



Original Research

Isolation and Molecular Diagnosis of Bacteria Isolated (*S. aureus*) From Patients with Post-Surgical Sinusitis

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Abstract

Background: Sinusitis is caused most often by bacteria and is one of the most serious potential complications of endoscopic sinus surgery. This disorder is a major cause of morbidity and cost of treatment; *S. aureus* is one of its major causal bacteria. The aim of this study was to isolate and identify *S. aureus* from clinically diagnosed post-surgical sinusitis patients, characterize virulence factors and drug susceptibility patterns. One hundred and twenty persons were included in the study who suffered from sinusitis after FESS. The patients were enrolled for 12 months. The middle meatus was the site of sterile collection for the nasal swabs. These were cultured on selective media and biochemical tests were performed. The molecular technique specifically targeted the *S. aureus* nuc gene and was performed using a polymerase chain reaction (PCR). Antibiotic susceptibility was determined using Clinical and Laboratory Standards Institute (CLSI) guidelines in the disc diffusion method. Amplification of the *mecA* gene and cefoxitin disc screening were used to discover methicillin resistance.

Results: A prevalence rate of 39.2% was determined based on the 47 patients out of 120 who tested positive for *S. aureus*. The results obtained by phenotypic techniques and nuc gene amplification were in perfect accord with each other. 40.4% (19 isolates) were (MRSA). High prevalence of resistance was observed for ciprofloxacin (55.3%), erythromycin (68.1%) and penicillin (91.5%). Nonetheless, vancomycin and linezolid were able to keep the isolates in check. All of the isolates had the *mecA* gene, and the 310 base pair amplicons showed that the genotype was resistant.

Conclusions: Sinusitis following surgery is often caused by *Staphylococcus aureus*, and this is especially true with methicillin-resistant strains. Routine molecular characterisation and antimicrobial



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susceptibility testing are necessary to guide suitable therapeutic strategies due to the significant frequency of antibiotic resistance. To reduce the impact of antibiotic

resistance in surgical settings, it is crucial to implement infection control measures and utilise antibiotics wisely.

Keywords: *Staphylococcus aureus*, post-surgical sinusitis, molecular diagnosis, antibiotic susceptibility, methicillin resistance, *mecA* gene.

Introduction

Functional endoscopic sinus surgery is the most successful surgical technique for non-medicated treatment of CRS. Despite the significant advances in the care of the patient in the period before and after surgery, the main clinical problem is still postoperative complications, which are most frequently bacterial infections [1]. Sometimes there is a condition after a surgical procedure, called post-surgical sinusitis. In this instance, the sinuses surrounding the nose become inflamed or infected causing signs and symptoms such as facial pain, and purulent discharge. Also, wound healing may be retarded [2]. An estimated 30% of healthy people have the multi-purpose opportunistic pathogen *Staphylococcus aureus* in their front noses [3,4]. Surgical procedures may also damage the mucosal surface, creating a hotspot for infection and the development of biofilms. *Staphylococcus aureus* bacteria are highly virulent and have a number of virulence factors including toxins and immune evasins that allow for the maintenance of the infection. The capacity for *S. aureus* to form biofilms on implanted medical devices and on mucosal surfaces is one major challenge to infection therapy. Infections then are sustained as a result of this [6]. One of the major challenges for infection therapy is the emergence and spread of (MRSA) and its significant role in the epidemiology and management of staphylococcal infections. Methicillin Resistant *Staphylococcus aureus* [7] seems to be driven by the *mecA* gene. This gene codes for a penicillin-binding protein that is resistant to beta-lactam medicines. In both the world and in other countries, the prevalence of methicillin resistance has been steadily increasing in HAI, including post-surgical complications, and differs by region [8]. Because of this pattern of resistance, doctors have little choice but to resort to using glycopeptides and oxazolidinones, which

