Iranian Journal of Microbiology
Volume 16 Number 4 (August 2024) 443-449

ORIGINAL ARTICLE

Phenotypic and genotypic characterization of methicillin resistant *Staphylococcus aureus* associated with pyogenic infections

Sharanya Krishnakumar[1*], Abdul Azeez Mohamed Khalid[2], Jothipandian Sowndarya[2], Lakshmi Krishnasamy[1], Paramasivam Nithyanand[2]

[1]Department of Microbiology, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamilnadu, India
[2]Biofilm Biology Laboratory, Centre for Research on Infectious Diseases (CRID), School of Chemical and Biotechnology, SASTRA Deemed University, Tirumalaisamudram, Thanjavur, Tamil Nadu, India

Received: April 2024**, Accepted:** June 2024

ABSTRACT

Background and Objectives: Staphylococcal infections are one of the major infectious diseases affecting globally in spite of advances in development of antimicrobial agents. Knowledge and awareness about the local pattern and prevalence of MRSA infections plays a key role in treatment. The aim of this study was to identify MRSA strains by phenotypic and genotypic methods and to analyze the antibiotic susceptibility pattern of MRSA strains from patients attending a tertiary care hospital.
Materials and Methods: This study was conducted over a period of 1 year, where 296 isolates of *Staphylococcus aureus* were isolated from various clinical specimens. The isolated strains were examined for antibiotic susceptibility by the modified Kirby Bauer disc diffusion method. Methicillin resistance was detected by cefoxitin disk diffusion test.
Results: A total of 104 isolates were found to be MRSA and 192 were found to be MSSA. Among the 104 MRSA isolates, 10 strains that were multidrug resistant were subjected to 16S rRNA gene sequencing analysis. All the 10 strains had a 99% match with *S. aureus* strains that were responsible for causing some serious biofilm mediated clinical manifestations like cystic fibrosis and device mediated infections. The biofilms were quantified using crystal violet staining and their ability to produce biofilms was analyzed using scanning electron microscopy and matched with the Genbank.
Conclusion: Hence these phylogenetic analysis aid in treating the patients and combating resistance to antibiotics.

Keywords: *Staphylococcus aureus*; Pyogenic infection; Methicillin-resistant *Staphylococcus aureus*; Biofilm; Multi drug resistance; Penicillin binding proteins; Healthcare associated infections

INTRODUCTION

Staphylococcal infections are one of the major infectious diseases affecting globally in spite of advances in evolution of various antibacterial agents. Of the various species of staphylococci, *Staphylococcus aureus* is the highly pathogenic species which can cause nosocomial and community acquired infections (1). Moreover, *S. aureus* is known to cause septicemia, skin and soft tissue infections, respiratory tract infections, urinary tract infections, bone infections and vaginal infections (2). Alterations in the antibi-

*Corresponding author: Sharanya Krishnakumar, MD, MBBS, Department of Microbiology, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamilnadu, India. Tel: +91-9443166001 Email: sharanyavinod87@gmail.com

otic susceptibility profile of the bacteria, makes the management of infections difficult (3). Increasing resistance patterns in the susceptibility profile of *S. aureus* to beta lactam antibiotics especially penicillin and cephalosporins have been reported (4). Various resistance mechanisms have been established for the occurrence of MRSA, namely synthesis of unique penicillin binding proteins with decreased likeness to beta lactam antibiotics determined by structural genes (mec R1, mec I), penicillin binding proteins with altered affinity to beta lactam antibiotics and production of penicillinase enzyme (5).

The pyogenic infections of MRSA may range from infections affecting skin and soft tissue, pneumonia, pyogenic endocarditis, bone and joint infections, toxic-shock syndrome, staphylococcal scalded skin syndrome (6) and otitis media (7). The occurrence of MRSA infections scales between 13% and 74% globally (8). The occurrence of MRSA in Europe ranges between 0.9% to 56% in 2014 (9). The Centre for Disease Control estimated approximately 50% of methicillin resistance among *S. aureus* causing health care associated infections in the high risk units like ICU's (10).

