Staphylococcus aureus virulence genes and methicillin-resistant gene detection and antimicrobial resistance profiles isolated from different infection sites

Rafal Ismael[a], Alaa K. Alhameedawi[b], Rajaa S. Abbas[c], Sarah M.S. Alsallameh[a], Halah Amer[d], Müge Firat[e], Maryam Karkhane[f]

[a]Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Gilgamesh Alhliya University, [b]Ministry of Education General Directorate for Education/Rusafa2, [c]Department of Medical Laboratory Techniques, AL-Ma'moon University College, [d]Department of Medical Laboratory Techniques, Al-Turath University College, Baghdad, Iraq, [e]Department of Biology, Faculty of Health Sciences, Cankiri Karatekin University, Cankiri, Türkey, [f]Department of Medical Biotechnology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

Correspondence to Sarah M.S. Alsallameh, MSc, Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Gilgamesh Alhliya University, Baghdad 10022, Iraq. Tel: +9647719758362; e-mail: sarahalsallameh@gau.edu.iq

Received: 20 December 2022
Revised: 15 February 2023
Accepted: 25 February 2023
Published: 27 June 2023

Egyptian Pharmaceutical Journal 2023, 22:265–271

Background
One of the most significant pathogenic bacteria is *Staphylococcus aureus*, and both adults and children are susceptible to this bacterium from the front of the nose. In the United States, the Centers for Disease Control and Prevention estimate that 80,461 invasive methicillin-resistant *S. aureus* (MRSA) infections and 11 285 related deaths occurred in 2011. In the UK, around 190 people passed away from MRSA disease in 2021. Australia, Hong Kong, Singapore, Japan, and Greece also have MRSA infections, along with the whole world. MRSA caused less than 2% of bacterial diseases in the United States in 1974, while the percentage rate jumped to 64% in 2004 only 10 years to increase the infection rate by 300%.
Objective
This study aimed to detect medication susceptibility patterns, staphylococcal enterotoxins A to C, toxic shock syndrome toxin-1, and methicillin-resistant genes.
Materials and methods
Ninety-eight *S. aureus* strains were isolated from different infection sites from Salah Al-Din Teaching Hospital. There have only been a few studies conducted on the epidemiology and virulence genes of *S. aureus* in Salah Al-Din city, Iraq.
Results and conclusion
The rates of drug resistance among *S. aureus* strains to routinely used antibiotics were found to be extremely high. In this study, the expression of toxic shock syndrome toxin, *sec B*, and *sec C* genes in *S. aureus* strains was not detected, unlike in previous studies. While all the strains were *sec A* gene positive, another gene found in bacterial cells that enables them to be resistant to antibiotics like methicillin and other vancomycin drugs is *mecA.*

Keywords:
antibiotic resistance, antimicrobial susceptibility test, methicillin-resistant *Staphylococcus* aureus, *Staphylococcus aureus*, virulence genes

Egypt Pharmaceut J 22:265–271

1687-4315

Introduction

Gram-positive coccal bacterium *Staphylococcus aureus* is facultative, aerobic, and easily contaminates meat, food, and the environment [1]. Serious animal disorders such as sepsis, pneumonia, arthritis, and urinary tract infection are all brought on by *S. aureus* [2,3]. This bacterium is a significant contributor to nosocomial bacteremia, postoperative wound infections, pneumonia, and food poisoning in humans [1].

Skin and soft-tissue infections, as well as lower respiratory tract infections, are significantly attributed to *S. aureus* in all examined regions [4]. *S. aureus* is frequently responsible for toxin-mediated illnesses such as toxic shock syndrome and foodborne diseases. This bacterium has several MSCRAMMs, or microbial surface components that recognize adhesive matrix molecules, on its surface that helps it connect to host tissues and begin colonization, which leads to infection [5]. These binding proteins include an A domain, which binds fibers such as fibrinogen and also interacts with histones, which causes the neutralization of antibacterial activity of these molecules. They also promote biofilm formation and platelet aggregation by enhancing cell-to-cell interaction [6].

Due to its high virulence and its outstanding resistance, *S. aureus* is considered to be of significant clinical value. Among the virulence components of *S. aureus* are surface proteins, enterotoxins, and enzymes. [7].