are alternative antibiotics but are more expensive and may be harmful to patients. To guide empirical antibiotic treatment and guaranty better patient outcomes, bacterial pathogen identification is crucial. The traditional methods are important in the identification of bacteria, however, molecular biology techniques are emerging as useful tools to identify bacterial infections rapidly, sensitively and specifically [9]. PCR based methods are an important tool in the clinical laboratory for the detection of species-specific genes, including those of *S. aureus* thermonuclease genes [10]. The method's importance has increased with direct detection from clinical samples, thus decreasing time to diagnosis, and resistance determinants such as methicillin resistance have been detected using *mecA* genes [11]. This study would be useful to meticulously review how *Staphylococcus aureus* is involved in the case of post-surgical sinusitis in patients, with consideration to the clinical significance of this condition and the emergence of antibiotic resistance in the staphylococcal infections field. The research is focused on finding out how common *S. aureus* is in individuals who have had sinus surgery, utilising molecular biology to validate its identity, analysing patterns of antibiotic resistance, and detecting methicillin resistance by phenotypic and genotypic methods, respectively. The results of this study will certainly add to what is already known and aid in the development of evidence-based procedures for the treatment of sinusitis in patients who have just undergone surgery.

Materials and Methods

The design of the study and patient selection process.

The prospective observational study was conducted between 1st January 2024 and December 2024 in the Department of

Otolaryngology of a tertiary care centre. Informed consent was obtained following ethical clearance from the subjects in the study. A total of 120 patients were sampled—all of them were diagnosed with chronic sinusitis, underwent FESS, and experienced sinusitis symptoms within 4 weeks of the surgery. Eligibility for participants included age 18 to 65 and a history of postoperative sinusitis (defined as having symptoms including purulent nasal discharge or pressure, nasal obstruction, fever, and/or surgical procedures). Patients were excluded if they had immunocompromising diseases, had taken an antibiotic within 2 weeks of sampling, were pregnant or medical records had been missing.

Samples will be transported and collected as necessary.

Sterile calcium alginate swabs were taken inside the middle meatus of each patient's nose under endoscopy to ensure an accurate sample from the site of infection. Samples were collected and placed in Amies medium and taken to the microbiological lab within 2 hours. The specimens were treated immediately on arrival to ensure that the bacteria were alive and that the flora contaminating these specimens did not become overgrown.

Identification and Isolation of bacteria

Swabs were inoculated on mannitol salt agar and blood agar, which are selective for the growth of staphylococci. Cultures were maintained for 2 or more days at 37 °C. Colonies that stood out due to their unique shape were chosen for additional analysis; for example, MSSA colonies were identified as golden yellow. Initially, catalase, Gramme staining and tube coagulase were used. Identification of *S. aureus* was made by presumptive identification method – Gram positive grouped in catalase and coagulase positive.

Extracting DNA

Pure bacterial cultures were treated with boiling water in order to obtain genomic DNA. 2-3 bacterial colonies were suspended in 300 microliter of distilled water and then heated at 100

°C for 10 minutes. Next, the mixture was centrifuged at 12,000 RPM for 5 minutes. The supernatant that contains the DNA was then placed in a new tube and stored at -20 °C until ready for the PCR.

Molecular Identification of *S. aureus*

S. aureus was molecularly confirmed by amplifying the thermonuclease gene (*nuc*) specific for the species. The amplification primers used were: forward 5'-GCGATTGATGGTGATACGGTT-3' and the reverse 5'-AGCCAAGCCTTGACGAACTAAAGC-3' yielding an amplification product of 270 base pairs. Polymerase chain reaction was performed in a 25 microliter reaction mixture consisting of 12.5 microliters master mix, 2 microliters template DNA, 1 microliter of each primer at 10 pmol/microliter and nuclease-free water up to the reaction volume. The DNA was denatured at 95 °C for 5 minutes to start the amplification process. Then, it was subjected to 35 cycles of denaturation, annealing, and extension at 72 °C for 30 seconds, 45 seconds, and 7 minutes, respectively. Amplification products were analysed by electrophoresis on 1.5% agarose gel stained with ethidium bromide under UV trans-illumination.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed according to the CLSI guidelines with the Kirby-Bauer disc diffusion method and on Mueller-Hinton agar medium. Organism suspensions were then added to the agar plates in the 0.5 McFarland standard. The antibiotics tested on the antibiotic discs were: penicillin (10 units), oxacillin (1 µg), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), ciprofloxacin (5 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), vancomycin (30 µg), and linezolid (30 µg). The plates were examined after 18 to 24 hours of incubation at 35 ± 1°C. Based on the CLSI breakpoints, the zone diameters were determined and classified as susceptible, intermediate, or resistant.

