



Open Access

A Survey of Methicillin-Resistance *Staphylococcus aureus* Isolated from Patients Hospitalized in the ICU, NICU and Surgical Departments of Tehran University of Medical Sciences Hospitals

Monireh Rahimkhani^{1*}, Zahra Rajabi²

¹Associated Professor, Department of Lab Medical Sciences, Faculty of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran

²PhD Microbiology, Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding Author: Monireh Rahimkhani, Associated Professor, Department of Lab Medical Sciences, Faculty of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran;
Email: rrahimkhani@sina.tums.ac.ir

Received Date: 12-10-2022; Accepted Date: 25-10-2022; Published Date: 03-11-2022

Copyright© 2022 by Rahimkhani M, et al. All rights reserved. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Abstract

Background and Aim: *Staphylococcus aureus* is an important pathogen that causes various infections. The presence of some pathogenic factors in this bacterium helps the bacteria to survive in response to the immune system and antimicrobial treatments. Efforts to treat infections caused by these strains have resulted in resistance to most antibiotics, especially methicillin and vancomycin resistance, during recent years. Therefore, the consequences of infections caused by this bacterium can be serious. In this study we aimed to survey methicillin-resistance *Staphylococcus aureus* isolated from patients hospitalized in the ICU, NICU and surgical departments of Tehran University of Medical Sciences hospitals.

Material and Method: 430 clinical samples of the patients were collected and identified as *Staphylococcus aureus* by phenotype and biotype tests. MRSA isolates were first screened by showing resistance to cefoxitin by disc diffusion method and finally confirmed by examining the *mecA* gene by PCR. The microbial resistance pattern of MRSA was also measured by disk diffusion method. Investigating the presence of a vancomycin resistance gene in isolated MRSA occurred by PCR.

Rahimkhani M | Volume 3; Issue 3 (2022) | JCIM-3(3)-057 | Research Article

Citation: Rahimkhani M, et al. A Survey of Methicillin-Resistance *Staphylococcus aureus* Isolated from Patients Hospitalized in the ICU, NICU and Surgical Departments of Tehran University of Medical Sciences Hospitals. J Clin Immunol Microbiol. 2022;3(3):1-10.

DOI: <http://dx.doi.org/10.46889/JCIM.2022.3303>

Results: Out of 430 clinical samples, 117 (27.20%) *Staphylococcus aureus* were isolated of which 42 (35.89%) were identified as MRSA. The most MRSA obtained from the ICU department with 25 cases (59.52%). The majority of patients infected with MRSA belonged to the age group of 40-65 years. The *mecA* gene was detected in all MRSA. The entire MRSA was 100% sensitive to vancomycin and linezolid, and 100% resistance to cloxacillin. Molecular detection of *vanA* gene in isolated MRSA showed 2 (4.76%) of them contained *vanA* gene.

Conclusion: The high prevalence of MRSA and its antibiotic resistance pattern to vancomycin and other antibiotic families can lead to treatment failures, uncontrolled nosocomial infections, and mortality. Although the PCR-based detection method is superior to other conventional techniques, a combination of these methods can provide diagnostic accuracy.

Keywords

Staphylococcus aureus; MRSA; VRSA; *mecA*; *vanA*; Nosocomial Infection

Introduction

Staphylococcus aureus (*S. aureus*) is a round, gram-positive, facultative anaerobic bacterium that is part of the natural micro flora of humans. This bacterium is one of the major causes of hospital- and community-acquired infections, opportunistic pathogens that can cause skin and mucosal infections, endocarditis, osteomyelitis and sepsis [1].

Antimicrobial therapy plays an important role in infection control caused by *S. aureus* but has become less effective due to widespread drug resistance. Antibiotic treatment is a major key for treatment of infections caused by *S. aureus* [2]. However, the abuse of antibiotics has led to the resistance of *S. aureus* to antibiotics in recent years, and the issue of multidrug resistance has become increasingly prominent. There is a relationship between bacterial resistance and drug resistance genes. The *mecA* gene is considered one of the major resistance genes that confer resistance to β -lactams [3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are currently a remarkable health problem. Due to the widespread outbreak of MRSA, the treatment of MRSA was changed to vancomycin. Since the 1980s, vancomycin has become the choice drug for the treatment of serious MRSA infections in many healthcare institutions [4]. Although vancomycin has been the most reliable therapeutic agent against infections caused by MRSA, there is an alarming emergence of *S. aureus* strains with reduced susceptibility to vancomycin and other glycopeptides [5].