In the case of life-threatening bacterial infections, the ability of *S. aureus*' to withstand antibiotics may become more problematic. [8,9]. Methicillin-resistant *Staphylococcus* aureus (MRSA) is a member of the Micrococcaceae family and belongs to the genus *Staphylococcus*, which in turn has more than 30 species, including *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and *Staphylococcus haemolyticus* [10]. *S. aureus* is the staphylococcal species that is most dangerous. However, different geographical areas have a variable prevalence of *S. aureus* nasal carriers [11].

Materials and methods

Sample collection and identification

Samples were collected from outpatients aged (from 6 to 50 years), who attended the Department of Salah Al-Din City General Hospital in salah al-Din, Iraq. The specimens were collected in the period between January and August 2022. The aim of this project was discussed and explained to the patients or the parents of children, and signed consent forms were obtained. A total of 98 *S. aureus* samples were collected for this study. Clinical specimens were isolated from the throat, ear, wound, and urine. Bacterial strains were identified using biochemical tests and conventional methods such as hemolysis and Gram staining, and it was determined that all strains belong to *S. aureus*.

Antimicrobial susceptibility test (AST)

Isolates were examined for antimicrobial susceptibility according to the recommendations of the CLSI, and AST was carried out using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar [12]. Rifampicin, Amoxicillin, Cefalexin, Teicoplanin, Cefotaxime, Ciprofloxacin, Tetracycline, Vancomycin, Meropenem, and Aztreonam were used as antibiotics. The sensitivity of the *S. aureus* samples for each antimicrobial agent was assessed after the incubation at 35°C overnight, and the results were interpreted in accordance with the NCCLS criteria [13].

Staphylococcus aureus DNA extraction

After 24 h of cultivation on blood agar, the isolates were resuspended in 100 ml of DW and then heated for 15 min at 99°C in a thermomixer. The mixture was then centrifuged at 1000 g at 14°C for 5 min. The supernatant and bacterial crude DNA extract were transferred to another tube after removing the precipitant [14].

PCR gene detection

The PCR amplification reaction mixture was prepared according to Wang *et al.* [15]. The toxic shock syndrome toxin (TSST-1), (SEa), (SEb), (SEc), and mecA genes were amplified using PCR. The reaction products were then electrophoresed in a 2% agarose gel with tris-acetate electrophoresis buffer, and a 100-bp DNA ladder was used as a molecular marker to determine the presence of PCR products [16]. A multiplex PCR test was used to investigate the genes.

Preparation of primers

Multiplex PCR was performed to detect the presence of virulence genes (SEA, SEB, SEC, mecA, and tsst) [16]. To amplify each of the target genes, appropriate primers were used. This experiment was carried out using primers usede in earlier investigations.

Table 1 displays the primer sequence and the size of the amplified product. Multiplex PCR is a fast and reliable technique for identifying and amplifying multiple genes at the same time using specific primers, and so allowed to save money, time, and energy. To carry out the multiplex PCR reaction, specific primers related to virulence genes with annealing temperatures in the same range were grouped together.

The reaction mixtures for each group of virulence genes were done in accordance to AubaisAljelehawy *et al.* [17]. To do this, the reaction mixture of each group of virulence genes was put into sterile microtubes, and PCR was performed after changing the temperature and time of the reaction. Following PCR amplification, PCR products were electrophoresed for 60 min on a 1% (w/v) agarose gel in ×1 TBE solution (Tables 1 and 2).

Results

In this study, 98 strains of *S. aureus were* isolated from clinical samples in Salah Al-Din Teaching Hospital, Iraq. Identification of clinical specimens using biochemical tests and conventional methods such as hemolysis and Gram staining showed that all strains

Table 1 Sequence of primers with related sizes

Primer	Oligopeptide sequence	Product size
mecA^{F}	TGTCCGGGGAAATAACTGAA	305
mecA^{R}	TGGCATGATTTCTTCTGCAA	
Se A^{F}	TCGATTGACCGAAGAGAAAAA	203
Se A^{R}	CGATTAATCCCCTCTGAACC	
Se B^{F}	CCTAAACCAGATGAGTTGCACA	404
Se B^{R}	CCATCTTCAAATACCCGAACA	
Se C^{F}	AAACATGAAGGAAACCACTTTGA	126
Se C^{R}	TTCTTGAGCTGTTGCACTTTTC	
Tsst-1^{F}	CTTGCGACAATCGCTACAGA	537
Tsst-1^{R}	TTTCCAATAACCACCCGTTT	

Tsst, toxic shock syndrome toxin.

belonged to *S. aureus* as shown in Fig. 1. The strains were isolated from the wound, urine, burns, otitis media infections, and throat, which accounted for 58, 9, 16, 8, 8, and 8% of the samples in each group, respectively, as shown in Fig. 2.