Methicillin Resistance Detection

Methicillin resistance was screened by cefoxitin disc. The species that were not responsive to methicillin were identified as those with zone diameters of 21 mm or less. Molecular validation was done by amplification of the *mec* gene using the PCR. The use of specific primers by a gene: forward primer 5'-AAAATCGATGGTAAAGGTTGGC-3' and reverse primer 5'-AGTTCTGCAGTACCGGATTTGC-3', yielding a 310 base pair amplicon. Reaction conditions were essentially the same as for *nuc* gene amplification, except that the annealing temperature was raised to 58 °C. Amplification products were then separated by gel electrophoresis and detected as described above.

Data Analysis by Statistic

The data was examined with the help of SPSS version 26.0. Data for categorical variables were presented as percentages and frequencies. A chi-square test was performed to determine the relationships of methicillin resistance with clinical and demographic factors. A *p* value < 0.05 was statistically significant. Using Cohen's kappa coefficient, we checked if the phenotypic and genotypic approaches were consistent with one another.

Results

The age range of the 120 patients with post-surgical sinusitis in the study was 22–63 years, with an average age of 42.5 years. There were 68 males and 52 females in the cohort. Initial signs were seen from 5 to 28 days after surgery, (median 14 days). The most common symptoms reported by the patients were fever (76), nasal blockage (88), facial pain (95) and purulent nasal discharge (112). FESS was performed in all patients with chronic rhinosinusitis (both patients with and without polyps).

Table 1. Demographic and clinical parameters of patients.

Characteristic	Value
Total patients (N)	120

Sex	
– Male	68
– Female	52
Age (years)	
– Range	22 – 63
– Mean	42.5
Time from surgery to symptom onset (days)	
– Median	14
– Range	5 – 28
Presenting symptoms	
– Purulent nasal discharge	112
– Facial pain	95
– Nasal obstruction	88
– Fever	76
Prior surgery (all patients)	Functional endoscopic sinus surgery for chronic rhinosinusitis (with or without polyps)

S. aureus Molecular Verification

A polymerase chain reaction (PCR) was performed to amplify the *nuc* gene for all 47 isolates previously suspected of being *S. aureus*, on the basis of conventional biochemical techniques. The identification results by phenotypic and genotypic techniques were in perfect agreement, with all the isolates giving the expected 270-base-pair product. The study not only illustrates the usefulness of molecular methods for confirmation, but it also backs up traditional identification methods.

Table 2. Shows the results of molecular confirmation of *S. aureus*.

Molecular Confirmation of <i>S. aureus</i>	Value
<i>S. aureus</i> isolates subjected to PCR (n)	47
Target gene amplified	<i>nuc</i> gene

Expected amplicon size (bp)	270
Isolates with <i>nuc</i> gene detected (n)	47
Concordance between phenotypic and genotypic identification	100%
Conclusion	Molecular methods confirm reliability of conventional biochemical identification

Patterns of Antimicrobial Resistance

Worrying resistance patterns for *S. aureus* samples were uncovered by antimicrobial susceptibility testing. The 43 strains that were resistant to penicillin (the most resistant antibiotic) accounted for the highest percentage. The other antibiotic resistance rates were: Trimethoprim-sulfamethoxazole (25.5%), Ciprofloxacin (55.3%), Gentamicin (36.2%), Clindamycin (46.8%) and Erythromycin (68.1%). Since all strains were sensitive to vancomycin and linezolid, these agents will probably continue to be used.

