-D-Lac in the peptidoglycan precursors of vancomycin-resistant strains), sequestration of the antibiotic (as observed with vancomycin and potentially

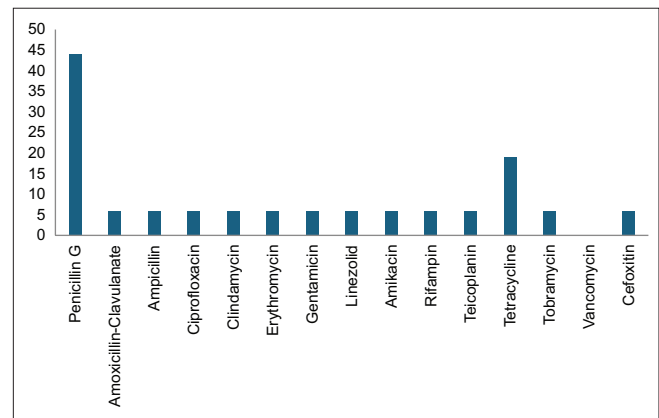


Figure 4: Antimicrobial resistance percentage in water samples of wastewater treatment plants and the Ciliwung River

daptomycin), and the action of efflux pumps (as seen with fluoroquinolones and tetracycline).²⁰ Urban rivers have transformed into major reservoirs for MDR bacteria and antibiotic-resistance genes²¹ due to the rapid urbanization and industrialization leading to the release of industrial, domestic, and hospital waste containing pharmaceuticals, heavy metals, and pesticides posing risks to community health and the environment.²² Those exposed to contaminated water sources, such as sewage workers, hospital staff, and even the general public enjoying recreational waterways, can get *S. aureus* by skin contact, ingestion, or inhalation. Once *S. aureus* has infected humans, it can spread by direct contact or fomites in hospital environments, leading to hospital-acquired illnesses.²³ In this study, no MRSA strains were detected in the Ciliwung River samples, indicating a low prevalence of MDR *S. aureus* in this water source. This could be attributed to various environmental factors such as dilution effects, natural microbial competition, or less exposure to antibiotics. However, resistance to Penicillin G was observed, highlighting persistent antibiotic contamination and underscoring the river's function as a reservoir for resistant bacteria. This aligns with findings indicating that the prevalence of MRSA in environmental water sources may be lower than in clinical isolates, such as in India's Yamuna River,²⁴ where MDR *S. aureus* strains have been associated with untreated wastewater, pharmaceutical effluents, and industrial discharge.

A study conducted in South Sulawesi, Indonesia, investigated the AMR profiles of *S. aureus* isolated from humans, animals, and Dangke revealed that penicillin showed the highest prevalence of resistance across all sources, followed by ampicillin, tetracycline, and erythromycin,²⁵ as revealed in this study as well. The identification of MDR VRSA in the WWTPs sample indicates the first detection of VRSA in Indonesia from a hospital WWTP. Vancomycin has long been the last-resort antibiotic for MRSA infections, and

Table 3: AST Results of the *S. aureus* isolates obtained from WWTPs and Ciliwung River

Antibiotics	WTP0001	WTP0002	WTP0003	WTP0004	WTP0005	WTP0006	WTP0007	WTP0008	WTP0009	WTP0010	WTP0011	WTP0012	WTP0013	WTP0014	CR0001	CR0002
PEN	R	R	S	S	S	S	R	R	S	S	S	R	R	S	R	R
AMC	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
AMP	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
CIP	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
CLI	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
ERY	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
FUS	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
GEN	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
LIN	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
MUP	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NIT	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S
OXA	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
AMIK	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
RIF	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
TEI	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
TET	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S	S
TOB	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
SEX	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
VAN	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
CEF	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S