Management of MRSA cases is quite challenging. Vancomycin, linezolid and daptomycin are the commonly used anti-MRSA agents (11). Currently, treatment of MRSA infections is often associated with higher risks imposing a challenge to the treating physicians. MRSA infections are global threat causing economic burden to a country when compared with MSSA infections (11). Knowledge and awareness about the local pattern and prevalence of MRSA infections would be helpful for the treating physicians, microbiologists, public health care officials and workers in the particular region. The information would be helpful in proper management of cases, formulating the hospital infection control policies and designing appropriate control measures. So, the main objective of the present study was to determine MRSA strains by phenotypic and genotypic methods and to assess the antibiotic susceptibility profile of the MRSA isolates.

MATERIALS AND METHODS

Strain isolation and characterization of its drug susceptibility pattern. The study was conducted in a tertiary care centre, Sree Balaji Medical college and Hospital for a period of 1 year between May 2021 to April 2022, after obtaining ethical clearance. 296 *Staphylococcus aureus* isolates of various clinical samples like pus, tissue fluids, skin, soft tissue and blood were included in the study. The isolated strains were tested for its drug susceptibility by the modified Kirby Bauer disc diffusion method against antibiotic discs (HiMedia; Mumbai) as per the CLSI (Clinical and Laboratory Standards Institute) guidelines recommended for Gram positive cocci (7). The following antibiotic discs were used; Penicillin (10 units), ciprofloxacin (10 µg), cotrimoxazole (25 µg), erythromycin (5 µg), clindamycin (2 µg), linezolid (30 µg), rifampin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg) and gentamycin (120 µg). For detecting methicillin resistance, cefoxitin disk diffusion test was done. Cefoxitin (30 µg) disc was used for the test and the interpretation of results were done based on latest CLSI guidelines (12). If the zone of inhibition of the *S. aureus* strains were ≤21 mm then it was considered to be methicillin resistant. If the zone of inhibition of *S. aureus* strains were ≥21 mm then it was considered to be sensitive (13). The results were also confirmed by Vitek-2 compac. 10 strains of MRSA that showed multi drug resistance were selected for genotypic analysis.

Molecular identification of MRSA strains by 16S rRNA gene sequence analysis. Identification of MRSA isolates were confirmed by 16S rRNA gene sequence analysis. The strains were grown in Soybean casein Digest (SCD) broth overnight at 37°C. The broth was rotated and the pellet was resuspended in sucrose TE. Lysozyme was additionally mixed to get a final concentration of 8 mg ml^{-1}. It was then incubated for about an hour at 37°C. 100 µl of 0.5 M EDTA (pH 8.0), 60 µl of 10% SDS and 3 µl of proteinase K from 20 mg ml^{-1} were added to the tube and was further incubated at 55°C overnight. The supernatant was extracted twice with phenol: chloroform (1:1) and once with chloroform: isoamyl alcohol (24:1) and ethanol precipitated. The DNA pellet was resuspended in sterile distilled water. Eubacterial specific primers (forward primer 24f—5'-AGAGTTTGATCCTGGCTCAG-3') and (reverse primer 1492r—5' ACGGCTACCTTGTTACG ACTT-3') were used to amplify 16S rRNA genes. PCR fragments were purified and sequencing of the rRNA gene was carried out. The full-length sequences that resulted were matched with the closest associated strain which were acquired by BLAST searches (14).

Biofilm quantification of MRSA strains. A crystal violet (CV) staining method was used for quantifying the biofilm forming ability of the 10 strains. All 10 strains were raised overnight and measured for an absorbance of 0.3. 1ml of SCD broth and 100 ul of each strain of bacterial culture was added in 10 test tubes with triplicates was incubated at 37°C and 50 rpm conditions for 24 hours for young biofilm quantification and repeated for further 24 hours incubation by removing the spent medium and adding 1 ml of fresh SCD broth to the wells of 24 well plate for mature biofilms. 0.4% of CV is used to stain the biofilms and 70% ethanol is used to solubilize the biofilms formed on the sides of test tubes. A spectrophotometer was used to measure absorbance of 595 nm (10). Visual confirmation of the biofilm formation was done by growing biofilms over 1 × 1 cm kept in 24 well microtiter plate for 24 hours. It was viewed under the light microscope.

Scanning electron microscopy analysis. The potential of some selected methicillin-resistant *Staphylococcus aureus* to produce biofilms was studied by using Scanning Electron Microscopy (SEM). The strains included were selected based on the source of the closest match in the Genbank. The biofilms were cultured on top of the glass slides of 1 × 1 cm which were kept on the 24-well microtiter plate. The plates were incubated for the first 24 hours and repeated by replacing the spent media with fresh SCD media and further incubation was done for another 24 hours. Afterwhich the slides containing the samples were layered with gold and examined (15).