S. aureus strains are definitive causes of nosocomial infections. High pathogenicity of *S. aureus* and especially MRSA strains and the general weakness of hospitalized patients make it necessary to assess their antimicrobial resistance in clinical infections [6]. Thus, the present

Rahimkhani M | Volume 3; Issue 3 (2022) | JCIM-3(3)-057 | Research Article

Citation: Rahimkhani M, et al. A Survey of Methicillin-Resistance *Staphylococcus aureus* Isolated from Patients Hospitalized in the ICU, NICU and Surgical Departments of Tehran University of Medical Sciences Hospitals. J Clin Immunol Microbiol. 2022;3(3):1-10.

DOI: <http://dx.doi.org/10.46889/JCIM.2022.3303>

study was done to survey the prevalence rate and distribution of antibiotic resistance among the MRSA strains isolated from patients hospitalized in the ICU and surgical departments.

Materials and Methods

Sampling: During 9 month, 430 clinical samples were collected from hospitalized patients in the ICU, NICU and surgical department of Tehran University of medical sciences hospitals. These samples referred to the laboratory for detection of *S. aureus*.

Identification Tests: Confirmatory tests of *S. aureus* including gram staining, catalase test, coagulase test, mannitol fermentation and DNase were performed.

Antibiotic Resistance Tests: Screening of MRSA was performed by cefoxitin disc diffusion method and then the antimicrobial resistant pattern of the MRSA was carried out by the disk diffusion agar method based on the CLSI 2021 guide line for antimicrobial tests. The used antibiotics consist of: amoxicillin, trimethoprim-sulfamethoxazole, cefazolin, ceftriaxone, ciprofloxacin, erythromycin, imipenem, cefotaxime, clindamycin, cloxacillin, vancomycin and linezolid. Also, for vancomycin, E- test was done [7].

Detection of mecA and vanA Genes in the Isolated MRSA: PCR was used to detect the mecA gene to confirm MRSA and detect the presence of a vanA gene in confirmed MRSA. DNA extraction of isolated MRSA was followed by the Pars Tous kit (code A101161) protocol. Then the appropriated concentration of extracted DNA was measured by a Nano-drop device (Thermo). Primers sequence of mecA and vanA genes are displayed in Table 1. Thermo cycler (peQlab) was used for PCR amplification. The PCR products were electrophoresed on a 1% agarose gel.

Primers	Sequence (5' to 3')	Amplicon Size (bp)	Ref
mecA-F	AAAATCGATGGTAAAGGTTGGC	533	(8)
mecA-R	AGTTCTGCAGTACCGGATTTGC		
vanA-F	GGCAAGTCAGGTGAAGATG	713	(9)
vanA-R	ATCAAGCGGTCAATCAGTTC		

Table 1: Primers sequence of mecA and vanA genes.

Results

During 9 months, out of 430 clinical samples, 117 (27.20%) samples were positive for *S. aureus*, whereas 42 (35.89%) of them confirmed as MRSA. Patients colonized with MRSA included 24(57.14%) men and 18 (42.86%) females by mean age 45.1 years old. No significant difference was observed between gender and MRSA infection ($P=0.05$). Clinical samples which contain MRSA included: 12 (28.58%) blood, 10 (23.81%) tracheal exudate, 8 (19.04%) wound, 4 (9.52%) CSF, 3(7.14%) urine and 3 (7.14%) abscess, 2 (4.77%) pleura. The hospital department where MRSA strains were isolated from hospitalized patients included 14 (33.33%) samples from the surgical clinic, 25 (59.52%) samples from the ICU and 3 (7.15%) samples from the NICU departments. Out of 117 isolates of *S.aureus* 42 (35.89%) were resistant to cefoxitin and considered as MRSA. The resistant or sensitivity pattern of MRSA is shown in Table 2. The entire MRSA system was resistant to cloxacillin and sensitive to vancomycin and linezolid. After that, the most resistance was shown to both erythromycin and ceftriaxone. 41 (97.62).

Antibiotic	Resistant	Sensitive
	Number (%)	
Amoxicillin	23 (54.76)	19 (45.24)
Trimethoprim- sulfamethoxazole	38(90.48)	4 (9.52)
Cefazolin	38(90.48)	4 (9.52)
Ceftriaxone	41 (97.62)	1(2.38)
Ciprofloxacin	39 (92.86)	3(7.14)
Erythromycin	41 (97.62)	1(2.38)
Imipenem	23(54.76)	19(45.24)
Cefotaxime	36(85.71)	6(14.29)
Clindamycin	40(95.24)	2(4.76)
Cefoxitin	42(100)	0(0)
Cloxacillin	42(100)	0(0)
Vancomycin	0(0)	42(100)
Linezolid	0(0)	42(100)

Table 2: The result of antibiotic resistance and sensitivity pattern of isolated MRSA.

The results of the E-test for Vancomycin showed that one of the samples Minimum Inhibitory Concentration (MIC) for *S. aureus* (MIC= 16) was in the vancomycin resistance range, whereas in the disc diffusion method, all of them were sensitive to vancomycin.

