Identification and Antibiotic Susceptibility Profiling of *Staphylococcus aureus* in Oral Samples from Students at Brac University

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A thesis submitted to the Department of Mathematics and Natural Science in partial fulfillment of the requirements for the Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences
Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Approval

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Ethics Statement

For the completion of this study, samples from students were collected following all the necessary precautions. All the experiments were done in the Brac University Laboratory. It should also be noted that no animal models were used or harmed in this study. Also, all student participants provided informed consent prior to the collection of samples. Ethical permission to conduct this thesis was obtained from the departmental review board of Brac University.

Abstract

This study investigated the prevalence of *Staphylococcus aureus (S. aureus)* in oral swabs collected from Brac University students and assessed the antibiotic resistance profiles of the isolates. A total of 50 samples were cultured on Mannitol Salt Agar and subsequently on Nutrient Agar. Of these, 32 samples exhibited bacterial growth, with *S. aureus* identified in 16 cases. The prevalence of *S. aureus* was higher among male students compared to female students. Polymerase chain reaction (PCR) analysis confirmed the presence of *S. aureus*, as indicated by distinct 275 bp bands in positive samples. Antibiotic susceptibility testing revealed that 75% of the isolates were resistant to both Ciprofloxacin and Levofloxacin, which are classified as Fluoroquinolones. In contrast, the majority of isolates remained sensitive to Linezolid, Chloramphenicol, and Doxycycline. These findings underscore the necessity for continuous surveillance of oral *S. aureus* to monitor resistance patterns and guide effective treatment strategies.

Keywords: *Staphylococcus aureus*, oral cavity, antibiotic susceptibility, fluoroquinolones, linezolid, resistance patterns

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List of Acronyms

AST	Antibiotic Susceptibility Test
AZM	Azithromycin
BSI	Bloodstream Infection
bp	Base Pair
C	Chloramphenicol
CBC	Complete Blood Count
CD	Clindamycin
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
DO	Doxycycline
E	Erythromycin
EDTA	Ethylenediaminetetraacetic Acid
EPS	Extracellular Polymeric Substances
erm genes	Macrolide resistance genes
F	Fleroxacin

*F*emA	Gene Marker for *S. aureus* Identification
K	Kanamycin
LEV	Levofloxacin
LZ	Linezolid
MCT	Microcentrifuge Tube
MSA	Mannitol Salt Agar
MHA	Mueller Hinton Agar
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MIC	Minimum Inhibitory Concentration
*M*ecA	Methicillin Resistance Gene
NA	Nutrient Agar
NCCLS	National Committee for Clinical Laboratory Standards
*N*uc	Thermostable Nuclease Gene (used for *S. aureus* confirmation)
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PABA	Para-Aminobenzoic Acid
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
SSTI	Skin and Soft Tissue Infection

sa442	Alternative molecular marker for *S. aureus* detection
TAE	Tris-Acetate-EDTA Buffer
UV	Ultraviolet
UNG	Uracil-N-Glycosylase
VRSA	Vancomycin-Resistant *Staphylococcus aureus*
VITEK	Automated microbial identification and antibiotic susceptibility system
23S rRNA	Ribosomal Gene Target of Macrolide Resistance

Glossary

Agarose Gel Electrophoresis	A molecular technique used to separate DNA fragments by size through an agarose matrix
Antibiogram	Laboratory test report showing the susceptibility of bacteria to different antibiotics
Amplicon	DNA fragment produced during PCR amplification
Antimicrobial Stewardship	Coordinated strategies to optimize antibiotic use and strains
Biofilm	A structured microbial community encased in a self-produced polymeric matrix that adheres to surfaces
Colony Morphology	Physical characteristics of microbial colonies grown on solid media (e.g., color, shape, texture)
Clonal Typing (MLST)	Multi-locus sequence typing used to distinguish bacterial strains
Commensal	An organism living symbiotically on a host without causing harm
Disc Diffusion (Kirby-Bauer method)	Standardized method for testing bacterial antibiotic susceptibility
Decolonization	Medical protocol using topical antibiotics/antiseptics to eliminate carriage of resistant bacteria

Efflux Pump	Cellular mechanism bacteria use to expel antibiotics and resist treatment
Facultative Anaerobe	An organism that can grow in both the presence and absence of oxygen
Gram - positive	Bacteria that can retain the crystal violet stain in Gram staining due to a thick peptidoglycan layer
MIC (Minimum Inhibitory Concentration)	The lowest concentration of an antibiotic that inhibits visible growth of a microorganism
McFarland Standard	Turbidity standard used to adjust bacterial inoculum for AST
Nosocomial infection	Hospital-acquired infection
Pathogenicity	The ability of an organism to cause disease
Phenol Red	pH indicator used in MSA media to detect mannitol fermentation
Primer-Dimer	Artifact in PCR where primers bind to each other instead of the target DNA, causing spurious bands
Primer (PCR)	Short synthetic DNA sequences that initiate DNA synthesis during PCR amplification
Septicemia	Blood infection caused by the presence and multiplication of pathogens

Superantigens	A class of antigens causing excessive immune response by non-specific T-cell activation
Uracil - DNA Glycosylase (UNG)	Enzyme used in PCR setups to prevent carryover contamination
Virulence factor	Molecules produced by microbes that enhance their ability to cause disease

Chapter 1

Introduction and Literature Review

1.1 Overview of *Staphylococcus aureus*: Characteristics, Infection Spectrum, and Clinical Significance

Staphylococcus aureus (S. aureus) is a widely prevalent bacterial pathogen that is found in healthcare facilities and in the community, associated with human skin, soft tissue, and severe invasive infections such as bacteremia or septicemia, with an indeterminate annual incidence of disease cases (Cheung et al., 2021). *S. aureus* is a gram-positive, cocci-shaped bacterium that looks like grape-like clusters under microscopic examination due to a division along numerous planes. The cells are non-motile, non-spore-forming, and generally range from 0.5 to 10 micrometers in diameter. Colonies of *S. aureus* on solid media typically exhibit a golden-yellow hue due to their production of carotenoid pigment. This characteristic influenced its species designation ("*aureus*" signifying golden) (Tong et al., 2015).

The prevalence of *S. aureus* carriage varies globally due to differences in regional epidemiology, healthcare practices and host factors. A recent meta-analysis estimated that 26% of the global population carries *S. aureus* as a common commensal with pathogenic potential (Locke et al., 2025). Community-based surveillance studies corroborate these findings. For example, Hu et al. (2024) reported 16.8% nasal carriage rate among healthy adults in China, and Congdon et al. (2023) observed 21% carriage rate in a healthy volunteer cohort. These results are consistent with earlier reports of 20-30% prevalence and demonstrate that carriage rates fluctuate by geography and over time. It is recognized as a primary contributor to skin and soft tissue infections (SSTIs) and bloodstream infections (BSIs). Nosocomial *S. aureus* infections continue to pose a substantial threat, with an estimated global incidence rate of 23.7

per 100,000 individuals for methicillin-resistant *S. aureus* (MRSA) bloodstream infections (Van Hal et al., 2012).