RESULTS

Of the 296 *Staphylococcus aureus* isolated from various clinical samples, 104 were found to be resistant to methicillin (MRSA) and the remaining192 isolates were found to be sensitive to methicillin (MSSA). The antibiotic susceptibility pattern of the MRSA isolates is shown in Fig. 1.

Most of the MRSA strains were isolated from pus (42.72%) samples. 16s rDNA sequence analysis revealed that all the strains had a closest match to *S. aureus* of various clinical samples. A majority of strains (LS1, LS2 and LS5) had a closest match with pus or wound isolates (Table 1). The sample distribution is shown in Fig. 2.

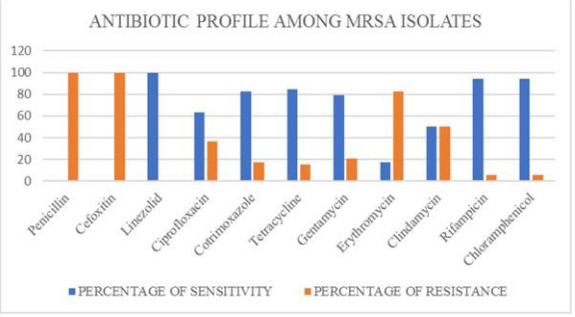

Fig. 1. Antibiotic profile among MRSA isolates

Visualization of biofilm formation by MRSA strains. Biofilm assay on 24 well microtiter plate showed the ability of all 10 selected MRSA strains to form biofilms. Amongst all, LS8 with mean absorbance of 2.357 measured at 595 nm showed the maximum biofilm formation (Fig. 3). Light Microscopic visualization also authenticated the biofilm forming ability of all the clinical isolates as there was a cluster of bacterial cells (Fig. 4).

SEM analysis of MRSA strains. Five MRSA strains were selected based on their closest match in the Genbank, each strain representing a different source. SEM images revealed that all the five strains showed a thick conglomeration of cocci which might be due to the presence of exopolysaccharide layer produced by the biofilm matrix.

DISCUSSION

The infections due to *S. aureus* and the prevalence of MRSA are major threats to the healthcare systems worldwide. Community acquired *S. aureus* (CA-MRSA) infections have been on the rise in the recent decades. The CA-MRSA is an alarming threat worldwide because of the multidrug resistant genes. The MRSA is a rising global threat due to the capability of the *Staphylococcus* strains to acquire the resistant genes overtime.

A highly sensitive and specific detection method of MRSA is much needed by the clinicians as well as microbiologists. The antimicrobial resistance action plan devised by World Health organisation (WHO) involves many approaches to battle the AMR including novel methods for identification of pathogens (16). Mec A gene identification by PCR is the gold standard in identification of MRSA (17).

Table 1. 16s gene sequence analysis of MRSA strains isolated from pus samples based on BLAST analysis

Strain	GenBank accession number	Most closely related hit in GenBank	Source of the closest match
LS1	OP010186	*Staphylococcus aureus* (MK346033)	Pus
LS2	OP010187	*Staphylococcus aureus* (MK346033)	Pus
LS3	OP010202	*Staphylococcus aureus* (MZ462056)	Ear swab
LS4	OP010912	*Staphylococcus aureus* (LN899816)	Biofilm former on medical device
LS5	OP012468	*Staphylococcus aureus* (MK346033)	Pus
LS6	OP012475	*Staphylococcus aureus* (OK285211)	Wound
LS7	OP012476	*Staphylococcus aureus* (MF144453)	Sputum of cystic fibrosis patient
LS8	OP012510	*Staphylococcus aureus* (OP364883)	Human Blood Samples
LS9	OP012702	*Staphylococcus aureus* (KR232862)	Cord blood unit
LS10	OP072205	*Staphylococcus aureus* (MF144453)	Sputum of cystic fibrosis patient