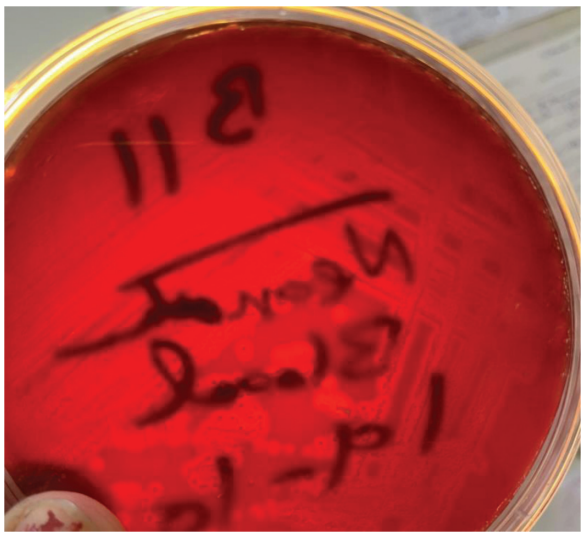
The susceptibility testing against *S. aureus* is demonstrated in Table 3 and Fig. 2, which clearly show that all isolates were vancomycin and amoxicillin resistant, with 100% resistance for all isolates, while the prevalence of *S. aureus* showed full resistance to the antibiotic Cefotaxime in the wound, burn, and urine samples, while otitis media samples showed the least resistance with 69%. Cephalexin is effective against 88% of *S. aureus* in urine samples, followed by Rifampicin for throat samples with only 13% resistance and 6% resistance to Teicoplanin.

All 98 *S. aureus* isolates were MRSA, and these entire collections tested positive for the *mecA* gene. The presence of toxin genes in the strains isolated from wound, burn, urinary, throat, and otitis media infections is shown in Table 4. All specimens showed 100% for the presence of the SeA gene and tested negative for SeB, SeC, and TSST-1 genes (Fig. 3). Multiplex PCR was performed to detect the specific genes, which is shown in Fig. 4.

Table 2 PCR program for genes (*sea*, *seB*, *seC*, *mec*A, toxic shock syndrome toxin-*1*)

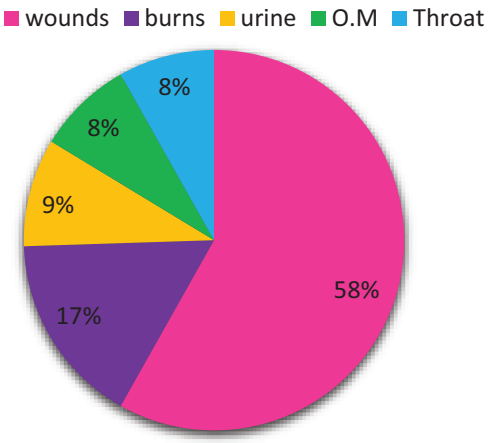
Stages	Time (s)	Temperature (°C)	Cycle
Denaturation	300	94	1
Denaturation	30	95	35
Annealing	45	56	
Elongation	60	72	
Final elongation	350	72	1

Figure 1



Identification of *Staphylococcus aureus* isolates using the hemolysis method.

Figure 2



Frequency of *Staphylococcus aureus* isolates in a clinical specimen.

Table 3 Antibiotic resistance frequency among clinical strains isolated from different parts of the body

	Wounds		Burns		Urine		Otitis media		Throat	
	R* %	S* %	R %	S %	R %	S %	R %	S %	R %	S %
Rifampicin	57	43	48	52	100		81	19	13	87
Amoxicillin	100		100		100		100		100	
Cephalexin	85	15	70	30	12	88	64	38	81	19
Teicoplanin	72	28	82	18	71	29	94	6	6	94
Cefotaxime	100		100		100		69	31	94	6
Ciprofloxacin	43	57	49	51	100		25	75	100	
Tetracycline	72	28	52	48	94	6	44	56	100	
Vancomycin	100		100		100		100		100	
Meropenem	44	56	64	36	82	18	94	6	19	81
Aztreonam	86	14	79	21	65	35	12	88	81	19

R, resistance; S, sensitive.