Table 3. Shows the pattern of susceptibility of *S. aureus* isolates (n = 47) to the different antimicrobial drugs.

Antibiotic Agent	Resistant n (%)	Susceptible n (%)
Penicillin	43 (91.5)	4 (8.5)
Oxacillin	19 (40.4)	28 (59.6)
Erythromycin	32 (68.1)	15 (31.9)
Ciprofloxacin	26 (55.3)	21 (44.7)
Clindamycin	22 (46.8)	25 (53.2)
Gentamicin	17 (36.2)	30 (63.8)
Trimethoprim-Sulfamethoxazole	12 (25.5)	35 (74.5)
Vancomycin	0 (0)	47 (100)

Linezolid	0 (0)	47 (100)
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The detection of Methicillin Resistance

As a result of phenotypic screening with the disc diffusion test with cefoxitin, 19 of the *S. aureus* isolates (or 40.4% of the total) were found to be resistant to methicillin. The genotypic characterisation of the 19 isolates by the *mecA* gene amplification approach yielded the expected 310 bp product, all of which were methicillin resistant. The 28 isolates however were confirmed as methicillin susceptible (MSS) negative for the *mecA* gene. In the case of cefoxitin disc diffusion testing, we can confirm that it is effective at identifying MR in clinical isolates, as the morphological and genotypic characterisation of the isolates agrees 100%. There was a considerable difference in the prevalence of antibiotic resistance between *Staphylococcus aureus* strains when comparing the two types of bacteria. All MR samples were resistant to penicillin, erythromycin, and ciprofloxacin, which means that they were multidrug resistant.

Table 4. Shows the results of the methicillin resistance test.

Antibiotic resistance pattern (MRSA vs. MSSA)	Value
MRSA resistant to penicillin, erythromycin, ciprofloxacin	100% (19/19)
MRSA – multidrug-resistant phenotype	Present
MSSA – resistance rates to multiple antibiotics	Significantly lower than MRSA

Relationships in Statistics

There was no relationship between age, gender or surgical operation type and methicillin resistance found by statistical study. Nevertheless, methicillin-resistant strains were considerably related with a lengthier postoperative hospital stay (8.2 days vs. 5.1 days) than methicillin-susceptible strains. The presence of MRSA infections after sinus surgery seems to result from nosocomial infections.

Table 5. Statistical associations

Statistical Associations	Value	Significance
MRSA vs. patient age	No significant association	ns
MRSA vs. patient gender	No significant association	ns
MRSA vs. type of surgical procedure	No significant association	ns
MRSA vs. post-operative hospital stay (mean days) – MRSA	8.2 days	—
MRSA vs. post-operative hospital stay (mean days) – MSSA	5.1 days	—
Association of longer hospital stay with MRSA isolation	Significant	*