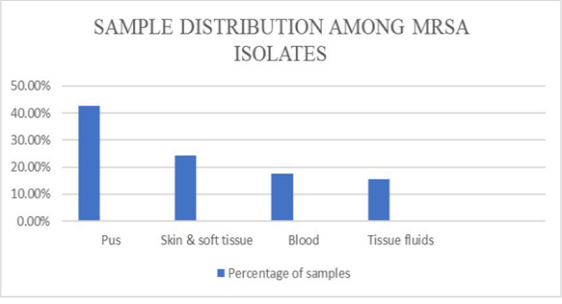

Fig. 2. Distribution of samples among MRSA isolates

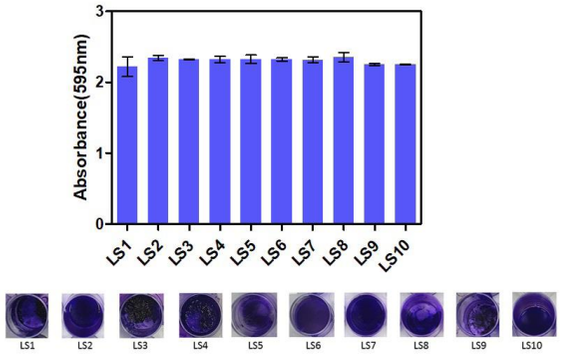

Fig. 3. Biofilm quantification of various MRSA strains.

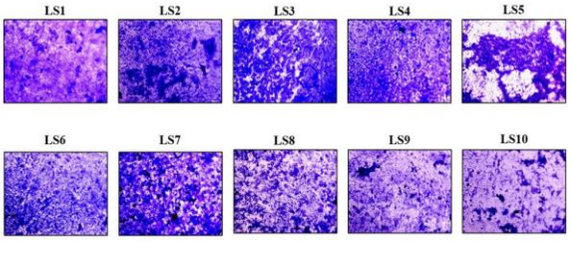

Fig. 4. Biofilm forming ability of various MRSA strains visualized by light microscopy.

The occurence of MRSA has been shown to be due to acquisition of *mec A* gene which is an important part of staphylococcal cassette chromosome mec (SCCmec). The penicillin binding protein (PBP2a), a peptidoglycan transpeptidase is coded *mec A* which holds beta lactam antibiotics resistant drugs like penicillins, cephalosporins and carbapenems (18). The presence of *mec A* or PBP 2a can be diagnosed using methicillin or oxacillin, hence the name MRSA is being used commonly (19). This study is done to analyse the presence of MRSA among *Staphylococcus aureus* isolates from both inpatients and outpatients of Sree Balaji Medical college and hospital, Chennai, India.

Since early times, MRSA is considered as a precursor of multidrug resistance and Hospital acquired infections in healthcare settings (20). Hence sepsis caused by MRSA are challenging to treat because of the multi drug resistance nature of *S. aureus* causing failure of treatment and adds to the complications (21). Recent decades show remarkable rise in MRSA percentage with emergence of healthcare-associated MRSA (HA-MRSA). It has emerged as an endemic in many countries causing high mortality and morbidity due to bloodstream infections and respiratory infections including pneumonia (20). Though there are newer development of antibiotics, adequate surveillance efforts and increased importance given to infection prevention, MRSA still seems to be a prominent pathogen and a leading cause of mortality (22).

According to our data, 296 *S. aureus* isolates were obtained from various clinical samples. MRSA isolation rate was found as 35.1%. There was no significant connection found among the Gender and the

MRSA isolation levels. However, majority of the strains (80%) analysed genotypically were between the age group of 30-59 years.

Studies show that the commonly used method currently employed in identification of MRSA is by cefoxitin disc diffusion test. It is superior to disc diffusion test using oxacillin disc and is found to be the surrogate marker of *mec A* gene. Cefoxitin disc is now being a routinely used technique in identification of MRSA strains and a good alternative to PCR in resource limited settings (23).

Many studies reported the presence or absence of MRSA (24-26). *Staphylococcus aureus* is regarded as a common causative agent of infectious diseases. Since it is usually a normal skin commensal, it has the ability to enter the body through cut wounds, abrasions, burns, surgical incisions and may cause pyogenic infections.This study shows that *S. aureus* was mostly isolated from pus samples, which was similar to studies by Onemu and Ophori (27). Which showed a prevalence of 72.7%. Another study, in Algeria showed 64.28 % (28), but its higher than results obtained by our study which showed 42.7% of pus samples hence this is comparable to a study in Maroc 19% (29). As expected, majority of the isolates in our study had a closest match to strains isolated from wound and pus samples. Moreover, it is noteworthy that all the 10 strains produced a prodigious amount of biofilms (Fig. 3).

