

Isolation of Penicillin resistant *Staphylococcus aureus* (PRSA) from sewage water and testing for Antibiotic susceptibility with protein synthesis inhibitor antimicrobials

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Research Article

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Abstract

Emergence of multidrug-resistant *Staphylococcus aureus* is a major challenge right now the microbial research community facing worldwide. *S. aureus* has developed wide resistance to a broad range of β -lactam antibiotics through expression of β -lactamase and emerging in to methicillin-resistant *Staphylococcus aureus* (MRSA). In this study, *S. aureus* isolated from the sewage water sample incidentally and are caught resistant to Penicillin (PRSA). Antibiotic susceptibility testing confirmed that these PRSA isolates remains to be susceptible to protein synthesis inhibitor antibiotics, including amikacin, streptomycin, chloramphenicol, and tetracycline. In addition, *Bacillus subtilis* isolated using same sewage sample exhibited susceptibility to tetracycline, although its susceptibility to remaining antibiotics were not evaluated in this study These findings highlight sewage wage as potent reservoir of PRSA and importance of evaluation of resistance patterns in non-clinical settings.

Novelty Statement

The novelty of research includes studies on exploitation of sewage water as a least explored environmental reservoir of penicillin-resistant *Staphylococcus aureus* (PRSA) and evaluation of its susceptibility specifically to antimicrobials like chloramphenicol, Tetracycline, Streptomycin and Amikacin (protein synthesis inhibitors), therefore considered alternative treatment strategies for multi drug resistant staphylococcal infections. PRSA has been extensively studied from contaminated objects of the clinical environment or clinical settings, limited information is available on PRSA presence and resistance to antimicrobials isolated from environmental matrices like sewage. By focusing on antimicrobial like protein synthesis inhibitors, this study highlights about effectiveness of non- β -lactam on therapeutic outcome against circulating strains of PRSA in environment and helps to identify resistance patterns or susceptibility to PSI's. The findings contribute studies of surveillance on antimicrobial resistance (AMR) from a One Health perspective, contributing to region-specific data that helps in environmental monitoring, public health risk assessment, and antibiotic stewardship strategies.

Introduction

Quick emergence of multidrug-resistant (MDR) strains of *Staphylococcus aureus* raises a major concern on public health and clinical management due to highly adaptions of the virulent pathogen that can evade host immune defences. Due to the ability to withstand high antimicrobial pressure, *S. aureus* infections will lead to higher morbidity, mortality, and medical expenses along with frequent treatment failures.

The emergence of *methicillin-resistant Staphylococcus aureus* (MRSA), is first documented in the year 1997 with increased resistance to vancomycin¹. Recent advances in science like monoclonal antibody therapies, CRISPR–Cas-based gene editing, bacteriophage treatment, plant-derived antimicrobials, nanomedicine applications, and drug restructuring approaches, will provide promising alternatives to

conventional antibiotics². However, the development and spread of resistance to antibiotics in both clinical and non-clinical settings is strongly associated with efficacy of these interventions.

Most investigations on antimicrobial resistance developed by *S. aureus* have reports from clinical isolates. For instance, Chew et al. documented on both phenotypic and genotypic characteristics of MRSA strains isolated from neonate samples³, while Onanuga et al. documented about high resistance rates of MDR *S. aureus* with 65.8% of isolates exhibiting multidrug resistance⁴. Although these may provide clinical insights on hospital acquired infections, limited information is available on environmental reservoirs¹¹ that contribute to the persistence, amplification, and reintroduction of MDR *S. aureus* strains into human populations.

Sewage systems is one of the significant yet underexplored interface between environmental and clinical interface, which receive microbial inputs from households, hospitals, and industrial sources. These systems serve as convergence points for antibiotic-resistant bacteria and resistance genes by acting as reservoir there by facilitating the transmission back to the health care communities and common public. Therefore, it is crucial to comprehend resistance patterns of *S. aureus* isolated from environment to understand the antimicrobial resistance and to design effective infection preventive and treatment strategies⁹.

