Original Research Article

Prevalence of Antibiotic-resistant *Staphylococcus aureus* from fomites in a tertiary institution in Ibadan, Oyo State, Nigeria

ABSTRACT

The increasing prevalence of antibiotic-resistant *Staphylococcus aureus* (ARSA) poses a significant public health threat, especially in environments with high human contact such as tertiary institutions. This study was justified by the need to address the role of fomites as vectors for the transmission of ARSA, particularly in communal settings where contamination can lead to widespread infections. The investigation aimed to isolate and identify *Staphylococcus aureus* from fomites in a tertiary institution in Ibadan, Nigeria, and assess the antibiotic resistance profiles of these isolates. Fifteen samples were collected from various fomites within the institution and were cultured on Mannitol Salt Agar (MSA). Biochemical tests, including catalase, coagulase, and hemolysis assays, were used for the identification of *Staphylococcus* species. Antibiotic susceptibility testwas conducted using the disk diffusion method to determine the resistance patterns of the isolates. Additionally, biofilm formation, which complicates infection control, was assessed using Congo red agar. The results revealed that 54% of the isolates were identified as *Staphylococcus aureus*, 33% as *Staphylococcus epidermidis*, and 13% as *Staphylococcus haemolyticus*. The isolates exhibited high resistance to commonly used antibiotics, including beta-lactams, with significant multi-drug resistance observed. Furthermore, all isolates demonstrated biofilm-forming abilities, which increase their virulence and resistance to environmental stressors. This study highlights the critical role of fomites in the transmission of antibiotic-resistant pathogens such as *Staphylococcus aureus* in communal settings. This finding underscores the urgent need for stringent hygiene practices, routine surveillance, and targeted interventions to control the spread of ARSA in environments with frequent human interaction.

Keywords: Antibiotic- resistance, *Staphylococcus aureus*, fomites, biofilm

Comment [P1]: Fifty samples are considered somewhat small, and the researcher should have studied larger samples, no less than 200 samples.

Comment [P2]: The number of isolates is needed to be written aside with percentages, in addition, I think the percentages are written inaccurately, there are decimal points not mentioned.

Comment [P3]: Were only three types of staphylococcus spp. diagnosed, do we understand that there were no other types?

Introduction

Antibiotic resistance represents one of the most severe threats to global public health (Collignon and McEwen, 2019; WHO, 2020). The misuse of antibiotics in healthcare and agriculture has accelerated the emergence of multidrug-resistant bacteria, complicating the treatment of infections. Among these pathogens, *Staphylococcus aureus* has drawn particular attention due to its adaptability and resistance mechanisms, including methicillin-resistant *Staphylococcus aureus* (MRSA). *S. aureus* is a common cause of both hospital-acquired and community-acquired infections, which range from mild skin infections to life-threatening conditions like pneumonia and septicemia (Akinrotoye*et al.*, 2019).

In environments such as tertiary institutions, fomites like door handles, tables, and chairs,beddings frominstitutionclinic serve as vectors for the transmission of pathogens, including *S. aureus*. Shared facilities and close human contact in such settings can foster the spread of antibiotic-resistant bacteria, posing a significant risk to public health (Omololu-Aso*et al.*, 2022).

This study aimed to determine the prevalence of antibiotic-resistant *S. aureus* on fomites within a tertiary institution in Ibadan, Nigeria. By investigating the resistance patterns and biofilm formation capabilities of the isolated bacteria, the study will provide valuable insight into the potential public health risks posed by these pathogens.

Materials and Methods

Study Location and Sample Collection

This study was conducted in Fist Technical University, Ibadan, Oyo State, Nigeria. Fifteen samples were collected from fomites (floors, door handles, tables, and chairs) in the institution's cafeterias and clinic. Sterile cotton swabs soaked in normal saline were used to swab the surfaces, and samples were immediately transported to the laboratory on ice (Musa *et al.*, 2023).

Isolation and Identification of Bacterial Isolates

The samples were cultured on Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hours. Distinct colonies were sub-cultured on Nutrient Agar for purification. The isolates were identified through standard biochemical tests including Gram staining, catalase, oxidase, urease, citrate, coagulase, and hemolysis tests (Buszewski*et al.*, 2018: Wang *et al.*, 2019: Groeneveld *et al.*, 2017: Dogra *et al.*, 2018: Zhang *et al.*, 2020: Kim *et al.*, 2017). Sugar fermentation tests were also performed to identify bacterial species based on their metabolic profiles (Charles and Morgan, 2019).

Hemolysis Testing

Comment [P4]: This test should be combined with the bacterial isolation and identification section; there was no need to separate this section.

The hemolytic activity of the isolates was determined by streaking onblood agar and incubated at 37°C for 24 hours. The plates were then examined for zones of hemolysis: beta-hemolysis (clear zones indicating complete lysis of red blood cells) and gamma-hemolysis (no lysis of red blood cells) (Oliveira *et al.*, 2018).