Table 4 The frequency of virulence genes and *mecA* gene among clinical strains isolated from different parts of the body

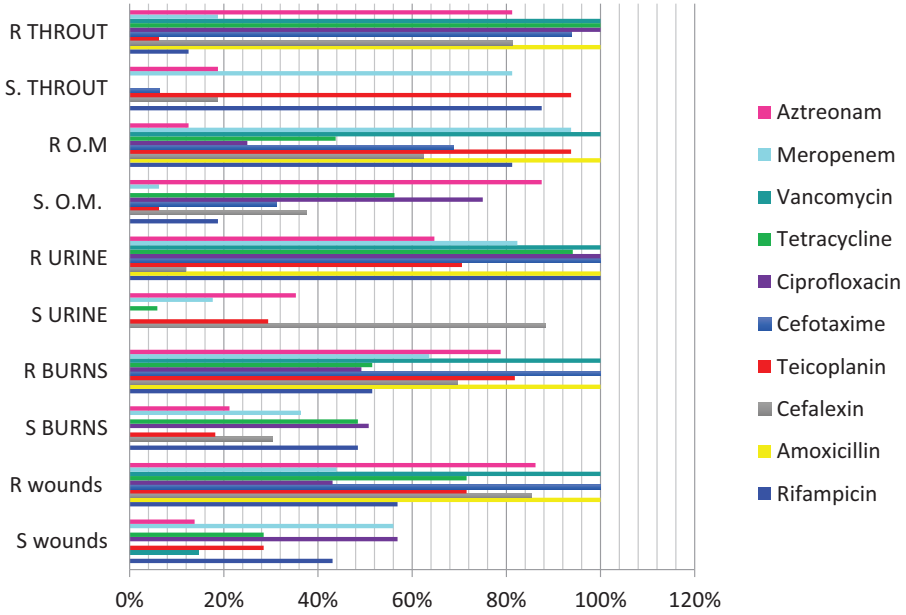
Samples	Sea (%)	SEB (%)	SEC (%)	TSST-1 (%)	mecA (%)
Wounds	100	0	0	0	100
Burns	100	0	0	0	100
Urine	100	0	0	0	100
Otitis media	100	0	0	0	100
Throat	100	0	0	0	100

TSST, toxic shock syndrome toxin.

Discussion

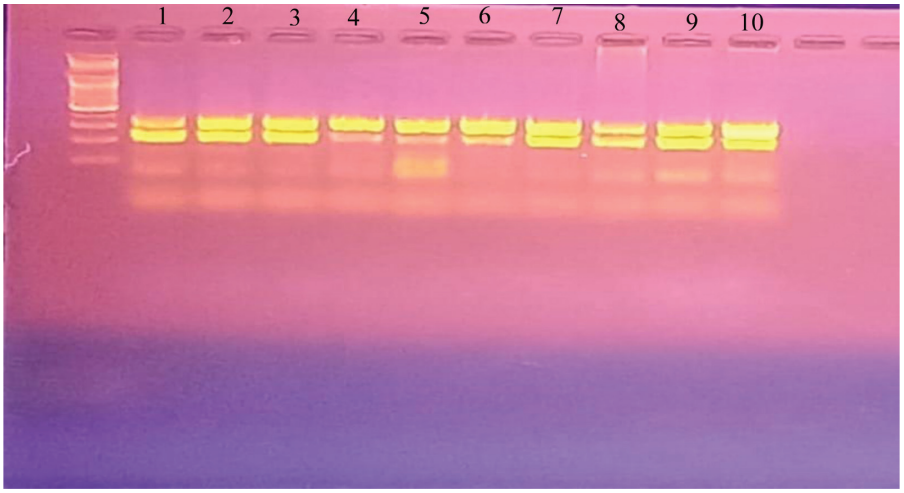
MRSA is most common in newborns, the elderly, and persons with chronic diseases such as burn survivors, organ transplant recipients, chemotherapy-treated cancer patients, steroid users, diabetics, intravenous drug users, and HIV patients [18–21]. Accumulation of microorganisms in the human body causes increased contamination levels. It can happen in the skin, perineum, throat, and gastrointestinal system, as well as the vagina [22].