In this context, the current study aims to bridge the gap between environmental–clinical research through studies on isolation and characterization of *S. aureus* from sewage water and evaluation on its antibiotic resistance profile. The isolates demonstrated 100% resistance to penicillin and moderate resistance (40–50%) to Tetracycline and Chloramphenicol, representing the presence of penicillin-resistant *S. aureus* (PRSA) strains in the environment. In order to investigate potential alternative treatments for potential therapeutic outcome pertinent to both clinical antimicrobial stewardship and environmental surveillance by evaluating the susceptibility of pathogenic strain *S. aureus* to protein synthesis inhibitor antimicrobials¹⁰.

The study is limited due to restricted number of antibiotics tested and lack of molecular characterization of resistance genes involved. Future investigations through incorporating larger sample sizes, genotypic analysis and comparative studies between environmental and clinical isolates to study the transmission dynamics and resistance evolution may further validate the work. Integrative environmental monitoring together with clinical surveillance under a One Health framework can help in upgrading in comprehensive strategies to mitigate the spread of multidrug resistance and can increase the therapeutic outcomes with existing and emerging technologies and therapeutic appliances.

Methodology

Antibiotic sensitivity test:

Bacteria are isolated from sewage water in pure form by using spread plate technique and is confirmed as *S.aureus* by Gram staining procedure and catalase confirmation test. Antibiotic sensitivity of *S.aureus* is tested using antibiotic disc diffusion method by using antibiotics like Penicillin (10 mcg), Streptomycin (10mcg), Tetracycline (30 mcg), Chloramphenicol (10 mcg) and amikacin (10 mcg).

Catalase Confirmation Test:

Few ml of 30% H₂O₂ is directly added to the antibiotic disc placed petri plate after required growth of bacteria and observed for oxygen evolution by air bubble formation.

Gram staining:

Few drops of water are placed on to the sterile glass slide and small portion of culture is added to the water and a thin smear is made and heat fixed. To the heat fixed smear few drops of crystal violet is added and incubated for 1 min. After the time period the slide is washed under running tap water and mordant gram's iodine is added and incubated for 1 min and washed after incubated time period. Decolouriser ethanol is added to the slide and incubated for 15–30 sec. After time period the slide is washed under running tap water and stained with safranin for 1 min. After the time period the slide is washed, air dried and observed under either 25X or 40X objective of Olympus microscope.

Results

Round shaped coccus isolated from sewage water is identified as Gram-positive *Staphylococcus aureus* and as shown in Figs. 1B and 1C, the isolated strain exhibited resistance to beta lactam antibiotic, penicillin and classified as Penicillin resistant *Staphylococcus aureus* (PRSA)., rod-shaped bacilli isolated from the sewage sample, is identified as a Gram-positive, catalase-positive bacterium *Bacillus subtilis* and is found to be sensitive to the antibiotic tetracycline.

Gram staining (Fig. 2) is used for genus-level characterization of the sample isolates. The cocci observed are identified as Gram-positive *S.aureus* by positivity to catalase test. *S.aureus* show characteristic diplococci arrangement as grape-like clusters observed under microscope using oil immersion objective lens. In contrast, Micro coccus species typically exhibit a tetrad arrangement. The rod-shaped bacterium isolated from sewage water shown to be susceptible to antibiotic tetracycline on the tested plate and identified as *Bacillus subtilis* with no metachromatic granules, in contrast *Corynebacterium* species is distinguished by the presence of metachromatic granules at their polar ends.

The *S. aureus* isolates of sewage sample exhibited multidrug resistance to antibiotics, like Penicillin (Complete) and Streptomycin (Partial). From the Table 1 the measured zones of inhibition are recorded as 0.9 cm for chloramphenicol, 0.7 cm for amikacin, 1.2 cm for streptomycin approximately with no clear zone, and 0.25 cm for tetracycline. The observed zone of inhibition is recorded as 0 cm for penicillin.