Biofilm formation is a major hurdle in treating wound infections as the chronic wounds do not heal easily (30). The composition of the biofilm i.e., extracellular matrix (ECM) plays a major role in mediating drug resistance among the pathogens. The present study also show that some selected strains formed a thick exopolysaccharide (EPS) layer. The EPS layer helps to attach the bacterial strains to the surrounding tissue which helps in the aggregation of bacterial cells (Fig. 5). It is important to note that the strain LS4 which produced a robust aggregation had a closest match to an *S. aureus* strain (LN899816) which was a biofilm former isolated from a medical device. Aggregation is an important property among strains involved in device mediated biofilm infection as a stable aggregation is the base form biofilms to develop in mature biofilms (27). This further thickens the EPS layer which in turn prevent the entry of antimicrobials thereby mediating drug resistance. It was interesting to note that 2 strains (LS7 and LS10) had a closest match to *S. aureus* strains recovered from the sputum of patients with cystic fibrosis (Table 1).

Recent studies show that *S. aureus* is the second most common next to *Pseudomonas aeruginosa* reported in cystic fibrosis patients. Pathogens present in the air way passage adapt to the hostile niche by producing a biofilm and myriad of virulence factors to overcome the host immune response, antibiotic therapy and the competition with coinfection of pathogens (31). Wound infections are also one such environment where biofilm formation protects the pathogens from conditions that are unfavourable to them. Overall, it is clearly evident that biofilm formation is crucial virulence factor in *S. aureus* strains across different clinical conditions which stresses the need for an antibiofilm therapy rather than mere

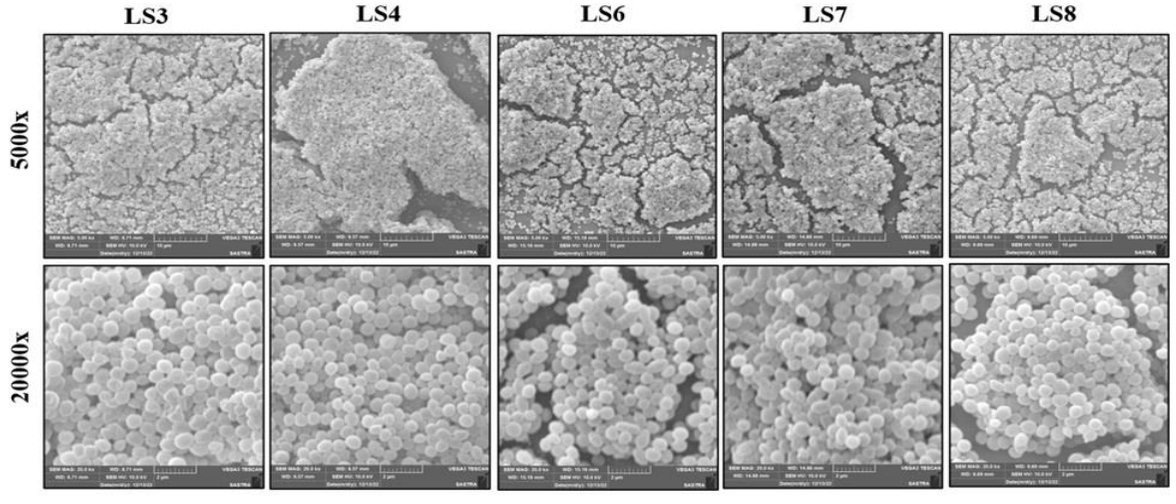

Fig. 5. Scanning Electron Microscopic images of biofilms formed by selected MRSA strains.

administration of antimicrobials. Moreover, as Quorum sensing (QS) has a major role in cell aggregation causing assembly of biofilms. Quorum sensing inhibitors (QSI) or quorum quenchers (QQ) should be formulated as topical agents to treat wound infections. Extensive knowledge about prevalence and incidence of MRSA strains in the local area is an important pre requisite for developing appropriate control and surveillance measures. In the present study, phenotypic antimicrobial susceptibility tests shows higher sensitivity to linezolid (100%), rifampicin (94.23%) and chloramphenicol (94.23%) which resembles a previous study by Gitau et al. (32) which shows high sensitivity to linezolid. Our study shows high levels of resistance to benzyl penicillin (100%) and erythromycin (82.69%) which is comparable to few Kenyan and African studies (32-34).