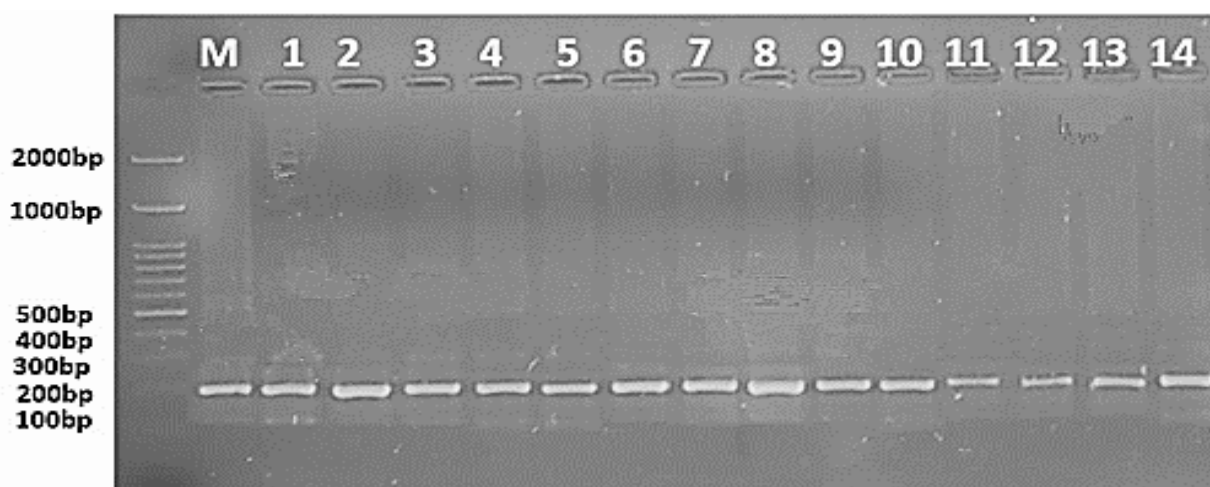


Figure 1. Shows the result of Agarose gel electrophoresis of nuc gene *S. aureus* isolates 270 bp.

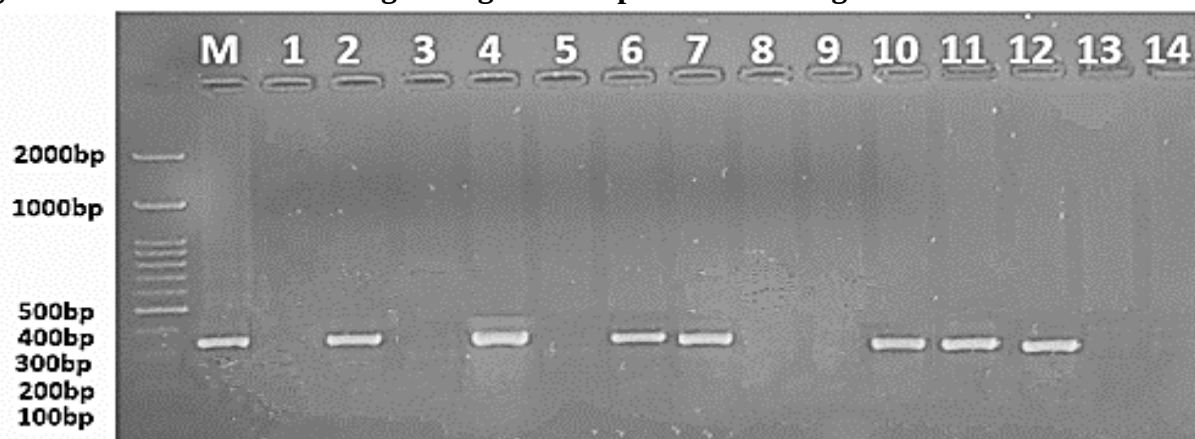


Figure 2: The mecA gene size of *S. aureus* isolates (310 bp) was determined using Agarose gel electrophoresis.

Discussion

Sinusitis can occur following surgery, and *Staphylococcus aureus* is a very common bacteria that can cause this. In this study, the frequency, molecular characteristics and antibiotic resistance of this bacterium was attempted to be

documented. Our study population was similar to other studies with an isolation rate of 39.2%, indicating that *S. aureus* is one of the most frequent bacteria isolated after sinus surgery. Considering its incidence, it is evident that it has an important role in the development of