From Figs. 2A, 2B, 2C, 2D and Figs. 3B, 3C, 3D, and 3E, the bacterial isolate was identified as *Staphylococcus aureus* a catalase positive bacterium while genus *Streptococcus* is characterized as Gram-positive cocci bacteria with catalase-negative, that distinguishes it from *Staphylococcus* species.

Table 1
Zones of diameter in cm for antibiotics Penicillin, Tetracycline, Streptomycin, Chloramphenicol and Amikacin tested on *S.aureus*

S.No	Antibiotic tested	Bacterial strain	Zone of diameter (cm)
1.	Penicillin	<i>S.aureus</i>	0 cm
2.	Tetracycline		0.25 cm
3.	Streptomycin		1.2 cm
4.	Chloramphenicol		0.9 cm
5.	Amikacin		0.7cm

Staphylococcus aureus is resistant to penicillin due to the production of the enzyme penicillinase and protein synthesis–inhibiting antibiotics such as streptomycin, chloramphenicol, amikacin, and tetracycline isolated from clinical settings. However, the data from environmental settings is limited and from the current study bacteria isolated from environmental matrices, remained susceptible to protein synthesis–inhibiting antibiotics such as streptomycin, chloramphenicol, amikacin, and tetracycline. Both amikacin and streptomycin are aminoglycosides that inhibits protein synthesis by binding irreversibly to the A-site of the 30S ribosomal subunit. While Tetracycline, a broad-spectrum antibiotic, inhibits protein synthesis and binds reversibly to the 30S subunit. Chloramphenicol binds to the 50S ribosomal subunit, and inhibits peptide bond formation and thereby blocks protein synthesis in prokaryotes.

Discussion

Staphylococcus aureus is recognised as one of a major human pathogen causing wide range of infections, from minor skin rashes to severe systemic diseases. Environmental sources, like sewage and wastewater, is reported to be major reservoirs of most of the antibiotic-resistant bacteria and in common penicillin-resistant *S.aureus* (PRSA). These strains usually carry resistance genes to multiple antibiotics, making them as a significant public health concern. Study on PRSA strains isolated from sewage water is important as it provides clues about the dissemination of resistant pathogens present in the environment and their potential entry in to human populations, reflecting both hospital and community-based usage patterns of antibiotics.

While β -lactam resistance trait is most commonly studied one, understanding susceptibility to various protein synthesis inhibitors such as lincosamides, macrolides, tetracyclines and aminoglycosides, is important equally to devise treatment strategies and surveillance on resistance-based evolution. Current study indicate that sewage isolates of *S. aureus* exhibit 100% resistance to penicillin consistently but

exhibit susceptibility variably to inhibitors of protein synthesis¹⁴. Studies on isolates of hospital wastewater have reported notable non-susceptibility to protein synthesis inhibitors like macrolides and lincosamides^{7,15} among MRSA isolates, while retain partial efficacy to some of the aminoglycosides and other protein synthesis inhibitors^{6,12,17,18}. These patterns suggest the existence of multidrug-resistant strains in the environment and also highlights the importance of protein synthesis inhibitors as one of the potential treatment options and also as indicators of evolving resistance.

Many recent studies on MDR strains have reported similar findings globally, demonstrating a high prevalence of penicillin resistant *S. aureus* from environmental isolates and the frequent occurrence of multidrug-resistant phenotypes. Wastewater treatment processes usually reduce the resistant bacterial load but does not limit the persistence of few resistant bacteria emphasizing the environmental reservoir of antibiotic resistance. However, difference in resistance patterns to protein synthesis inhibitors across different regions, exist likely due to variation patterns in antibiotic usage, wastewater processing efficiency, and local microbial ecology. There are reports on some studies with lower resistance to aminoglycosides and clindamycin, while others report higher rates, which indicates complex regional differences. In addition, genotypic resistance profiles do not always correspond with phenotypic susceptibility, there by urgent need for integrated molecular and functional analyses.