CONCLUSION

The present study throws light into the diverse distribution and resistance pattern of MRSA. Though the study is limited by lesser numbers of isolates, it furnishes a base for monitoring the occurrence of *S. aureus* and observing the resistance patterns that aids to analyse risk factors for *S. aureus* infections. The study will further intensify our basic knowledge of interactions among pathogen-drug and pathogen-host in case of MRSA infections. Further in-depth studies are required in newer areas of drug development wherein focus should be more on anti-infective agents to cope with the challenges of these life-threatening infections.

ACKNOWLEDGEMENTS

PN acknowledges the financial support from Indian Council of Medical Research (ICMR), New Delhi (OMI/18/2020-ECD-I). The Fellowship to SJ in the form of JRF by ICMR is thankfully acknowledged.

REFERENCES

1. Francois P, Pittet D, Bento M, Pepey B, Vaudaux P, Lew D, et al. Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. *J Clin Microbiol* 2003; 41: 254-260.
2. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* 2015; 28: 603-661.
3. Bukhari MH, Iqbal A, Khatoon N, Iqbal N, Naeem S, Qureshi GR, et al. A laboratory study of susceptibility of methicillin-resistant *Staphylococcus aureus. Pak J Med Sci* 2004; 20: 229-233.
4. Orrett FA. Antimicrobial sensitivity patterns of aerobic bacterial blood isolates: experience at a University Hospital in Trinidad. *Int J Antimicrob Agents* 2001; 17: 75-77.
5. Fruit AC, Wielders CL, Verhoef J, Schmitz FJ. Epidemiology and susceptibility of 3,051 *Staphylococcus aureus* isolated from 25 University Hospitals participating in the European SENTRY Study. *J Clin Microbiol* 2001; 39: 3727-3732.
6. Ostojic M, Hukic M. Genotypic and phenotypic characteristics of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains, isolated on three different geography locations. *Bosn J Basic Med Sci* 2015; 15: 48-56.
7. Algammal AM, Hetta HF, Elkelish A, Alkhalifah DHH, Hozzein WN, Batiha GE, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infect Drug Resist* 2020; 13: 3255-3265.
8. Köck R, Becker K, Cookson B, van Gemert-Pijnen JE, Harbarth S, Kluytmans J, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. *Euro Surveill* 2010; 15: 19688.
9. Hassoun A, Linden PK, Friedman B. Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment. *Crit Care* 2017; 21: 211.
10. Salgado CD, Farr BM, Calfee DP. Community-acquired methicillin-resistant *Staphylococcus aureus:* a meta-analysis of prevalence and risk factors. *Clin Infect Dis* 2003; 36: 131-139.
11. Kong EF, Johnson JK, Jabra-Rizk MA. Community-associated methicillin-resistant *Staphylococcus aureus:* an enemy amidst us. *PLoS Pathog* 2016; 12(10): e1005837.
12. Clinical and Laboratory Standards Institute (CLSI). M100 Performance Standards for Antimicrobial Susceptibility Testing. M100-Ed31, Clinical and Laboratory Standards Institute (CLSI). 2021.
13. Gebremeskel FT, Alemayehu T, Ali MM. Methicillin-resistant *Staphylococcus aureus* antibiotic susceptibility profile and associated factors among hospitalized