S. aureus is accountable for a diverse array of diseases, ranging from minor, localized infections to serious systemic diseases, such as septicemia, endocarditis, and necrotizing pneumonia. A defining characteristic of *S. aureus* is its capacity to produce biofilms, which are complex patterns made up of extracellular polymeric substances (EPS) that encase bacterial cells. Biofilms are crucial for survival on medical devices like catheters and prosthetics, presenting considerable obstacles to antimicrobial treatment (Otto, 2008). Methicillin-resistant *S. aureus* strains are particularly concerning, as MRSA constitutes a substantial percentage of nosocomial infections and demonstrates resistance to many drug classes (Lakhundi & Zhang, 2018).

The pathogenicity of *S. aureus* is propelled by its array of toxins and immune evasion mechanisms, such as hemolysins, leukocidines, and superantigens. The bacterium is also associated with animal illnesses, including bovine mastitis, which impacts the dairy industry (Fitzgerald, 2014).

Due to its significant prevalence and worldwide impact, initiatives to address *S. aureus* encompass enhanced surveillance, innovative therapeutic strategies aimed at biofilm-related infections, and the advancement of vaccine research (Tong et al., 2015).

1.2 Role of *S. aureus* in Biofilm Formation, Infection Development, and Microbial Interactions in Oral Cavity

S. aureus is a facultative anaerobic, gram-positive bacterium that frequently inhabits human skin and mucosal surfaces, such as the oral cavity. Although it is regarded as a commensal bacterium in several individuals, it can become harmful under specific conditions, leading to various oral infections. *S. aureus* can often be found in dental plaque, saliva, and mucosal surfaces, especially in people exhibiting inadequate oral hygiene or pre-existing systemic conditions (Zomorodian et al., 2018). The presence of *S. aureus* in the oral cavity is affected by multiple factors, including the usage of antibiotics, immunity, and alterations in oral health. It can develop biofilms on teeth, prosthetic devices, and mucosal surfaces, rendering it highly resistant to antimicrobial treatments and host immunological responses (Kaplan et al., 2019). These biofilms provide *S. aureus* with a protective barrier, increasing its ability to stay in the oral spaces and leading to infections like periodontitis, tooth abscesses, and angular cheilitis (Tong et al., 2015).

A significant concern regarding *S. aureus* in oral health is its involvement in periodontal disorders. Research indicates that *S. aureus* can be extracted from subgingival plaque in individuals with periodontitis, especially in severe stages of the disease (Lang & Lindhe, 2015). Additionally, *S. aureus* has been recognized as a possible secondary invader in periodontal pockets, intensifying the inflammatory response and worsening the severity of periodontal disease. Furthermore, *S. aureus* can induce dental abscesses, which are localized accumulations of pus arising from bacterial infiltration of the dental pulp or adjacent tissues. Untreated abscesses may result in intense discomfort, edema, and systemic consequences (Khamisi et al., 2022).

Methicillin-resistant *S. aureus* (MRSA) poses significant concerns in oral health. MRSA exhibits resistance to beta-lactam medicines, complicating the treatment of infections. MRSA has been found in the oral cavities of dental patients, particularly those with a history of hospitalization, longer antibiotic courses, or impaired immune systems (Selvan & Ganapathy, 2016). In dental clinics, MRSA may transmit via contaminated equipment, aerosols, or close contact between patients and healthcare providers (Khamisi et al., 2022).

The capacity of *S. aureus* to colonize the oral cavity initiates with its adhesion to epithelial surfaces, dental plaque, and prosthetic materials, including dentures and implants. Recent studies confirm that *S. aureus* surface proteins, such as fibronectin-binding proteins and clumping factors, play a crucial role in enabling the bacteria to attach to host tissues. For example, Camaione et al. (2025) demonstrated that protein A can directly bind to fibronectin, thereby strengthening bacterial adhesion to human cells. Upon adherence, *S. aureus* proliferates by creating microcolonies that subsequently evolve into full biofilms, offering protection against mechanical disruption and immunological responses (Khamisi et al., 2022). The biofilm growth phase not only shields *S. aureus* from antimicrobial drugs but also promotes horizontal gene transfer, resulting in the dissemination of virulence and antibiotic resistance genes throughout bacterial populations. The adaptability enables *S. aureus* to flourish in the oral cavity, despite the antimicrobial therapies (Lang & Lindhe, 2015).

Another parameter affecting the development of *S. aureus* in oral health is its capacity to adjust to the fluctuating conditions of the oral cavity. The oral cavity experiences continual variations in temperature, pH, and nutrient availability as a result of dietary consumption and salivary secretion (Abrahamian & Goldstein, 2011).

Interbacterial associations within the oral microbiota significantly influence the emergence of *S. aureus* in oral health. The bacteria often cohabitate with the oral pathogens, such as

Streptococcus mutans and *Candida albicans*, creating polymicrobial communities that boost their survival and pathogenicity (Peters et al., 2010). *S. aureus* has been observed to form synergistic associations with *Candida albicans* in biofilms, enhancing resistance to antifungal and antimicrobial treatments (Harriott & Noverr, 2009).

Overall, the development of *S. aureus* in oral health is a complex process characterized by adhesion, biofilm formation, adaptive ability to the environment, resistance to antibiotics, and interactions among microbes. These features combine to increase the persistence and virulence, raising an increasing concern for oral healthcare practitioners.

1.3 Prevalence of S. aureus in Oral Infections in Bangladesh and Worldwide

Detailed data regarding the prevalence of oral *S. aureus* colonization in Bangladesh is scarce; global research offers insights into its possible role in oral health (Apu et al., 2019).

The rise of methicillin-resistant *S. aureus* presents a considerable public health challenge in Bangladesh. Research at Dhaka hospitals indicated a 4.35% incidence of MRSA in blood samples, underscoring the bacterium's involvement in systemic illnesses (Rahman, A., Raton, 2021). While specific research connecting MRSA to oral health in Bangladesh is limited, the worldwide correlation of MRSA with oral infections indicates possible dangers.

Several studies have shown how common and concerning *S. aureus* is in oral health environments. Manisha et al. (2019) found *S. aureus* in 64 dental infection samples using morphological, cultural, and biochemical assays. They stressed the prevalence of antibiotic-resistant strains and the risk of methicillin-resistant *S. aureus* (MRSA) in oral health care. The presence of *S. aureus* in the mouth varies around the world and is affected by factors such as oral hygiene, the use of prosthetics, and demographics.

Azmi et al. (2020) showed that 40% of 140 healthy people in Malaysia had *S. aureus* in their mouths. People who use prostheses were almost 5 times more likely to have the bacteria than people who did not. McCormack et al (2015) also showed a prevalence of approximately 30% in dental plaque samples in the UK. They also acknowledged its involvement in denture stomatitis, especially in the elderly, and emphasized the difficulty of addressing infections resulting from its coexistence with *Candida albicans* in resistant polymicrobial biofilms.

In conclusion, the prevalence of *S. aureus* in the oral cavity varies worldwide, determined by factors such as oral hygiene practices, the use of dental prosthesis, and demographic features. The presence of *S. aureus* in the oral cavity presents considerable difficulties to oral and systemic health, demanding specific therapies and ongoing surveillance to limit related hazards.