postoperative sinusitis and precautions may need to be taken to prevent it. The nuc gene amplification was 100% in agreement with conventional biochemical testing when used as a confirmation for the identification of *S. aureus* as part of PCR reactions. The nuc gene (encoding a thermostable nuclease) is recommended for species identification, and has been verified and has been shown to be an unambiguous marker for *S. aureus* [10]. The use of molecular biology has enhanced the reliability of the traditional methods that are readily available and low cost. This is especially the case in unusual situations and research experiments where the answer is crucial. This is much quicker than more traditional methods, giving results in only a few hours. The alarming drug resistance trends of our study emphasize an important problem in the epidemiology of staphylococci. The extremely high rate of penicillin resistance (more than 90%) is important and is consistent with global investigations that have demonstrated near universal resistance to penicillin among clinical strains of *S. aureus* as a result of the widespread dissemination of beta-lactamase genes [13]. In post-surgical infections, it is important that cultures are obtained to help inform antibiotic selection, because of the high antibiotic resistance rate to commonly used fluoroquinolone and macrolide antibiotics, with limited options available. The high prevalence of methicillin-resistant *S. aureus* (MRSA) in the isolates (40.4%) is an urgent public health issue. This rate is similar to the rate of HAI among similar patients, but higher than the rate of *S. aureus* typically associated with community-acquired infections [14]. The staphylococcal cassette chromosomal mec gives bacteria resistance to all beta-lactam antibiotics except newer generations of cephalosporins containing a mecA gene that is capable of resisting methicillin resistant *S. aureus* (MRSA) [15]. Phenotypic and genotypic detection methods of mecA-mediated resistance are totally concordant, and clinical microbiology laboratories should routinely perform cefoxitin disc screening as a surrogate marker for mecA-mediated resistance. In this group of patients, resistance

could be either because of selection pressure for the antibiotic or because of the presence of resistance factors that are associated with mobile genetic elements; the multidrug-resistant phenotype is most common in methicillin-resistant isolates. This study supports the notion that nosocomial transmission participates to the prevalence of resistant disease, indeed isolates of MRSA and extended stay at hospital were identified. To minimise the risk of transmission in surgical patient populations, there are some strict infection control measures that should be taken, including strict hand hygiene, screening patients for signs of infection, and restricting contact with known carriers. Fortunately, options for treatment of severe and resistant infections still exist due to the continuing susceptibility of vancomycin and linezolid. However, these drugs should not be used only, as it will create resistance. Although susceptibility and heteroresistance are more common in other countries, vancomycin is still an important drug for the treatment of methicillin-resistant *S. aureus* (MRSA) [16]. Linezolid is expensive and may have side effects, so it should only be used when other medicines have not been successful in treating an infection. It is very effective in combating biofilm and intracellular microorganisms. A number of caveats should be taken into account in the present study: The major limitations of the study are the single centre design, which may limit its generalisability to other centres and healthcare systems, due to different resistance epidemiology; and the small sample size of 63 cases and 46 controls. Other organisms have been associated although it is important to note that in the current study only *S. aureus* was studied. Although these genes (virulence genes and typing) would have provided insight into the infection's pathogenic processes and clonal spread, they were not used in this study. Further studies using whole genome sequencing techniques could clarify the origin, evolution and spread of MRSA in surgical patients. The present study sheds light on the treatment and management of sinusitis following surgery, which is a major practical consideration. *S. aureus* should be treated according to the local

level of resistance and drugs used to treat the infection should be effective against methicillin-resistant strains, especially in regions where the disease is endemic. The antibiotics vancomycin and linezolid might be helpful to treat high-risk infections, which include extended hospital stays after surgery, and prior colonisation with MRSA or exposure to health care, and serious infection. Once the drug susceptibility and culture results are available, the treatment should be limited to smaller spectrum drugs.

Conclusion

An key pathogen in the development of post-surgical sinusitis, characterised by worrying drug resistance patterns and considerable prevalence, is *S. aureus*, according to this study. Of special interest is the methicillin-resistant variety of *S. aureus*. Molecular methods, such as nuc gene amplification to identify species and mecA gene testing to determine antibiotic resistance, are critical and reliable tools for accurate species identification and characterisation of infections. The need for immediate surveillance of antibiotic resistance pattern, promotion of antimicrobial stewardship and the issue of alarmingly high multi-drug resistance is critical. Institutional policies regarding antibiotic prophylaxis in the perioperative period and routine molecular detection of MRSA in high risk surgical patients should be considered, taking into account the local epidemiology of antibiotic resistance. Known carriers who are undergoing surgery should take special precautions to prevent infection, including preoperative decolonization. Important measures in controlling and preventing the spread of the infection include improving environmental infection control and consistently applying contact precautions in the surgical wards. More work should be done in the future on epidemiological studies including: the evolution of antibiotic resistance over time, the pathogenic virulence factors in the post-operative setting, possible alternatives to antibiotics and other treatments, and the impact of antibiotic resistance on outcomes and cost-effectiveness. To effectively tackle the issue of antibiotic resistant staphylococcal infections in surgical patients, it is