Figure 3



Frequency of virulence genes among *Staphylococcus aureus* strains isolated from Salah Al-Din Teaching Hospital. Among the strains, *sea and mecA* genes with 100% frequency showed the highest prevalence among pathogenic genes. *Seb*, *Sec, tsst-1*genes with 0%. tsst, toxic shock syndrome toxin.

Figure 4



Multiplex PCR of virulence and resistance genes among *Staphylococcus aureus* strains. 1, 2 are from wounds, 3, 4 are burns, 5, 6 are urine samples, 7, 8 from otitis media, and 9, 10 from the throat

The location of infection in the body influences treatment options and antimicrobials used for people with MRSA [23]. Only four medications demonstrated activity *in vivo* against MRSA, Quinupristin, dalfopristin, minocycline, daptomycin, linezolid, and vancomycin. Rifampicin is an efficient antibiotic against staphylococci, although its effectiveness in MRSA infections has yet to be demonstrated. MRSA's antibacterial activity changes in response to trimethoprim-sulfamethoxazole [24–29].

We reported 100% resistance in urine samples to cefotaxime, compared with the Salman *et al.* [30] report, which showed no resistance that formed 37.5% of all urine samples. Ansari *et al.* [31] observed a 36.7% prevalence of ciprofloxacin resistance. Previous research in Nepal found no resistance to vancomycin or linezolid; our findings revealed 100% resistance [32]. However, among commonly used antibiotics, vancomycin and amoxicillin had the highest rate of resistance to *S. aureus*, and the highest rate of susceptibility to Cephalexin (88%) in urine samples, followed by Rifampicin (87%) and Teicoplanin (94%) in throat samples, and Aztreonam (88%) in OM samples, implying the possibility of using these drugs for preliminary treatment of *S. aureus* infections in our settings. *S. aureus* isolates were resistant to penicillin, ampicillin, and amoxicillin in 77.2%, but susceptible to co-amoxiclav in 81.8% and cephradine in 86.3%. [33]. In developing countries like Iraq, the low cost of these drugs will be an extra benefit.

By PCR methods, all of the isolates in our investigation were identified as MRSA, which is considered the highest when compared with Kshetry *et al.* [34] findings (37.6%) and Sanjana *et al.* [32] (39.6%). However, Subedi and Brahmadathan [35] (15.4%) MRSA isolates and in 2011, Baral *et al.* [36] (26%) reported lower prevalence, while in 2017, another study by Baral and Khanal [37] reported MRSA isolates to be 41%, whereas Jha [38] reported MRSA incidence to be 68% and Tiwari *et al.* [39] reported a higher prevalence (69.1%). The difference in isolation rates for MRSA between studies can be due to differences in study regions and time periods, differences in hygienic conditions maintained in different hospitals, healthcare facilities provided by the hospital, infection control program implementation, and rational use of antibiotics, which may vary from hospital to hospital [40].

Enterotoxins (SEs) belong to a large superantigen family of acidic toxins that cause vomiting. The classic SESs (sea, seb, sec, sed, and see) were found in studies of *S. aureus* species involved in *S. aureus* botulism transmission (SFP) and were classified into different serotypes. In the current study, the most prevalent gene for virulence was associated with the seA gene, which was present in 100% of bacterial samples isolated from patients. In a report by Iran Kamarehei *et al.* [41], the frequency of this gene was determined to be around 50.4% in *S. aureus* strains.

In the above report, the prevalence of the tsst-1 gene was relatively high; however, its frequency was not detectable in the results obtained in our study. The product of this gene is the main cause of tsst-1, which is produced in the second type of *S. aureus* isolates (MRSA) [42]. Thus, the production of this toxin is a variable genetic trait. It is known as an acute disease that is associated with symptoms such as fever, skin rash, high blood pressure, and dysfunction of multiple systems [43]. In the present study, the frequency of the tsst-1 gene among *S. aureus* strains was about 0%. Nagao *et al.* [44] (Japan) found that the prevalence of this gene among 152 MRSA strains was about 75%. Xie *et al.* [45] estimated the frequency of this gene among the 108 *S. aureus* strains isolated from 16 different hospitals in 14 provinces of China to be about 48.1%, which was the most common virulence gene among the studied genes. In the study of Pakbaz *et al.* [46], the frequency of the mentioned gene among 98 samples was obtained from Tehran University of Medical Sciences, with a total of 21 strains containing the *tsst* gene.