1.4 The Growing Challenge of Antibiotic Resistance in S. aureus Infections

The increasing prevalence of antibiotic resistance in *S. aureus* is a significant concern. While antibiotics are vital for treating bacterial infections, *S. aureus* can adapt and develop resistance, making these treatments less effective or even futile. As a result, infections caused by resistant strains are more difficult to manage and may lead to more prolonged illness, complications, or even death. (Cheung et al., 2021)

S. aureus shows remarkable adaptability, with many strains now resistant to multiple antibiotics. Methicillin-resistant *S. aureus* (MRSA) is a well-known example, showing resistance to beta-lactam antibiotics such as penicillin and cephalosporins (Gurung et al., 2020). Since penicillin is mainly ineffective, other drugs such as cefazolin, nafcillin, oxacillin, linezolid, chloramphenicol, and doxycycline are often used. In more severe cases, intravenous agents like vancomycin, daptomycin, ciprofloxacin, or levofloxacin may be required, though

these treatments carry a higher risk of side effects (Mayo Clinic, 2018). Vancomycin, in particular, remains an important option for serious *Staphylococcal* infections due to resistance to many other drugs. However, the emergence of vancomycin-resistant *S. aureus* has created additional challenges for treatment (H., M.D., 2024). These intravenous antibiotics are given directly into the bloodstream to reach high drug levels at the infection site. However, their use is often linked with risks such as toxicity, allergic reactions, or complications from prolonged therapy. However, in the following section, we will focus on the resistance patterns of the antibiotics used in this study.

The antibiotics called 'Ciprofloxacin', from the fluoroquinolone antibiotic group, is used for the treatment of certain *S. aureus* infections. This antibiotic showed excellent antibacterial potency, whilst having minimum side effects. The drug is usually administered orally with a dosage of 500 mg every 12 hours to treat odontogenic infections (Ahmadi et al., 2021). It is also an antibiotic with good penetrating activity (Abdullah et al., 2024). However, the most common side effect of ciprofloxacin is gastrointestinal problems, including nausea, vomiting, and diarrhea. Dental practitioners should take the patient's history or else the drug interaction could result in severe consequences. The initial signs of theophylline toxicity in these patients are nausea and vomiting, which should not be confused with the side effects of ciprofloxacin (Ahmadi et al., 2021).

Fleroxacin, another Fluoroquinolone antibiotic, has exhibited in vitro efficacy against *S. aureus*, encompassing methicillin-resistant strains. However, studies have shown that during therapy, *S. aureus* can swiftly develop resistance to Fleroxacin (Huynh et al., 2023). The possibility for faster resistance development renders the use of Fleroxacin for the prevention or treatment of *S. aureus* oral health issues inadvisable. There have been some first-line

antibiotics, such as Amoxicillin or Penicillin, that are more preferable for the treatment of oral infections (Ahmadi et al., 2021).

Levofloxacin, also a Fluoroquinolone antibiotic, showed efficacy against *S. aureus* including MRSA. Nonetheless, its application in addressing *S. aureus*-associated including MRSA-associated oral health concerns is constrained by the rapid emergence of resistance during treatment. Studies have shown that resistance can develop fast, frequently at a high rate (Kistler et al., 2020). However, serious side effects can occur during treatment with this medicine, without any warning. Concern over possible adverse effects, including tendinitis and peripheral neuropathy, has resulted in the cautious usage of Levofloxacin. (Drugs.com, 2024)

Kanamycin, an aminoglycoside antibiotic, has demonstrated effectiveness against a range of gram-negative and some gram-positive bacteria, including *S. aureus*, particularly in infections where other antibiotics fail. It is generally active against susceptible strains in oral infections, but monitoring is essential due to emerging resistance (DrugBank, n.d.). However, resistance to Kanamycin can develop through enzymatic modification of the drug, particularly by aminoglycoside-modifying enzymes. Additionally, concerns regarding nephrotoxicity and ototoxicity have led to restricted clinical use of Kanamycin, especially when safer alternatives are available. (Medscape. (2024).

Clindamycin, a Lincosamide antibiotic, has demonstrated potent activity against *S. aureus*. It is widely used for dental and soft tissue infections caused by *S. aureus*. (Abdullah et al., 2024) It is usually effective but requires D-test screening to rule out inducible resistance. (Janas-Naze et al., 2022) However, the risk of severe adverse effects, such as Clostridioides difficile-associated diarrhea, has led to careful consideration when prescribing Clindamycin, especially for prolonged use (Therapeutics Initiative, 2024).

Chloramphenicol, an Amphenicol antibiotic, has shown broad-spectrum activity against *S. aureus*, including oral isolates. Despite its effectiveness, its use in treating *S. aureus*-related oral infections is limited due to serious safety concerns. Resistance can emerge via enzymatic inactivation by Chloramphenicol acetyltransferase, reducing its clinical utility. Furthermore, the risk of severe adverse effects, such as aplastic anemia and bone marrow suppression, has led to a highly cautious and restricted use of Chloramphenicol in clinical practice. Hence, it remains an option when other treatments are unsuitable. (Przybylski et al., 2024)

Azithromycin is a macrolide antibiotic that exhibits activity against *S. aureus*. It is widely used in treating *S. aureus*-associated oral infections and is favored due to its high tissue penetration and prolonged half-life. (Rai, S., & Solanki, R.2023). However, resistance can emerge through target site modification mediated by erm genes, reducing its effectiveness during therapy. (DrugBank.2025). Additionally, concerns about potential adverse effects such as QT interval prolongation and hepatotoxicity have prompted cautious use of Azithromycin, particularly in patients with underlying cardiac conditions. (Elkhider, H. 2015)

Erythromycin, another macrolide antibiotic, has shown effectiveness against *S. aureus*, especially in penicillin-allergic patients (DermNet NZ, n.d.). However, its clinical utility is often limited by the rapid development of resistance, primarily through methylation of the 23S rRNA target site by erm genes (Mahfouz et al., 2023). Moreover, gastrointestinal side effects and potential drug interactions, have led to cautious use of Erythromycin in both dental and general medical practice (DrugBank, n.d.).

Doxycycline, a tetracycline antibiotic, has demonstrated broad-spectrum activity and efficacy against *S. aureus*, making it a potential option for managing *S. aureus*-related oral infections (Agarwal, Sharma, & Bhardwaj, 2023). It is often used when beta-lactams are contraindicated. However, its clinical effectiveness can be undermined by the development of resistance

through efflux pumps and ribosomal protection proteins. Additionally, concerns regarding side effects such as photosensitivity, gastrointestinal disturbances have limited its widespread use in specific populations (Pearson et al., 2025).

Linezolid, an oxazolidinone antibiotic, is highly effective against *S. aureus*, including multidrug-resistant strains such as MRSA and VRSA. Its use in *S. aureus*-associated oral infections is supported by excellent oral bioavailability and tissue penetration (Zou et al., 2024). Nonetheless, resistance may develop via mutations in the *23S rRNA* gene, although it remains relatively uncommon (Hashemian et al., 2018). Additionally, prolonged use of Linezolid has been associated with serious adverse effects such as bone marrow suppression, peripheral neuropathy, and serotonin syndrome, necessitating careful monitoring during treatment (Hashemian et al., 2018).

1.5 Treatment and Prevention Strategies for *S. aureus*

When *S. aureus* infects the oral cavity, effective treatment requires a combination of targeted antibiotics, antiseptic rinses, and supportive care.