Even though with these advances, several research gaps remain as most of the studies are primarily focused on resistance to β -lactamase gene, leaving behind the mechanisms of resistance to protein synthesis inhibitors unexplored¹⁶. Comparative analysis though exploring genotypic and phenotypic resistance patterns in sewage PRSA isolates, and reports on development of resistance genes to PSI drugs like macrolide, lincosamide, aminoglycoside, and tetracycline, from environment sources remains limited. Furthermore, longitudinal monitoring of horizontal resistance patterns in environmental waters is limited, and the impact of sewage treatment on the existence and persistence of protein synthesis inhibitor-resistant strains is not fully understood. To address these gaps there is a requirement of comprehensive approaches that together combine isolation, phenotypic testing, and molecular characterization of resistance determinants in bacteria is required.

The primary aim of this research is to isolate and characterize PRSA from sewage water and evaluate their antibiotic susceptibility profiles against various protein synthesis inhibitors like streptomycin, Amikacin, Tetracycline and chloramphenicol under suitable conditions. Through integrating phenotypic susceptibility testing with genotypic detection of resistance genes in future, we can identify factors contributing to the persistence or reduction of resistance in environmental reservoirs. Such investigations are critical for understanding the environmental dissemination of MDR *S. aureus*¹³ and devising strategies to mitigate the wide spread of antimicrobial resistance in both clinical and environmental settings^{8,13}.

Conclusion

Emergence of multidrug-resistant (MDR) *Staphylococcus aureus*, that is commonly associated with the nosocomial and respiratory infections, majorly poses significant risk to public health. Developing alternative therapeutic agents that prevent spread of MDR pathogens has become a critical concern. Zhang and colleagues employed a wide bioinformatic tools to design a series of antimicrobial peptides (AP138 derivatives), among which peptide A24 exhibited both strong antimicrobial activity and remarkable stability against *S. aureus*. A24 contains recorded hydrophobicity of approximately 35%, provided with 100% survival in a mouse model and proven anti-inflammatory properties ⁵.

Genetic analysis on bacterial resistant genes revealed the presence of several key genes like *nuc* (identification), *icaA* and *icaD* (biofilm formation), *mecA* and *blaZ* (resistance), and *sfaD* and *sbnD* (siderophore production). In conclusion, the majority of *S. aureus* strains are multidrug-resistant, with susceptibility to various protein synthesis inhibitor drugs like Tetracycline, Amikacin, Chloramphenicol and Streptomycin with partial resistance.