Antibiotic Sensitivity Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The antibiotics tested included amoxicillin-clavulanate, cefotaxime, ceftriaxone, ciprofloxacin, gentamicin, erythromycin, and others. Results were interpreted based on Clinical Laboratory Standards Institute (CLSI, 2024) guidelines.

Biofilm Formation

Biofilm formation was assessed using Congo Red Agar (CRA). The medium was prepared by adding Congo red dye to nutrient agar containing sucrose. The plates were inoculated and incubated at 37°C for 24 hours. The formation of black colonies with a dry crystalline appearance was interpreted as positive for biofilm production (Peng *et al.*, 2022).

Results

Prevalence of *Staphylococcus aureus* on Fomites

Fifty- four (54%)of the isolates were identified as *S. aureus*. Other identified species included *Staphylococcus epidermidis* (33%) and *Staphylococcus haemolyticus* (13%). The high prevalence of *S. aureus* on fomites highlights their potential role in pathogen transmission in communal environments.

Antibiotic Resistance Profiles of *Staphylococcus aureus*isolates

The antibiotic susceptibility tests revealed widespread resistance to multiple antibiotics. All *S. aureus* isolates showed resistance to cefotaxime, ceftriaxone, and cefuroxime. Additionally, resistance to macrolides, including erythromycin and azithromycin, was observed. Ciprofloxacin and gentamicin demonstrated some efficacy, with certain isolates exhibiting intermediate susceptibility.

Hemolytic Activity

The hemolysis test results revealed that all *S. aureus* and *S. haemolyticus* isolates exhibited beta-hemolysis, indicating their strong virulence potential. In contrast, *S. epidermidis* isolates showed gamma-hemolysis, signifying lower virulence. Beta-hemolysis is indicative of the bacteria's ability to lyse red blood cells, contributing to their pathogenicity in severe infections.

Comment [P5]: Need to add sample numbers not just percentages

Comment [P6]: This phrase is written in the discussion, not in the results.

Comment [P7]: The results of this test (hemolysis test) are diagnostic and do not reflect the virulence of the bacteria, as *Staphylococcus aureus* is always of the alpha type, and does not change with changes in its virulence.

Comment [P8]: This phrase is written in the discussion, not in the results.

Table 1: Antibiotic Susceptibility Pattern of *S. aureus* Isolates

Isolate/ Codes	CTX	CRO	IMP	CXM	OFX	ZEM	LBC	CIP	ERY	GN	AZN	AUG

Staphylococcus aureus (CMF)	R	R	R	R	S	R	S	S	R	S	I	R
Staphylococcus aureus (CLF)	R	R	R	R	R	R	S	R	I	I	S	R
Staphylococcus aureus (CLDH)	R	R	R	R	S	R	S	I	R	R	R	R
Staphylococcus aureus (CMDH)	R	R	R	R	R	R	R	R	I	S	I	R
Staphylococcus aureus (CLT)	R	R	R	R	R	R	R	R	I	R	R	R
Staphylococcus aureus (CLBH)	R	R	R	R	R	R	S	R	I	S	R	R
Staphylococcus aureus (CMC)	I	I	R	R	R	R	R	R	I	I	R	R
Staphylococcus aureus (CET)	I	S	R	R	R	S	S	R	I	S	R	I

Comment [P9]: What do these (abbreviations) refer to?

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Keys:

R= Resistant, S= Susceptible, I= Intermediate,AUG= Amoxicilin Clavulanate, CTX= Cefotaxime, CRO= Ceftriaxone Sulbactam, IMP= Imipenem/Cilastatin, CXM= Cefuroxime, OFX= Ofloxacin, ZEM= Cefexime, LBC= Levofloxacin, CIP= Ciprofloxacin, ERY= Erythromycin, GN= Gentamycin, AZN= Azithromycin.