To begin with, antibiotic therapy is the mainstay of treatment, as it has consistently shown remarkable effectiveness in combating *S. aureus* in the medical field. Antibiotics help eliminate the bacteria responsible for conditions such as oral abscesses, periodontal infections, cellulitis, mucosal ulcers, and post-surgical infections. Commonly used agents like Amoxicillin–clavulanic acid, Clindamycin, and Doxycycline penetrate oral tissues well and cover both *S. aureus* and mixed oral flora. For sensitive *S. aureus* strains, first-line options include Amoxicillin–clavulanic acid, which targets β-lactamase-producing strains, and Cephalexin. This first-generation Cephalosporin serves as an effective oral option (Abdullah et al., 2024). If methicillin-resistant *S. aureus* (MRSA) is suspected, alternative antibiotics are required.

Clindamycin is often preferred because of its excellent oral penetration and additional anaerobic coverage, while Doxycycline may be used in less severe infections. For more serious or systemic infections, Vancomycin is administered intravenously, and Linezolid may be given either orally or intravenously in critical cases. (Brown et al., 2021)

In all scenarios, it is essential to perform a culture and sensitivity test (antibiogram) before finalizing antibiotic therapy, as *S. aureus* can often be multidrug resistant. This approach ensures that the most effective antibiotic is chosen, thereby reducing the risk of treatment failure and preventing further resistance. When antibiotic therapy is combined with drainage of pus and the maintenance of good oral hygiene, the chances of successful healing are greatly improved. At the same time, the risk of systemic spread is minimized. (Ardila & Granada, 2022)

Another important approach is the use of antiseptic mouth rinses, which help reduce the overall bacterial load in the oral cavity. This decreases the risk of reinfection and promotes faster healing. Among these, chlorhexidine gluconate (0.12%–0.2%) is widely recommended, typically used two to three times daily, as it is highly effective in controlling bacterial burden and maintaining oral hygiene. Povidone–iodine, a broad-spectrum antimicrobial, can also be used as a mouth rinse to target a wide range of oral pathogens (McGrath et al., 2023).

Lastly, 'Supportive Therapy' plays a vital role in ensuring patient comfort and complete recovery. Pain relief is essential, with non-steroidal anti-inflammatory drugs (NSAIDs) such as Ibuprofen or paracetamol frequently prescribed to reduce pain and inflammation, thereby allowing patients to eat and function more comfortably. Nutritional support must also be considered, particularly when oral intake is difficult; maintaining hydration and providing a soft diet can prevent malnutrition and support tissue healing. Continuous monitoring is equally

critical- regular follow-up visits enable healthcare providers to track healing progress, confirm the resolution of infection, and identify any complications or recurrence early. (ADA, 2024)

Since *S. aureus* frequently colonizes the nose, throat, and oral cavity without immediately causing disease, prevention strategies are aimed at limiting colonization and preserving the integrity of the oral barrier. Maintaining proper oral hygiene plays a central role, which includes brushing twice daily with fluoride toothpaste, flossing to eliminate food particles and plaque, and, in high-risk individuals, the occasional use of antiseptic rinses such as Chlorhexidine (Ontario Health Quality, 2022). Alongside self-care, professional dental support is essential to regular check-ups every six months and professional cleaning (scaling) helps remove biofilm and calculus, thereby reducing bacterial reservoirs. In high-risk patients, such as those preparing for major surgery or admitted to hospitals, decolonization protocols are sometimes employed. These involve applying topical Mupirocin ointment inside the nostrils for five days and using chlorhexidine baths and mouthwash daily over the same period, effectively reducing *S. aureus* load on the skin, mucosa, and oropharynx (Ontario Health Quality, 2022).

Infection control measures in hospitals and dental clinics further strengthen prevention, with strict hand hygiene, adherence to sterile techniques during oral surgeries, and thorough disinfection of dental instruments between patients being essential practices (CDC, 2024). Managing patient-specific risk factors is equally important; for instance, controlling diabetes is vital since high blood sugar impairs immune defenses, while smoking cessation improves oral immunity and tissue healing. Currently, no approved vaccine exists against *S. aureus*, though ongoing research is actively exploring vaccine development, particularly to prevent methicillin-resistant strains (MRSA) (Popovich et al., 2023). In summary, effective management combines treatment of active infections with the correct antibiotics, pus drainage,

and antiseptic mouthwashes. At the same time, prevention is best achieved through consistent oral hygiene, targeted decolonization when necessary, and control of underlying health risks.

1.6 Objectives:

- To detect the presence of *S. aureus* in the oral cavity of Brac University students.
- To analyze the antibiotic susceptibility profile of *S. aureus* isolated from the oral cavity.

Chapter 2

Materials & Methods

2.1 Study Place, Duration and Population

Between April 2024 and August 2024, 50 oral swab samples were collected from male and female students at BRAC University for this research. The study population consisted of 14 males and 36 females within the age range of 19 to 25 years old students of both genders without visible symptoms of oral infection. The research involved the acquisition of data by collecting swab samples from the oral cavity as well as from the gums. According to the student's statement, they had brushed their teeth before coming to university and had eaten at least 2 hours prior to the sample collection.

2.2 Sample Collection of *S. aureus* from Oral Cavity

A swab sample collection method of *S. aureus* from the oral cavity was performed. At first, saliva samples were taken from all over the mouth with the help of a sterile autoclaved cotton swab, and direct streaking of the samples on Mannitol Salt Agar (MSA) agar plates was quickly performed. While samples were being collected from the students, the sterile cotton swabs were thoroughly rubbed inside the cheek, gum area, and under the tongue.

2.3 Isolation of *S. aureus* from Oral Cavity Swab Samples

For this thesis, a direct streaking method was followed on Mannitol Salt Agar (MSA) agar medium. Prior to sample collection day, the MSA media was prepared, the necessary equipment was autoclaved, and labels were applied accordingly. After the preparation of the media, it was poured into sterilized petri dishes inside the laminar air flow. On the day of sample collection, freshly sterilized cotton swabs were used to collect saliva samples from various parts of the mouth, and direct streaking of the samples was performed on MSA agar

plates. To avoid any touch or cross-contamination, gloves were used. While samples were being collected from the students, the sterile cotton swabs were thoroughly rubbed inside the cheek, gum area, and under the tongue. All of the samples were then incubated at 37 °C for 24 to 48 hours. The above process was carried out following a guideline (Aryal, 2022).

Figure 1: Swab sample collection of *S. aureus* from oral cavity

2.4 Media Used for Isolation

We used two types of media for the isolation. These are MSA media (Mannitol Salt Agar) and Nutrient media.