The molecular results of the *mecA* gene showed that all the isolates that were resistant to the antibiotic cefoxitin by the agar disk diffusion method also contained the *mecA* gene in the PCR test. Two (4.76%) of the MRSA studied had vancomycin resistance genes. Electrophoresis results of the *mecA* and *vanA* genes are shown in Fig. 1.

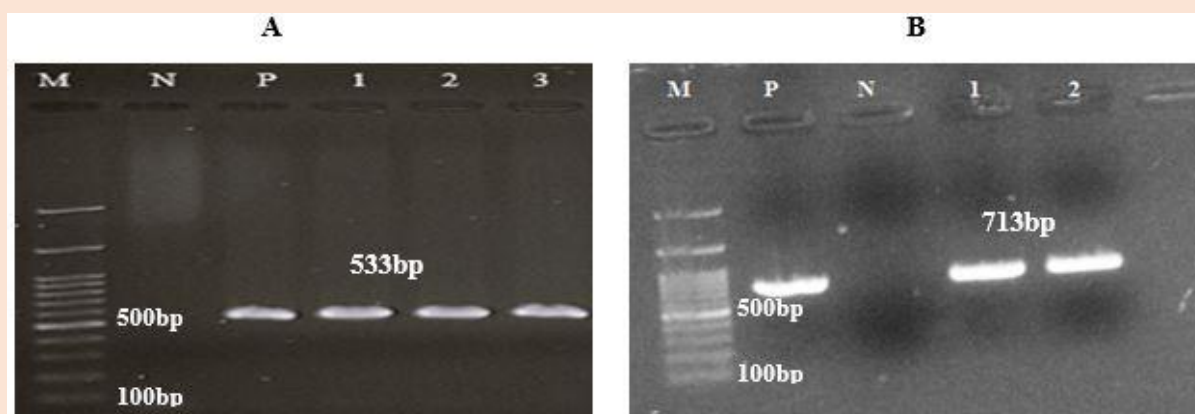


Figure 1: Gel electrophoresis of PCR products of genes from isolated MRSA: a, *mecA* (533 bp); b, *vanA* (733 bp). M, Marker (100 bp); P, positive control *S. aureus* ATCC 25923 for *mecA* and *Enterococcus faecalis* ATCC 29212 for *vanA*; N, negative control (the tube containing distilled water, gene primers, and Master Mix). The numbers indicate the samples expressing the target genes.

SPSS (IBM SPSS Statistics 24) software was used to check the significant relationship between the obtained results and the relationship with MRSA.

Discussion

MRSA is one of the major causes of hospital-acquired infections, especially in developing countries. Nowadays, MRSA has increasingly become a global problem in health care facilities [10]. *S. aureus* is considered as an opportunistic pathogen responsible for high mortality in humans [11]. The pathogenesis of this bacterium is further accentuated by the prevalence of antibiotic resistance among *S. aureus* isolates, often necessitating the use of more sophisticated treatment options [12].

The present study carried out on methicillin- resistance *Staphylococcus aureus* isolated from patients hospitalized in the ICU, NICU and surgical departments of Tehran University of Medical Sciences hospitals. During 6 months, out of 430 clinical samples, 117 (27.20%) samples were positive for *S. aureus* which 42 (89.35%) samples confirmed as MRSA. For primary confirmation of MRSA, we used cefoxitin disk by disk diffusion agar method. 24 (57.14%) MRSA isolated from men and 18 (42.86%) from women. No significant relationship between gender and MRSA infection was seen ($P=0.05$). The mean average age of hospitalized patients was 45.1 years old, the youngest patient was 4 months old in NICU and the oldest patient was 73 years old in ICU. Blood with 12 (28.58%) cases and pleura with 2 (4.77%) cases were the most and least samples obtained from the studied departments, respectively. There was no significant relationship between samples and MRSA ($P=0.01$).

Besharati, et al., Sasan, et al., Garoy, et al., and many similar studies, have shown different frequency of *S.aureus* in clinical samples [13-15]. In some studies, no significant relationship has been observed between gender and the rate of MRSA infection but in some other studies, they have stated that this infection is more common in young people, and in some other studies, they have found this to be the case in the elderly [16,17]. Although it is possible in different regions, the percentage report of isolated MRSA is slightly different, which is due to the regional, cultural, health and type of referrals hospital differences. But all the results indicate the high prevalence of MRSA among clinical samples.

In this study, ICU included the highest number of isolates with 25(59.52%) samples containing MRSA. In a study by Razin, et al., the distribution of MRSA positive cultures was the highest in the ICU department with 60 cases (42%) and the wounds with 28% were the most abundant samples [18]. Sabouni, et al., reported the most isolation of MRSA from pediatric intensive care units [19]. A study in Congo showed the prevalence of 63.5% of MRSA among surgical patients hospitalized and Rodrigues, et al., in 2015 reported the medical clinic and adult ICU, had the highest MRSA isolate rates [20,21].