In most of the above reports, the expression of the tsst-1 gene in *S. aureus* strains was higher than in the present study. *MecA* is a gene discovered in bacteria that permits them to withstand medications like methicillin and other penicillin-like drugs. *MecA* is involved in the coding of the PBP2A protein (a transpeptidase that promotes bacterial cell wall construction) as well as antibiotic resistance [47]. In this study, the frequency of the *mecA* gene among samples was about 65%. The frequency of this gene in the studies of Galdiero *et al.* [48] (Italy-2003) among 43 MRSA strains was estimated to be about 46.5%.

The frequency of this gene among coagulase-negative S. *aureus* and MRSA was described in a report published by Choi *et al.* [49] and determined to be 98% and 81%, respectively. In the study of Askari *et al.* [50], the prevalence of this gene among 2690 strains was estimated to be about 52.7%. The prevalence of the *mecA* gene in the above reports was different from the results of the present study.

Conclusions

Substantial rates of drug resistance among *S. aureus* strains to routinely used medicines were found in this study. MRSA isolation from community-acquired disease was shown to be common in Iraq. In the present study, the expression of *tsst*, *sec B*, and *sec C* genes in *S. aureus* strains were not detected, unlike in previous studies. While all the strains were *sec A* gene positive, another gene found in bacterial cells that enables them to be resistant to antibiotics like methicillin and other vancomycin drugs is *mecA*. *mecA* is involved in the coding of PBP2A protein (a transpeptidase that promotes bacterial cell wall formation) and has the ability to resist some antibiotics. This study was limited to Salah Al-Din Teaching Hospital and therefore more investigations are required to evaluate virulence factors of isolated *S. aureus* strains from other antibiotic-resistant patients. Also, new efficient techniques should be applied to determine the presence of virulence genes among antibiotic-resistant strains.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Sidhu MS, Oppegaard H, Devor TP, Sørum H. Persistence of multidrug-resistant *Staphylococcus haemolyticus* in an animal veterinary teaching hospital clinic. Microb Drug Resist 2007; 13:271–280.

2 Foster TJ. Potential for vaccination against infections caused by *Staphylococcus aureus*. Vaccine 1991; 9:221–227.

3 Witte W. Antibiotic resistance in gram-positive bacteria: epidemiological aspects. J Antimicrob Chemother 1999; 44(suppl_1): 1–9.

4 Stein GE, Wells EM. The importance of tissue penetration in achieving successful antimicrobial treatment of nosocomial pneumonia and complicated skin and soft-tissue infections caused by methicillin-resistant *Staphylococcus aureus*: vancomycin and linezolid. Curr Med Res Opin 2010; 26:571–588.

5 Kadariya J, Smith TC, Thapaliya D. Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. Biomed Res Int 2014; 2014:827965.

6 Speziale P., Pietrocola G. The multivalent role of fibronectin-binding proteins A and B (FnBPA and FnBPB) of *Staphylococcus aureus* in host infections. Front Microbiol 2020; 11:2054.

7 Arvidson S, Tegmark K. Regulation of virulence determinants in *Staphylococcus aureus*. Int J Med Microbiol 2001; 291:159–170.

8 Aljelehawy Q, Karimi N, Alavi M. Comparison of antibacterial and cytotoxic activities of phytosynthesizedZnONPs by leaves extract of Daphne mucronata at different salt sources. Mater Technol 2021; 36:747–759.

9 Alavi M, Karimi N. Antibacterial, hemoglobin/albumin-interaction, and molecular docking properties of phytogenic AgNPs functionalized by three antibiotics of penicillin, amoxicillin, and tetracycline. Microb Pathog 2022; 164:105427.

10 Becker K, Skov RL, von Eiff C. Staphylococcus, Micrococcus, and other catalase-positive cocci. Manual Clin Microbiol 2015; 15:354–382.