essential to continue intensive research, monitor the situation closely and act on the recommendations provided by evidence.

References

1. Orlandi, R.R., Kingdom, T.T., Smith, T.L., Bleier, B., DeConde, A., Luong, A.U., 2021. International consensus statement on allergy and rhinology: rhinosinusitis 2021. *Int. Forum Allergy Rhinol.*, 11(3): 213-739.
2. Kennedy, J.L., Hubbard, M.A., Huyett, P., Patrie, J.T., Borish, L., and Payne, S.C., 2013. Sino-nasal outcome test (SNOT-22): a predictor of postsurgical improvement in patients with chronic sinusitis. *Ann. Allergy Asthma Immunol.*, 111(4): 246-251.
3. Wertheim, H.F., Melles, D.C., Vos, M.C., van Leeuwen, W., van Belkum, A., Verbrugh, H.A., and Nouwen, J.L., 2005. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect. Dis.*, 5(12): 751-762.
4. Salim, D.K., Al-Doori, M.M., and Mohammed, K.J., 2025. Evaluation of Chloroxylonol, Ethanol, Chlorine, and Povidone-iodine against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Egypt. J. Med. Microbiol.*, 34(3): 385-391.
5. Foster, T.J., Geoghegan, J.A., Ganesh, V.K., and Höök, M., 2014. Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. *Nat. Rev. Microbiol.*, 12(1): 49-62.
6. Otto, M., 2018. Staphylococcal biofilms. *Microbiol. Spectr.*, 6(4): <https://doi.org/10.1128/microbiolspec.GPP3-0023-2018>
7. Lowy, F.D., 2003. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J. Clin. Invest.*, 111(9): 1265-1273
8. Diekema, D.J., Pfaller, M.A., Shortridge, D., Zervos, M., and Jones, R.N., 2019. Twenty-year trends in antimicrobial

- susceptibilities among *Staphylococcus aureus* from the SENTRY Antimicrobial Surveillance Program. Open Forum Infect. Dis., 6(Suppl 1): S47-S53.
9. Patel, R., 2015. MALDI-TOF mass spectrometry: transformative proteomics for clinical microbiology. Clin. Chem., 61(1): 100-111.
 10. Brakstad, O.G., Aasbakk, K., and Maeland, J.A., 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J. Clin. Microbiol., 30(7): 1654-1660.
 11. Zhang, K., McClure, J.A., Elsayed, S., Louie, T., and Conly, J.M., 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol., 43(10): 5026-5033.
 12. Jiang, R.S., Hsu, C.Y., and Liang, K.L., 2016. Bacteriology of endoscopic sinus surgery. J. Laryngol. Otol., 130(2): 148-153.
 13. Chambers, H.F., 2001. The changing epidemiology of *Staphylococcus aureus*. Emerg. Infect. Dis., 7(2): 178-182.
 14. David, M.Z. and Daum, R.S., 2017. Treatment of *Staphylococcus aureus* infections. Curr. Top. Microbiol. Immunol., 409: 325-383.
 15. Katayama, Y., Ito, T., and Hiramatsu, K., 2000. A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother., 44(6): 1549-1555.
 16. Howden, B.P., Davies, J.K., Johnson, P.D., Stinear, T.P., and Grayson, M.L., 2010. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin. Microbiol. Rev., 23(1): 99-139.