Trimethoprim-Sulfamethoxazole=SEX; Fusidic Acid=FUS; Vancomycin=VAN; Cefoxitin=CEF; Tobramycin=TOB; Tetracycline=TET; Rifamycin=RIF; Amikacin=AMIK; Oxacillin=OXA;
 Penicillin=PEN; Ciprofloxacin=CIP; Clindamycin=CLI; Erythromycin=ERY; Gentamycin=GEN; Linezolid=LIN; Mupirocin=MUP; Nitrofurantoin=NIT; Amoxicillin-Clavulanate=AMC;
 Teicoplanin=TEI; S=Susceptible; R=Resistant; I=Intermediate

the emergence of VRSA presents an alarming development with implications for treatment options and public health safety. This finding aligns with the studies conducted in India²⁶ and the USA.²⁷ Furthermore, its presence in the inlet of IPAL2 indicates that this MDR strain may originate from either hospital patients or the environment, thereby posing a potential risk of public transmission. The high resistance to penicillin observed in water isolates corresponded with findings from other areas of Indonesia, including South Sulawesi and Iran,²⁸ where all *S. aureus* isolates from water sources were reported to be penicillin resistant. A recent year-long study conducted in Malaysia, a neighboring country to Indonesia, on hospital wastewater reported a significant prevalence of multidrug-resistant *S. aureus*, particularly vancomycin-resistant strains, shows a 50% resistance to cefoxitin, indicating a substantial presence of MRSA.²⁹ However, the current study does not reflect this trend, likely due to differences in wastewater treatment efficiency and regional variation in resistant strains. This also indicates that WWTPs serve as reservoirs and dissemination points for resistant pathogens. A significant observation from the RSCM sample collected on November 12 was that all isolates, with the exception of one, along with the sole isolate from August 27, 2024, did not demonstrate beta-hemolysis, whereas beta-hemolysis was a prevalent characteristic in clinical isolates, warranting further investigation through genomic studies centered on the distribution of virulence genes. Recent research has shown that environmental stresses, such as those in wastewater, might cause the collective expression of *S. aureus*'s hemolysin genes to be downregulated or eliminated.^{30,31} MRSA prevalence in RSCM was reported at 11% in 2022.³² This high prevalence highlights the selective pressure exerted by broad-spectrum antibiotic use within healthcare facilities, likely contributing to the VRSA emergence in this study. The findings in Sweden³³ and the USA³⁴ show that the presence of ARB in hospital effluent is notably lower, a trend also observed in this study. However, despite reduced resistance at specific treatment points, the detection of resistant strains downstream highlights that current wastewater treatment processes are insufficient to eliminate resistant bacteria. A clinical isolate from December showed similar antimicrobial susceptibility patterns to two *S. aureus* wastewater isolates (JKT0002 and JKT0012) collected in November. This correlation suggests a potential link between environmental *S. aureus* strains and clinical infections, highlighting the role of environmental reservoirs in the spread of *S. aureus*.³⁵ This study has a limited sample size that may not sufficiently represent the overall prevalence of ABR *S. aureus* in the region. In addition, sampling was restricted to a designated timeframe,

potentially neglecting seasonal or environmental fluctuations that affect resistance patterns. Longitudinal studies should be conducted throughout different sections of the Ciliwung River and WWTPs to capture seasonal and geographical fluctuations, along with the monitoring of the prevalence of MRSA in hospitals through molecular and high-throughput sequencing methods.

CONCLUSION

This study highlights the antibiotic resistance patterns of *S. aureus* across diverse environments, including clinical settings, river water, and hospital wastewater. Although the prevalence of MRSA was relatively low, the detection of VRSA in hospital wastewater and MDR strains in clinical samples underscores the critical need for ongoing AMR surveillance. This study suggests an epidemiological link between environmental and clinical *S. aureus* strains, but to strengthen and generalize these observations, future studies should prioritize larger, more representative datasets encompassing clinical, environmental, and community-derived isolates. Comparative analyses of resistance profiles between inlet and outlet wastewater samples would provide valuable insights into the capacity of WWTPs to reduce the burden of resistant *S. aureus* strains.

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Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflicts of interest

There are no conflicts of interest.

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