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Figures

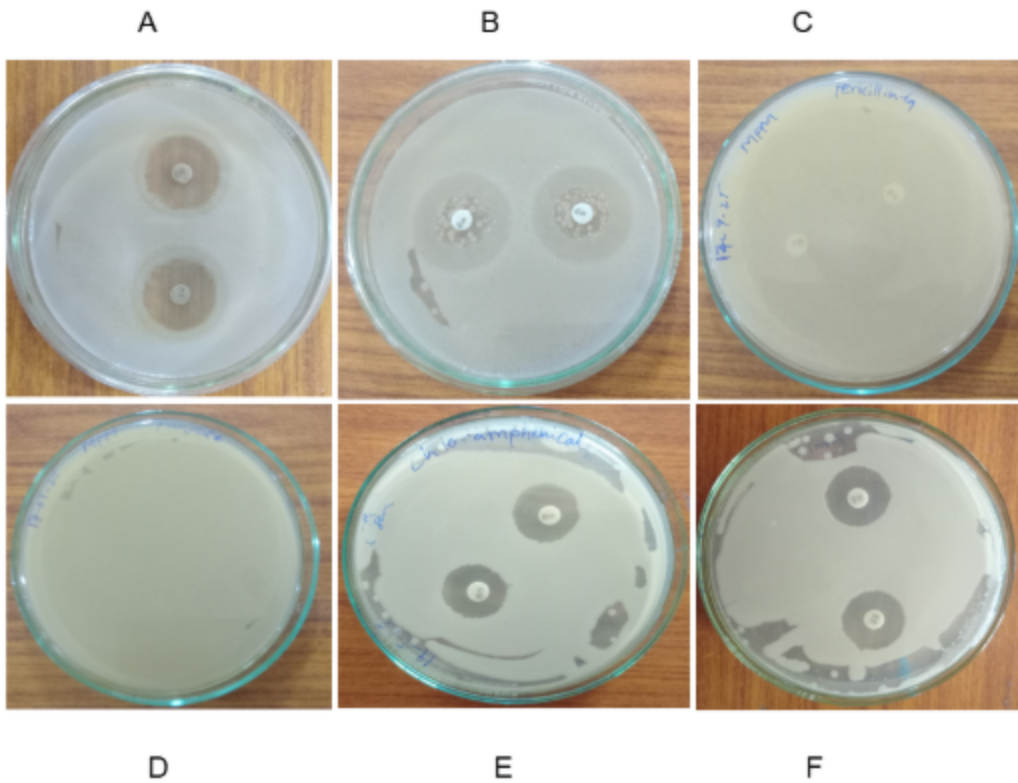


Figure 1

Antibiotic sensitivity test for multi drug resistant *S. aureus*. A. Tetracycline; B. Streptomycin; C. Penicillin; D. Positive control (Pour plate without antibiotic discs); E. Chloramphenicol; F. Amikacin

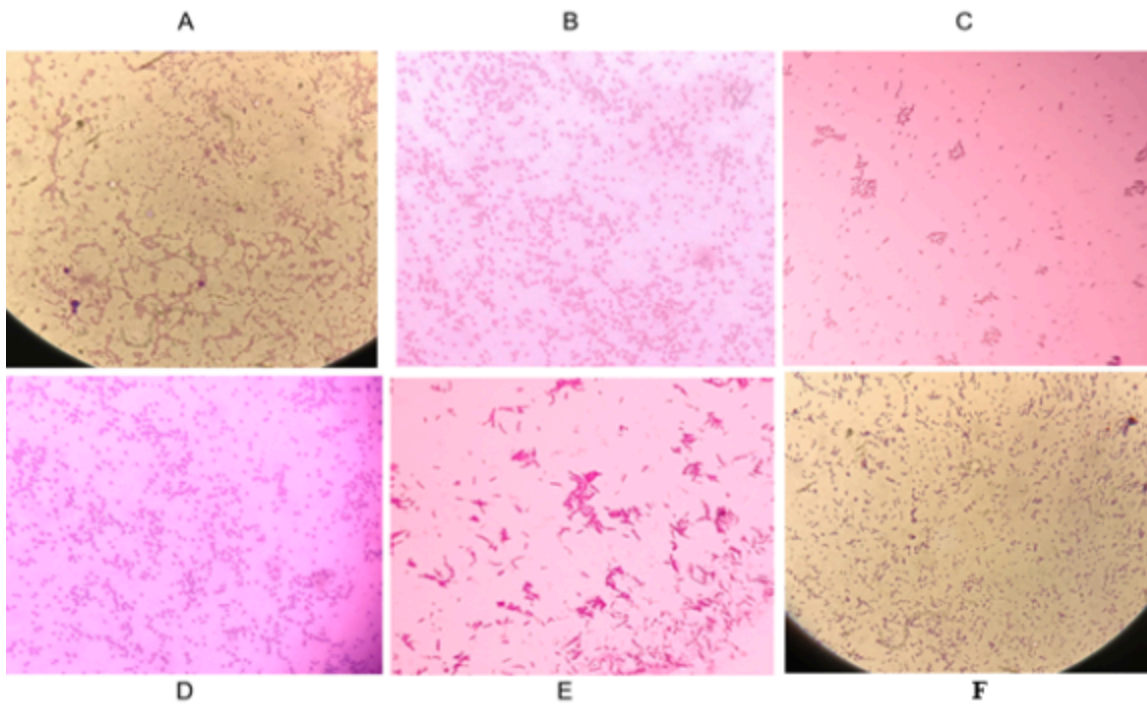


Figure 2

Gram staining of bacteria on the antibiotic tested plates isolated by pour plate method. A. *S.aureus* (Pencillin resistant) B. *S.aureu* (Chloramphenicol) C. *S.aureus* (Streptomycin) D. *S.aureus* tested using Amikacin E. *Bacillus* (Tetracycline sensitive), F. *S.aureus* (Tetracycline sensitive).