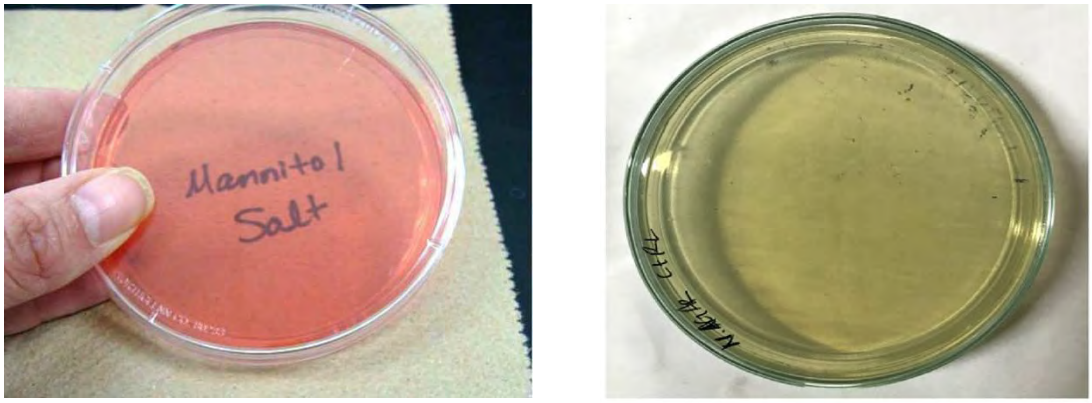

Figure 2: Mannitol Salt Agar and Nutrient Agar used for isolation of *S. aureus*

2.4.1 MSA Media

To isolate and identify *S. aureus* from both clinical and non-clinical materials, mannitol salt agar (MSA) is utilized as a selective and differential medium. Due to the presence of high concentrations of sodium chloride, the media selectively inhibits the growth of non-halophilic bacteria, for example, *Escherichia coli* or *Enterococcus faecalis*. On the other hand, it allows halophilic organisms to grow, such as Staphylococcal species. The presence of beef extract and proteose peptone in mannitol salt agar makes it incredibly nutrient-dense, as they supply vital growth factors and trace nutrients like nitrogen, vitamins, minerals, and growth-promoting amino acids. Bacterial species other than Staphylococci are wholly or partially inhibited by the 7.5% sodium chloride concentration in the medium. Mannitol is a fermentable source of carbohydrates that produces acid when it ferments. Likewise, MSA media shows differential aspects based on the organism's ability to ferment mannitol. Therefore, *S. aureus* forms yellow colonies through the fermentation of mannitol while growing in this medium. Other species of *Staphylococcus*, including *Staphylococcus epidermidis*, do not ferment mannitol (Aryal, 2022). As a result, bacteria that grow on the medium but fail to produce acid, thus the medium remains red or turns slightly pink. Additionally, the medium contains phenol red, which acts as a pH indicator and reacts to the medium's pH, giving the colonies and the medium their color. If a species ferments mannitol and produces acid, the pH of the media decreases, causing phenol red to turn yellow.

Mannitol salt agar (MSA) was used for direct streaking purposes. The process being followed is the MSA medium components were dissolved in distilled water according to the manufacturer's specifications. Then it was autoclaved at 121°C for 15 minutes to sterilize the medium. After the autoclave, the media was allowed to cool down and was poured into the

Petri dish. To prevent contamination, each step was carried out inside a laminar airflow and was later kept inside an incubator for 24-48 hours at 37°C.

2.4.2 Nutrient Media

Nutrient agar is a versatile general-purpose medium that supports the growth of a wide range of non-fastidious organisms. In this study, it was utilized to isolate and subculture *S. aureus*. The medium contains peptone, beef extract, and agar, all of which supply essential nutrients that satisfy the minimum nutritional requirements for bacterial growth. Additionally, sodium chloride is incorporated to maintain osmotic balance and stabilize pH throughout the growth of the bacteria. During media preparation, distilled water was used to dissolve the nutrients, facilitating easier bacterial absorption. Agar is the solidifying ingredient that gives the organism a firm surface on which to develop and allows for the observation of colony morphology. By adding other biological fluids, such as serum, egg yolk, or the blood of a horse or sheep, the media can be made suitable for the growth of other fastidious organisms (Sapkota, 2022).

Nutrient agar was used for subculturing *S. aureus*. At first, the nutrient agar powder was dissolved in distilled water according to the manufacturer's specifications. Then the medium was sterilized by autoclaving at 121°C for 15 minutes. After autoclaving, the liquid was transferred into the petri dish inside the laminar air flow and allowed to solidify. Throughout the process, the agar was prepared in a sterile setting to avoid contamination from the environment.

2.5 Molecular Identification of *S. aureus*

2.5.1 DNA Extraction

DNA from the bacterial isolates was extracted using the boiling method which is a physical method. The boiling method is a common technique for extracting DNA from cells because it is easy to use and can produce adequate amounts of DNA. It can be used to extract DNA from a variety of samples, including bacteria, and since we are working with bacteria, this method is efficient for us. Here is the procedure we follow for DNA Extraction-

First, to extract DNA, we took a loop full of colony (1ul) from previously sub-cultured organisms on an NA plate and suspended it in 400 microliters of TE buffer in an Eppendorf. The mixture was Vortexed for 15 seconds and then centrifuged at 13000 RPM for 10 minutes at 25°C. After that, the supernatant was discarded, and the pellet was mixed with 400 microliters of TE buffer. Vortexed the mixture again for 15 seconds. Then, we put the sample in the water bath for 10 minutes at 100°C to lyse the cells. After that, the mixture needs to be kept at room temperature for (5-10 minutes), to cool down. We centrifuged it once more at 13000 RPM for 10 minutes at 25°C. Lastly, transferred the supernatant to a 1.5 microliter MCT tube and stored it at (-20° C) for further use.

2.5.2 PCR

In this paper, the Polymerase Chain Reaction (PCR) technique was utilized to amplify a sequence of the *Nuc* gene, which encodes the thermostable Nuclease of *S. aureus*. To initiate, we followed the process below:

13µL mixtures of PCR components were prepared in sterile tubes for each organism. These mixtures consisted of 6µL of Master Mix, 1µL each of forward and reverse primers, 3µL of nuclease-free water, and 2µL of extracted DNA. The DNA templates were added immediately

after combining the other components. Subsequently, the tubes underwent a brief centrifugation, either in a microplate spinner or a microcentrifuge, to eliminate any air bubbles. The prepared samples were then loaded into the PCR instrument, with the settings adjusted according to the target gene. Finally, upon completion of the PCR procedure, the amplified products were stored at -20°C in a culture freezer, prior to being subjected to agarose gel electrophoresis.

A particular primer pair targeting the *Nuc* gene was employed to detect *S. aureus*. The forward primer (*Nuc*F) had the sequence 5′-GCGATTGATGGTGATACGGTT-3′, whereas the reverse primer (*Nuc*R) had the sequence 5′-AGCCAAGCCTTGACGAACTAAAGC-3′. This primer combination amplified a 275 bp fragment specific to *S. aureus*. The PCR protocol comprised an initial denaturation at 94°C for 4 minutes, followed by 32 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. A concluding extension phase was conducted at 72°C for 10 minutes. (Karimzadeh and Ghassab, 2022).