patients at Hawassa University Comprehensive Specialized Hospital, Ethiopia. *IJID Reg* 2022;3:129-134.
14. Gawlik D, Ruppelt-Lorz A, Müller E, Reißig A, Hotzel H, Braun SD, et al. Molecular investigations on a chimeric strain of *Staphylococcus aureus* sequence type 80. *PLoS One* 2020; 15(10): e0232071.
15. Jothipandiyan S, Suresh D, Sankaran SV, Thamotharan S, Shanmugasundaram K, Vincent P, et al. Heteroleptic pincer palladium(II) complex coated orthopedic implants impede the AbaI/AbaR quorum sensing system and biofilm development by *Acinetobacter baumannii*. *Biofouling* 2022; 38: 55-70.
16. Hoffman SJ, Outterson K, Røttingen J-A, Cars O, Clift C, Rizvi Z, et al. An international legal framework to address antimicrobial resistance. *Bull World Health Organ* 2015; 93: 66.
17. Pillai MM, Latha R, Sarkar G. Detection of Methicillin Resistance in *Staphylococcus aureus* by Polymerase Chain Reaction and Conventional Methods: A Comparative Study. *J Lab Physicians* 2012; 4: 83-88.
18. Stapleton PD, Taylor PW. Methicilin resistance in *Staphylococcus aureus*: mechanisms and modulation. *Sci Prog* 2002; 85: 57-72.
19. Velasco D, del Mar Tomas M, Cartelle M, Beceiro A, Perez A, Molina F, et al. Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus*. *J Antimicrob Chemother* 2005; 55: 379-382.
20. Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, et al. Survey of infections due to *Staphylococcus species*: Frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY antimicrobial surveillance program, 1997–1999. *Clin Infect Dis* 2001; 32 Suppl 2: S114-S132.
21. Hirao Y, Ikeda-Dantsuji1 Y, Matsui H, Yoshida M, Hori S, Sunakawa K, et al. Low level ß-lactamase production in methicillin resistant *Staphylococcus aureus* strains with ß-lactam antibiotics-induced vancomycin resistance. *BMC Microbiol* 2012; 12: 69.
22. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nat Rev Microbiol* 2019; 17: 203-218.
23. Madhavan A, Sachu A, Balakrishnan A, Vasudevan A, Balakrishnan S, Vasudevapanicker J. Comparison of PCR and phenotypic methods for the detection of methicillin resistant *Staphylococcus aureus. Iran J Microbiol* 2021; 13: 31-36.
24. Delport JA, Mohorovic I, Burn S, McCormick JK, Schaus D, Lannigan R, et al. Rapid detection of methicillin-resistant *Staphylococcus aureus* bacteremia using a combined three-hour short-incubation matrix-assisted laser desorption/ionization time-of-flight MS identification and the Alere culture colony PBP2a detection test. *J Med Microbiol* 2016; 65: 626-631.
25. Panda RK, Mahapatra A, Mallick B, Chayani N. Evaluation of genotypic and phenotypic methods for detection of methicillin resistant *Staphylococcus aureus* in a tertiary care hospital of Eastern Odisha. *J Clin Diagn Res* 2016; 10: DC19-DC21.
26. Thapa S, Sapkota LB. Changing trend of neonatal septicemia and antibiotic susceptibility pattern of isolates in Nepal. *Int J Pediatr* 2019; 2019: 3784529.
27. Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. *Front Cell Infect Microbiol* 2023; 13: 1137947.
28. Abdeen EE, Mousa WS, Abdelsalam SY, Heikal HS, Shawish RR, Nooruzzaman M, et al. Prevalence and characterization of coagulase positive *Staphylococci* from food products and human specimens in Egypt. *Antibiotics (Basel)* 2021; 10: 75.
29. Percival SL, McCarty SM, Lipsky B. Biofilms and wounds: an overview of the evidence. *Adv Wound Care (New Rochelle)* 2015; 4: 373-381.
30. Rumpf C, Lange J, Schwartbeck B, Kahl BC. *Staphylococcus aureus* and cystic fibrosis-a close relationship. what can we learn from sequencing studies? *Pathogens* 2021; 10: 1177.
31. Gitau W, Masika M, Musyoki M, Museve B, Mutwiri T. Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates from clinical specimens at Kenyatta National Hospital. *BMC Res Notes* 2018; 11: 226.
32. Maina EK, Kiiyukia C, Wamae CN, Waiyaki PG, Kariuki S. Characterization of methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections in patients in Nairobi, Kenya. *Int J Infect Dis* 2013; 17(2): e115-e119.
33. Aiken AM, Mutuku IM, Sabat AJ, Akkerboom V, Mwangi J, Scott JA, et al. Carriage of *Staphylococcus aureus* in Thika level 5 hospital, Kenya: a cross- sectional study. *Antimicrob Resist Infect Control* 2014; 3: 22.
34. Schaumburg F, Alabi AS, Peters G, Becker K. New epidemiology of *Staphylococcus aureus* infection in Africa. *Clin Microbiol Infect* 2014; 20: 589-596.