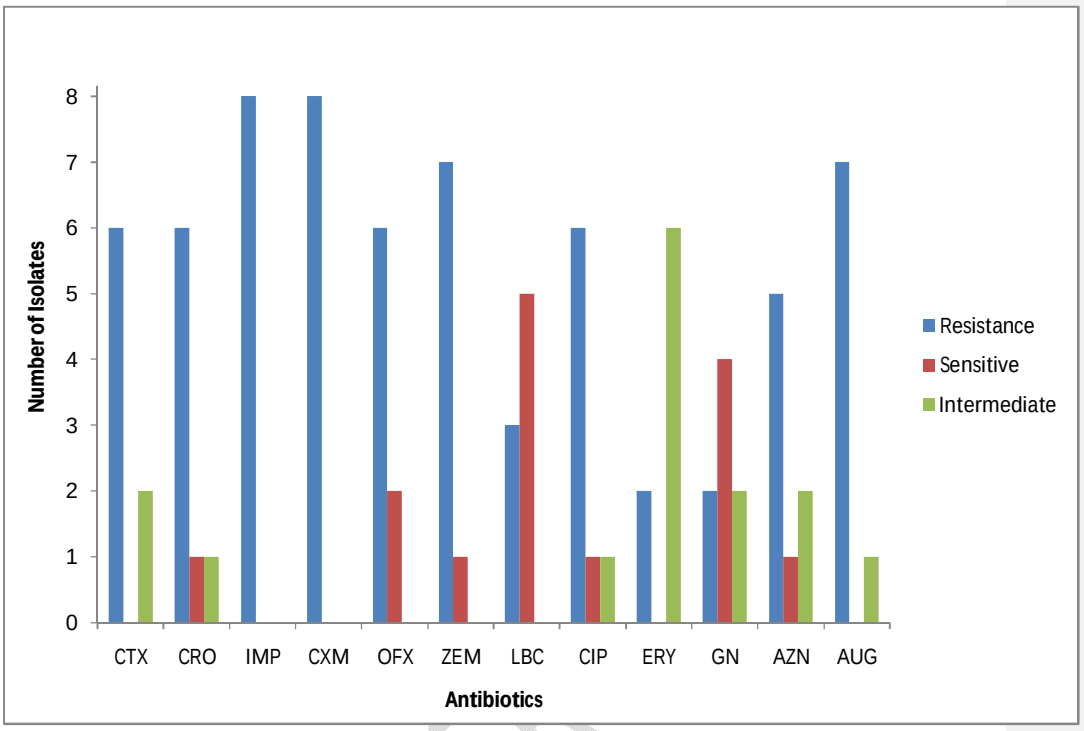

Figure 1: Antibiotics susceptibility profile of *Staphylococcus aureus*

Keys: AUG= Amoxicilin Clavulanate, CTX= Cefotaxime, CRO= Ceftriaxone Sulbactam, IMP= Imipenem/Cilastatin, CXM= Cefuroxime, OFX= Ofloxacin, ZEM= Cefexime, LBC= Levofloxacin, CIP= Ciprofloxacin, ERY= Erythromycin, GN= Gentamycin, AZN= Azithromycin.

Biofilm Formation

All isolates of *S. aureus*, *S. epidermidis*, and *S. haemolyticus* exhibited biofilm formation. Biofilms confer a survival advantage, protecting bacteria from environmental stressors and contributing to antibiotic resistance.

Discussion

The results of this study underscore the significant presence of *S. aureus* on fomites within the institution, highlighting the risk of indirect transmission of antibiotic-resistant pathogens. The high rate of antibiotic resistance observed in this study, particularly to beta-lactams and macrolides, is consistent with global trends in antibiotic resistance (Collignon *et al.*, 2018).

The hemolysis test results indicate that the beta-hemolytic activity of *S. aureus* and *S. haemolyticus* contributes to their pathogenic potential, as they can lyse red blood cells and evade host immune responses (Otto, 2022). The gamma-hemolysis of *S. epidermidis* suggests that it is less virulent, although it still poses a risk of opportunistic infections, particularly in immunocompromised individuals.

Biofilm formation is a critical factor in the persistence of *S. aureus* on surfaces. Biofilms protect bacteria from environmental stress, including antibiotic exposure, which may explain the observed multidrug resistance. These findings align with previous studies reporting that biofilm-forming *S. aureus* isolates are more resistant to antibiotics than their planktonic counterparts (Foster, 2021).

The resistance to cefotaxime, ceftriaxone, and other beta-lactams is of particular concern as these antibiotics are commonly used to treat *S. aureus* infections. The limited susceptibility to ciprofloxacin and gentamicin offers some hope, although the emergence of intermediate resistance calls for cautious use of these antibiotics (Akinrotoye*et al.*, 2019).

Recommendation

Implementation of stringent hygiene and disinfection protocols, particularly in high-contact areas such as cafeterias and clinics should be encouraged. Secondly, establishing routine surveillance programs to monitor fomite contamination and antibiotic resistance patterns is highly advised.Students and staff education on the importance of hand hygiene and the risks of fomite-mediated transmission of pathogens is very key to reduce the spread of antibiotic-resistant pathogen in tertiary institution.

Conclusion

This study highlights the prevalence of antibiotic-resistant *S. aureus* on fomites in a tertiary institution, with all isolates forming biofilms. The widespread resistance to key antibiotics underscores the urgent need for enhanced infection control measures. Regular disinfection of

communal areas, routine monitoring of antibiotic resistance patterns, and strict antibiotic stewardship are critical in reducing the spread of resistant *S. aureus* strains.

DECLARATION

Ethics approval:

Not Applicable

Consent for publication:

Not Applicable

Availability of data materials

All data generated or analyzed during this study are included in this article.

Statement of Competing Interest

The authors have no competing interests

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Authors contribution

This work was carried out in collaboration among all authors. ELO conceived and performed the methodology, ELO performed the data curation and wrote the original manuscript, TEO performed the data analysis, TOA and AM performed the Reviewing and Editing while all Authors gave the Resources, read and approved the manuscript.

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