2.5.3 Gel Electrophoresis

The last step of the molecular identification, Agarose Gel Electrophoresis, was applied to ensure the separation and investigation of nucleic acids. The high gel strength of agarose enables the efficient separation of large DNA fragments, typically ranging from 100 base pairs to 25 kilobases in size. The agarose matrix can be chemically modified through hydroxyethylation, resulting in low-melting agarose, which reduces the packing density and pore size of the gel. Ethidium bromide is employed as a loading dye to enhance the density, color, and migration patterns of DNA fragments, facilitating the estimation of their size based on a DNA standard. To conduct the experiments, an agarose gel is prepared by boiling the required agarose powder with 50X TAE buffer and distilled water. After cooling, ethidium bromide, an intercalating agent used as a fluorescent tag, is added. The previously amplified

PCR products are then loaded into the agarose gel using sterile pipette tips. The DNA samples are electrophoresed using 1X TAE as the running buffer, which allows the negatively charged DNA molecules to migrate toward the positive electrode. Agarose gel electrophoresis is typically performed using 5µl of a 100 bp ladder and 100 V of electricity. The applied electric current causes the DNA to move through the strongly cross-linked agarose substrate, with the negatively charged DNA phosphates migrating toward the positive pole. MIDORI Green is used to stain the DNA, enabling the tracking of its progress through the gel. After the DNA has been separated, it is stained with a substance that specifically binds to DNA molecules, either reflecting a specific color in visible light or fluorescing under UV light. The band sizes are then determined by comparing the sample DNA to the DNA ladder.

2.6 Antibiotic Susceptibility Test (AST)

In this study, we used the Kirby-Bauer disc diffusion method to test the antibiotic susceptibility of *S. aureus* which involves evenly spreading standardized bacterial suspension on agar plate. Besides that, small paper discs containing antibiotics were placed on the surface (Synbiosis, 2024). After inoculation, clean zones around each disc are called zones of inhibition, which reveals whether the bacteria are susceptible or resistant to each antibiotic. Our objective of performing AST was to establish the antibiotic resistance profile of the isolates to guide effective clinical treatment. Therefore, we carried out AST on *S. aureus* on Mueller-Hinton Agar (MHA), the Clinical and Laboratory Standards Institute (CLSI)-standardized medium for disk diffusion testing. This medium provides ideal conditions for bacterial growth and reliable antibiotic diffusion, making clear observation of inhibition zones.

To ensure reliable results, we prepared each plate carefully, as differences in agar thickness can affect accuracy and lead to misinterpretation of result. Before inoculation, cultures were grown to their exponential phase and diluted to match a 0.5 McFarland standard to achieve

consistent cell density. Using sterile cotton swabs, we spread the suspension onto fresh MHA plates. After that, we placed the antibiotic discs with sterile forceps, leaving enough space to prevent overlapping zones. Finally, the plates were labeled and incubated at 37 °C for 18-24 hours before measuring the zones of inhibition to determine the susceptibility profiles.

2.6.1 Mueller Hinton Agar

Mueller Hinton Agar is the standard medium for antibiotic susceptibility profiling of non-fastidious organisms using the Kirby-Bauer disk diffusion method. Because it is both non-selective and non-differential in nature, that makes it ideal for AST. The medium contains beef extract, acid hydrolysis of casein, starch, and agar. Beef extract and casein provides nitrogen, vitamins, carbon, amino acids, sulfur and other essential nutrients. Whereas, starch helps to absorb harmful bacteria by products and produces dextrose for energy, while agar serves as the solidifying agent. This loose agar allows nearly all organisms to grow and promotes enhanced antibiotic diffusion, giving clear and accurate zones of inhibition measurements (Aryal, 2022). Due to MHA's reliable performance, it has earned recognition by the NCCLS as the standard medium.

For this study we prepared MHA by dissolving agar powder in distilled water according to the manufacturer's instructions. The medium was autoclaved at 121 °C for 15 minutes, cooled to room temperature, and poured into a sterile petri dish to solidify inside the laminar air flow. Each and every step was performed under sterile conditions to prevent contamination, and the plates were stored at 2-8 °C until use (Aryal, 2023).

2.6.2 Disc Diffusion

The Kirby-Bauer disk diffusion susceptibility test analyzes the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria (Hudzicki, 2009). This method involves the cultivation of pathogenic organisms on Mueller-Hinton agar with antimicrobial filter paper disks. The presence or absence of bacterial development around antibiotic disks indicates a compound's capacity to inhibit the organism. The Kirby-Bauer disk diffusion method is time-efficient and was used to determine the antimicrobial susceptibility profiles of the *S. aureus* isolates for the oral samples. The assessment of bacterial resistance to antimicrobials is a crucial aspect and helps health officials in selecting various treatment alternatives.

2.6.3 List of Antibiotics

Table 1: Zone of Inhibition Standards According to CLSI Guidelines

Antibiotic Group	**Antibiotic**	**Disc Code**	**Disc Potency (µg)**	**Sensitive (mm)**	**Intermediate (mm)**	**Resistance (mm)**
Aminoglycosides	Kanamycin	K	30	≥18	14-17	≤13
Amphenicol	Chloramphenicol	C	30	≥18	13-17	≤12
Clindamycin	Clindamycin	CD	2	≥21	15-20	≤14
Macrolides	Azithromycin	AZM	15	≥18	14-17	≤13
	Erythromycin	E	15	≥23	14-22	≤13
Fluoroquinolones	Ciprofloxacin	CIP	5	≥21	16-20	≤15
	Fleroxacin	F	5	≥19	16-18	≤15
	Levofloxacin	LEV	5	≥17	14-16	≤13
Tetracycline	Doxycycline	DO	30	≥16	13-15	≤12
Oxazolidinones	Linezolid	LZ	30	≥21	–	–

Chapter 3

Results

3.1 Isolation of *S. aureus* by Culturing on MSA and NA Media

The isolation of *S. aureus* by culturing on MSA media results is presented in figure 4. The picture shows the growth of bacterial isolates from male and female students on selective media (Mannitol Salt Agar). The collected swab samples were streaked on MSA agar plates. On MSA media *S. aureus* produces smooth, round colonies that typically appear yellow. This distinguishing color appears due to *S. aureus* ability to ferment mannitol and acid production that changes the surrounding agar from red to yellow. Out of the 50 students who participated in the study, 32 samples demonstrated positive results for the presence of organisms where Males (n=14) showed yellow colony growth in 14 samples, with no white or no growth observed. Females (n=36) showed yellow colony growth in 18 samples, with no white or no growth observed. Based on this morphological assessment we obtained 32 presumptive *S. aureus* positive colonies.

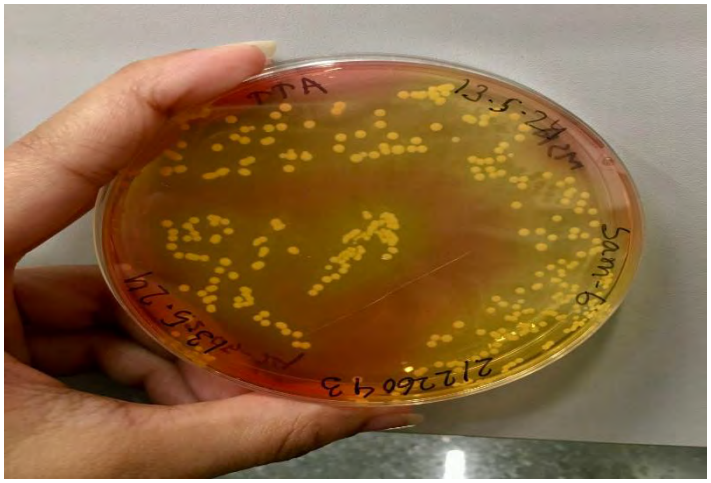

Figure 3: Growth of *S. aureus* on MSA media.