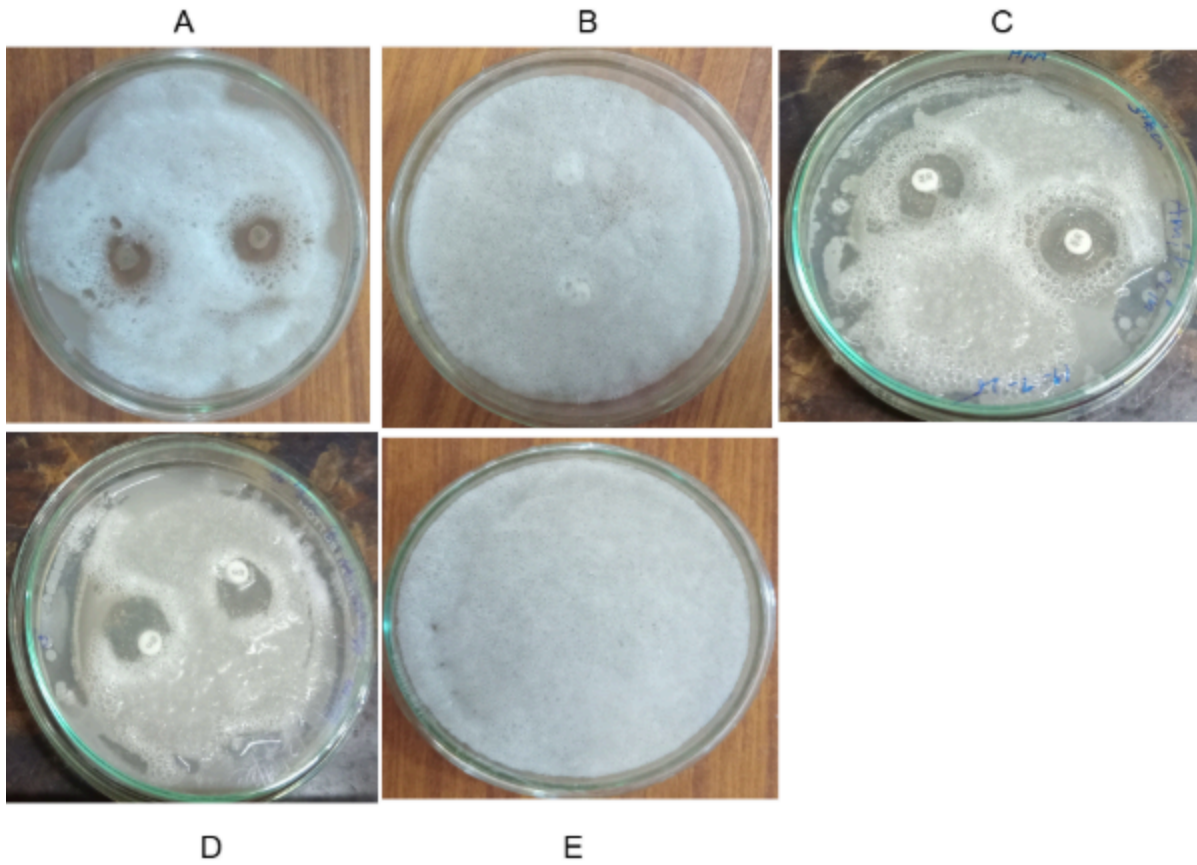


Figure 3

Catalase test for positive confirmation as *S. aureus* tested by antibiotic sensitivity test. A. Tetracycline; B. Streptomycin; C. Amikacin; D. Chloramphenicol; E. Penicillin.