11 Diekema DJ, Pfaller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M, SENTRY Participants Group. 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 (Supplement_2):S114–S132.

12 Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 30th (ed) CLSI supplement M100 Clinical and Laboratory Standards Institute, Wayne, PA. 2020.

13 https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria.

14 Yang L, Wang S, Cheng C, Pan C, Li F, Deng X. A simple and rapid method for extracting bacterial DNA from intestinal microflora for ERIC-PCR detection. World J Gastroenterol 2008; 14:2872–2876.

15 Wang TY, Guo L, Zhang JH. Preparation of DNA ladder based on multiplex PCR technique. J Nucleic Acids 2010; 2010:421803.

16 Mehrotra M, Wang G, Johnson WM. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol 2000; 38:1032–1035.

17 AubaisAljelehawy QH, HadiAlshaibah LH, Abbas Al-Khafaji ZK. Evaluation of virulence factors among *Staphylococcus aureus* strains isolated from patients with urinary tract infection in Al-Najaf Al-Ashraf teaching hospital. Cell Mol Biomed Rep 2021; 1:78–87.

18 D'Agata EM, Webb GF, Pressley J. Rapid emergence of co-colonization with community-acquired and hospital-acquired methicillin-resistant *Staphylococcus aureus* strains in the hospital setting. Math Model Natl Phenom 2010; 5:76–93.

19 Archer GL. *Staphylococcus aureus*: a well-armed pathogen. Rev Infect Dis 1998; 26:1179–1181.

20 Herman R.A., Kee V.R., Moores K.G, Ross MB. Etiology and treatment of community-associated methicillin-resistant Staphylococcus aureus. Am J Health Syst Pharm 2008; 65:219–225.

21 Heymann DL. Control of communicable diseases manual. Washington: American Public Health Association; 2008.

22 Cheung GY, Bae JS, Otto M. Pathogenicity and virulence of *Staphylococcus aureus*. Virulence. 2021; 12:547–569.

23 Van Cleef BA, Broens EM, Voss A, Huijsdens XW, Züchner L, Van Benthem BH, *et al.* High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in The Netherlands. Epidemiol Infect 2010; 138:756–763.

24 Finch RG. Antibacterial activity of quinupristin/dalfopristin. Drugs 1996; 51:31–37.

25 Lamb HM, Figgitt DP, Faulds D. Quinupristin/dalfopristin. Drugs 1999; 58: 1061–1097.

26 Cunha BA. Oral antibiotic treatment of MRSA infections. J Hosp Infect 2005; 60:88–90.

27 Lin L, Wang X, Wang W, Zhou X, Hargreaves JR. Cleaning up China's medical cabinet—an antibiotic take-back programme to reduce household antibiotic storage for unsupervised use in rural China: a mixed-methods feasibility study. Antibiotics 2020; 9:212.

28 Hassan D, Omolo C.A., Fasiku V.O., Elrashedy A.A., Mocktar C., Nkambule B., Soliman M.E.S., Govender T. Formulation of pH-responsive quatsomes from quaternary bicephalic surfactants and cholesterol for enhanced delivery of vancomycin against methicillin resistant *Staphylococcus aureus*. Pharmaceutics 2020; 12:1093.

29 Cunha BA. Antibiotic essentials. Boston: Jones & Bartlett Publishers; 2010.

30 Salman HA, Alhameedawi AK, Alsallameh SM, Muhamad G, Taha Z. Prevalence of multi-antibiotic resistant bacteria isolated from children with urinary tract infection from Baghdad, Iraq. Microbiol Biotechnol Lett 2022; 50:147–156.

31 Ansari S, Gautam R, Shrestha S, Ansari SR, Subedi SN, Chhetri MR. Risk factors assessment for nasal colonization of *Staphylococcus aureus* and its methicillin resistant strains among pre-clinical medical students of Nepal. BMC Res Notes 2016; 9:1–8.

32 Sanjana RK, Shah R, Chaudhary N, Singh YI. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) in CMS-teaching hospital: a preliminary report. J Coll Med Sci Nepal 2010; 6:1–6.

33 Aslam MA, Ahmed Z, Azim R. Microbiology and drug sensitivity patterns of chronic suppurative otitis media. J Coll Phy Surg Pak 2004; 14:459–461.