Later we subcultured presumptive *S. aureus* positive colonies on Nutrient agar, a general-purpose medium. This enables the organism to be stored in the pure culture for further testing or long-term storage without the inhibitory effects of selective agents. On the NA media males (n=14) showed golden-yellow colony growth in 12 samples, with no creamy-white or no growth observed. On the other hand, females (n=18) showed golden-yellow colony growth in 4 samples and creamy-white growth in 3 samples, with no growth observed.

3.2 Identification of *S. aureus* by PCR following by Gel Electrophoresis

In order to detect the *S. aureus* specific genes, the expected bands were then identified in the Agarose Gel Electrophoresis, which is a *S. aureus* confirmation test. Later, out of all the positive isolates, a total of 16 were chosen for PCR analysis to check the availability of virulence genes.

The presence of *S. aureus* was confirmed through PCR amplification of the *Nuc* gene, a well-characterized genetic marker encoding the thermostable Nuclease specific to *S. aureus*. After amplification, the PCR amplicons were subjected to agarose gel electrophoresis to confirm the presence and size of the expected amplicon.

Gel electrophoresis analysis revealed distinct bands corresponding to the expected size of 275 bp in the test samples (Figure 4). These results indicate successful amplification of the *Nuc* gene, confirming the presence of *S. aureus* in the tested isolates.

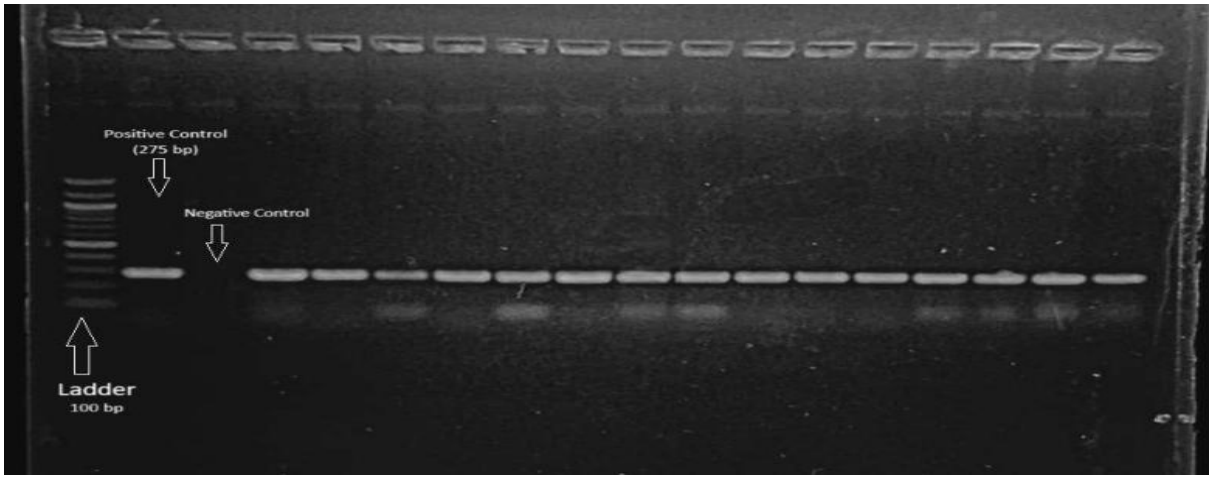

Figure 4: Agarose gel electrophoresis and visualization of *S. aureus* DNA bands

Agarose gel electrophoresis was performed to analyze the PCR amplification of the *Nuc* gene. It has been done to confirm the presence of *S. aureus*. Lane 1 featured a 100 bp DNA ladder for size reference, while lanes 2 and 3 comprised the positive and negative controls. Lanes 4 through 18 were loaded with various test samples. The identification of specific DNA bands at 275 bp in the test samples confirmed the adequate amplification of the *Nuc* gene, signifying the presence of *S. aureus*.

3.3 Prevalence of *S. aureus* on the Student Sample

Out of a total 50 oral swab samples from students, 16 isolates were confirmed as *S. aureus* based on their typical yellow or golden-yellow colony morphology and PCR verification of the *Nuc* gene. The result demonstrated that approximately one-third of the sampled students carried *S. aureus* in their oral cavities, with a greater proportion of confirmed isolates from male participants than female participants establishing a 3:1 prevalence ratio of males to females in this study.

3.4 Antibiotic Susceptibility Profiling

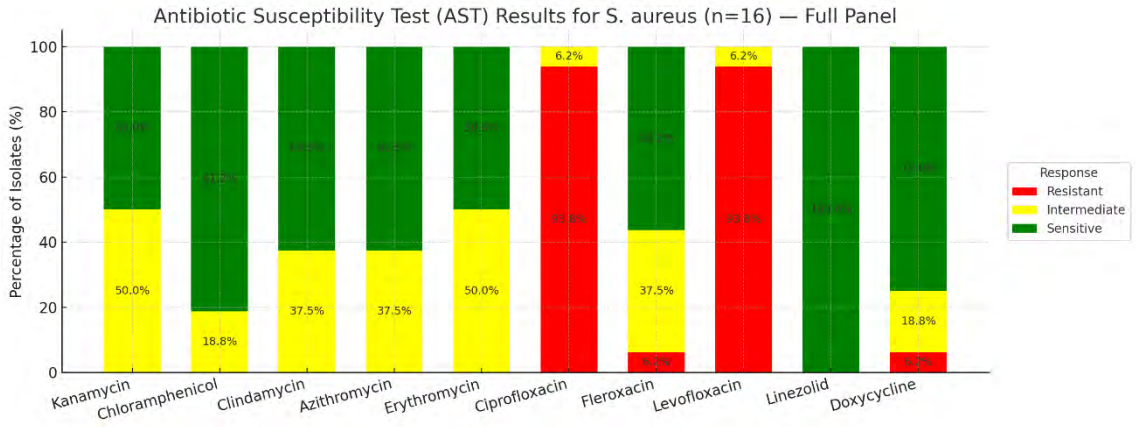

Figure 5: Resistance pattern of *S. aureus* against different antibiotics

Antibiotic susceptibility test (AST) results of *S. aureus* isolates are presented in figure 5 and the isolates (n=16) revealed distinct resistance, intermediate, and sensitivity patterns across tested antibiotics.

Among all the tested antibiotics Ciprofloxacin and Levofloxacin demonstrated the highest resistance, with 15 out of 16 isolates resistance (≈93.7%) and only one intermediate response. This finding indicates very limited therapeutic value of fluoroquinolones against oral *S. aureus* isolates.

Moderate resistance to Doxycycline and Fleroxacin was found. Fleroxacin exhibited low resistance (6.25%), with most isolates either sensitive (56.2%) or intermediate (37.5%). In

addition, Doxycycline showed only one resistant isolate (6.25%), with the majority being susceptible (75%).

The highest sensitivity was recorded for Linezolid and Chloramphenicol. Linezolid has been found to be the most effective antibiotic with 100% sensitivity against every isolate. Chloramphenicol also demonstrated strong activity (81.2% sensitive, 18.7% intermediate, and no resistance).