34 Kshetry AO, Pant ND, Bhandari R, Khatri S, Shrestha KL, Upadhaya SK, *et al.* Minimum inhibitory concentration of vancomycin to methicillin resistant *Staphylococcus aureus* isolated from different clinical samples at a tertiary care hospital in Nepal. Antimicrob Resist Infect Control 2016; 5:1–6.

35 Subedi S, Brahmadathan KN. Antimicrobial susceptibility patterns of clinical isolates of *Staphylococcus aureus* in Nepal. Clin Microbiol Infect 2005; 11:235–237.

36 Baral R, Khanal B, Acharya A. Antimicrobial susceptibility patterns of clinical isolates of *Staphylococcus aureus* in Eastern Nepal. Health Renaissance 2011; 9:78–82.

37 Baral R, Khanal B. Inducible clindamycin resistance in *Staphylococcus aureus* strains isolated from clinical samples. Int J Biomed Res 2017; 8:81–84.

38 Jha L. Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) among skin infection cases at a hospital in Chitwan. Nepal Med Coll J 2010; 12:224–228.

39 Tiwari HK, Sapkota D, Sen MR. High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. Infect Drug Resist 2008; 1:57.

40 Cho SY, Chung DR. Infection prevention strategy in hospitals in the era of community-associated methicillin-resistant *Staphylococcus aureus* in the Asia-Pacific region: a review. Clin Infect Dis 2017; 64(suppl_2):S82–S90.

41 Kamarehei F, Ghaemi EA, Dadgar T. Prevalence of enterotoxin A and B genes in *Staphylococcus aureus* isolated from clinical samples and healthy carriers in Gorgan City, North of Iran. Indian J Pathol Microbiol 2013; 56:265.

42 Koosha RZ, Hosseini HM, Aghdam EM, Tajandareh SG, Imani Fooladi AA. Distribution of tsst-1 and mecA genes in *Staphylococcus aureus* isolated from clinical specimens. Jundishapur J Microbiol 2016; 9: 29057.

43 Kreiswirth BN, Projan SJ, Schlievert PM, Novick RP. Toxic shock syndrome toxin 1 is encoded by a variable genetic element. Rev Infect Dis 1989; 11 (Supplement_1):S83–S89.

44 Nagao M, Okamoto A, Yamada K, Hasegawa T, Hasegawa Y, Ohta M. Variations in amount of TSST-1 produced by clinical methicillin resistant *Staphylococcus aureus* (MRSA) isolates and allelic variation in accessory gene regulator (agr) locus. BMC Microbiol 2009; 9:1–5.

45 Xie Y, He Y, Gehring A, Hu Y, Li Q, Tu SI, Shi X. Genotypes and toxin gene profiles of *Staphylococcus aureus* clinical isolates from China. PLoS ONE 2011; 6:e28276.

46 Pakbaz Z, Sahraian MA, Sabzi S, Mahmoodi M, Pourmand MR. Prevalence of sea, seb, sec, sed, and tsst-1 genes of *Staphylococcus aureus* in nasal carriage and their association with multiple sclerosis. Germs 2017; 7:171.

47 Thalsø-Madsen I, Torrubia FR, Xu L, Petersen A, Jensen C, Frees D. The Sle1 cell wall amidase is essential for β-lactam resistance in community-acquired methicillin-resistant *Staphylococcus aureus* USA300. Antimicrob Agents Chemother (Bethesda) 2019; 64:e01931–19.

48 Galdiero E, Liguori G, D'Isanto M, Damiano N, Sommese L. Distribution of mecA among methicillin-resistant clinical staphylococcal strains isolated at hospitals in Naples, Italy. Eur J Epidemiol 2003; 18:139–145.

49 Choi SM, Kim SH, Kim HJ, Lee DG, Choi JH, Yoo JH, *et al.* Multiplex PCR for the detection of genes encoding aminoglycoside modifying enzymes and methicillin resistance among Staphylococcus species. J Korean Med Sci 2003; 18:631–636.

50 Askari E, Soleymani F, Arianpoor A, Tabatabai SM, Amini A, NaderiNasab M. Epidemiology of mecA-methicillin resistant Staphylococcus aureus (MRSA) in Iran: a systematic review and meta-analysis. Iran J Basic Med Sci 2012; 15:1010.