Intermediate responses were observed for Clindamycin, Azithromycin, Erythromycin and Kanamycin. Among that, Clindamycin as well as Azithromycin showed 62.5% sensitivity and 37.5% intermediate with no resistance. Whereas, Erythromycin displayed a balanced pattern (50% sensitive, 50% intermediate). Although no resistance was found, Kanamycin showed varied outcomes, with 50% intermediate susceptibility and 50% sensitivity.

The overall result suggests that Fluoroquinolone resistance is a growing concern, but Doxycycline, Linezolid, and Chloramphenicol can be effective therapeutic choices. The fact that intermediate susceptibility is consistently present across several antibiotics highlights the significance of continuous antimicrobial surveillance, antibiotic usage optimization, and cautious stewardship in preventing the emergence of new resistance.

Chapter 4

Discussion

The objective of this study was to detect the presence of *S. aureus* in the oral cavity of Brac University students and to evaluate the antibiotic susceptibility profiling of the isolates. Among the 50 students sampled, phenotypic and molecular assays confirmed the presence of *S. aureus* in 16 isolates from a subset of oral cavity isolates.

This investigation found a higher prevalence of *S. aureus* among male students than female students. All male isolates produced yellow colonies on Mannitol Salt Agar, and 12 also exhibited yellow-golden colonies on Nutrient Agar, confirming typical *S. aureus* morphology. In contrast, only four female isolates showed positive growth on Nutrient Agar, with three producing white colonies, suggesting the presence of other staphylococcal species. The occurrence of different colony morphologies among female samples may indicate co-colonization or co-infections involving non-aureus *Staphylococcus* species. In the present study, the participant pool exhibited a marked gender imbalance, with females comprising 82.1% of the sample and males only 17.9%, resulting in an approximate female-to-male ratio of 4.6:1 (Fernandez et al., 2023).

When compared to global findings, the prevalence observed in Brac University students falls within the range reported in recent studies, though precise rates vary widely across settings. Locke et al. (2025) estimated that asymptomatic colonization at nasal and throat sites affects approximately 26.4% of the general population. Oral-specific studies report variable prevalence, with Kawayanagi et al. (2025) detecting 12.3% oral carriage in Japanese dental patients, and Nishihama et al. (2025) documenting 31.6% carriage in elderly home health care recipients. A student-focused investigation by Chmielewski et al. (2024) found that 27.7% of

young adults had dual oral and nasal carriage. Taken together, these findings highlight that oral *S. aureus* carriage is influenced by population demographics, site of sampling, and health status. The prevalence detected among Bangladeshi university students aligns with these international data, underscoring the oral cavity as a potential reservoir for *S. aureus*.

Another objective of this study was to determine the antimicrobial susceptibility profile of the isolated *S. aureus* strains. The analysis revealed significant resistance to Fluoroquinolones, with a substantial number of isolates exhibiting resistance to both Ciprofloxacin and Levofloxacin. In contrast, Linezolid, Doxycycline, and Chloramphenicol remained highly effective. These results reflect global concerns about the declining efficacy of Fluoroquinolones, which have been widely used in human and veterinary medicine throughout South Asia. The oral cavity, typically regarded as a commensal environment, may therefore represent an underrecognized reservoir of resistant clones that could contribute to future opportunistic infections.

Comparable observations have been documented in recent global studies. Huynh et al. (2023) demonstrated that repeated exposure of *S. aureus* to sub-MIC concentrations of Fluoroquinolones rapidly produced highly resistant strains; yet, susceptibility to Tetracycline class agents, including Doxycycline, remained largely preserved. Aiesh et al (2024) reported similar findings in a tertiary hospital setting, highlighting the widespread Fluoroquinolones resistance among clinical *S. aureus* isolates, which underscore the decline of this drug class as a therapeutic option.

Other studies examining oral *S. aureus* have reported variable resistance profiles. Koukos et al. (2015) detected *S. aureus* in 18% of healthy adult's oral samples but did not report significant resistance to Fluoroquinolones. In contrast, a study of denture wearers (2018) found resistance patterns influenced by repeated antibiotic exposure in the elderly. Our findings

suggest that Fluoroquinolone resistance may already be prevalent among oral carriage strains in our sampled population. This elevated resistance may be attributable to factors such as the overuse and misuse of fluoroquinolones in livestock, particularly bovines.

Overall, the evidence indicates that Fluoroquinolones resistance is prevalent in both clinical and community samples, including among healthy young individuals. Still, antibiotics like Doxycycline, Chloramphenicol, and Linezolid are often effective. These results underscore the importance of careful antibiotic use and regular monitoring to maintain the effectiveness of these treatment options.

Chapter 5

Limitations and Future Directions

This study presents data on the prevalence of *S. aureus* and its antibiotic susceptibility in oral samples from students at Brac University. Nevertheless, several limitations should be acknowledged.

There were limitations on Sample size and Population scope. Since this study analyzed only 50 oral swab samples from a single university, the findings may not be representative of the broader Bangladeshi population. The results might also differ for other age groups, regions or clinical settings. Moreover, the study gathered only basic demographic information and did not include full clinical histories, such as previous antibiotic use, health conditions, or oral hygiene habits. These factors could affect colonization and resistance patterns. While PCR targeting the *Nuc* gene confirmed the presence of *S. aureus*, using only one genetic marker may not be specific enough. Adding other markers, such as *MecA* or *femA*, or using sequencing, could improve species identification and help detect methicillin resistance. Additionally, morphological tests may not fully distinguish between similar *Staphylococcus* species, which could lead to some misclassification.

Furthermore, the study used the Kirby-Bauer disc diffusion method, which gives qualitative results like sensitive, intermediate, or resistant. Using more advanced methods, such as Minimum Inhibitory Concentration (MIC) testing could offer more precise results. Also, the study tested only a small number of antibiotics. Including more antibiotics would provide a more comprehensive picture of resistance patterns.

These limitations outline several opportunities for improvement.

Future Directions:

Future research should focus on overcoming current limitations and expanding our understanding of oral *S. aureus* colonization and antibiotic resistance. Utilizing additional molecular markers beyond *Nuc*, such as *femA* and *mecA*, could confirm *S. aureus* strains and identify methicillin or multidrug resistance with greater accuracy. Also, Complementing the disc diffusion method with Minimum Inhibitory Concentration (MIC) assays or automated systems (e.g., VITEK) would allow more precise determination of resistance levels. Furthermore, testing more antibiotics, including newer agents and combination therapies, would provide a broader resistance profile and inform treatment guidelines for oral and systemic infections. Also, long-term studies that track how *S. aureus* persists, returns, or changes with the seasons in the mouth could help us better understand its behavior and resistance patterns. Researchers should also explore methods to prevent *S. aureus* in the mouth, such as using probiotics, specialized mouth rinses, or vaccines. These studies could demonstrate how these options interact with antibiotics to reduce colonization and resistance.

Chapter 6

Conclusion

This research identified *S. aureus* in oral swabs from Brac University students using selective media and PCR targeting the *Nuc* gene. *S. aureus* showed notable prevalence, with high resistance to Ciprofloxacin and Levofloxacin, and effectiveness of Linezolid, Chloramphenicol, and Doxycycline. These results underscore the importance of ongoing resistance monitoring and targeted strategies against *S. aureus* infections.

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