Considering that the ICU department needs the highest hygiene conditions in terms of the specific conditions of the patients, considering the high frequency of MRSA in the ICU, the necessity of consideration and accuracy and correct observance of sterilization and hygiene principles by the personnel seems necessary and important. MRSA causes various infections, including urinary tract infections, in hospitalized patients, especially in ICU department [22,23].

The *mecA* gene is highly conserved among methicillin-resistant *S. aureus* and is contained in a Staphylococcal mobile chromosomal cassette (SCCmec). The *mecC* gene has been detected in MRSA and other staphylococcus species in several countries [24]. MRSA strains are currently a very significant health problem. Due to the widespread outbreak of MRSA, the treatment for MRSA was changed to vancomycin.

The high prevalence of *S. aureus* strains and, as a result, the use of vancomycin as an antimicrobial drug in many hospitals, the lack of control over the use of microbes and the inappropriate conditions of public health institutions, has been led to the emergence of medium sensitivity (VISA) or Vancomycin Resistant Strains [VRSA] [25]. In our study, all the strains that were resistant to cefoxitin disc contained the *mecA* gene by PCR and the results of the disc diffusion test were the same as the molecular results. In the case of the vancomycin gene, all MRSA were sensitive to vancomycin by disc diffusion method, the E-test strip in one of the samples showed suspicious conditions of resistance to vancomycin. But molecular detection of the vancomycin gene showed two samples (4.76%) containing the vancomycin resistance gene.

Bokaian, et al., Floum, et al., used cefoxitin disk for initial confirmation of MRSA and Rocchetti, et al., investigated the *mecA* gene in MRSA and showed that most of the isolates that are resistant to disk also have the *mecA* gene in the molecular method [26-28]. These studies reported the frequency of MRSA from 40% to 78/7%. In 2016, out of 1360 samples examined in Ethiopia, 34 (17.5%) were MRSA, and 10 (5.1%) showed vancomycin resistance [29]. In a case report study in 2003, a VRSA with MIC >32 µg per milliliter was identified [30]. Although the regional, economic and health conditions of the studied community can play a role in the difference in frequencies, the results indicate the abundance of MRSA strains and the presence of vancomycin-resistant strains, is a bad prognosis for the development of multidrug-resistant strains.

The E-test result for vancomycin showed the disk diffusion is not reliable for studying the sensitivity or resistance to vancomycin and does not differentiate VRSA and VSSA. So it seems that it is more reliable to perform the MIC test and then check the resistance gene with a molecular method. Although the molecular method is better and more accurate. Because there may be human error in the disc diffusion method or even MIC when doing lawn culture.

In the present study, the antimicrobial resistance pattern of MRSA isolates was investigated by an agar disk diffusion method based on the list of antibiotics available in CLSI 2021. Our results showed the highest sensitivity of MRSA to vancomycin and linezolid antibiotics (100%) and the highest resistance to cloxacillin antibiotics 100% and then to erythromycin and ceftriaxone, 97.62%. In similar studies, Kosha reported the highest sensitivity of MRSA to vancomycin by disk diffusion method and in Kavosi's study, all MRSA obtained were sensitive to vancomycin and had the highest resistance seen to erythromycin with 84.4% [31,32]. Gun in 2021 reported the highest resistance to macrolides like erythromycin (87.29 %) [33]. In Nepal in 2021, all MRSA were resistant to penicillin, followed by erythromycin (94.9%) and none of MRSA was resistant to vancomycin [34]. The high rate of MRSA isolation and resistance to penicillin, ampicillin, erythromycin, etc. indicate that these antibacterial agents are ineffective against MRSA. Vancomycin seems to be an antimicrobial agent that has shown high sensitivity against *S. aureus* in some studies. Hence, vancomycin may be used as the choice drug for the treatment of MDR-MRSA infections.

However, vancomycin susceptibility monitoring and routine testing should be performed, both by determining the MIC and checking for the vancomycin resistance gene in isolates. Regular monitoring of nosocomial infections, especially antibiogram monitoring of MRSA and MSSA, and formulating the policy of using the appropriate antibiotic for treatment, may be useful in reducing the incidence of MRSA infection.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

RM designed the study, performed the experiments, interpreted the data and revised the manuscript. ZR performed the experiments, analyzed the data and drafted the manuscript.

Ethics Approval and Consent to Participate

This study was approved by local conventional manner and by the ethical committee of Tehran University of Medical Sciences by number: IR.TUMS.SPH.REC.1400.187.

Conflict of Interest

The authors declare that they have no competing interests.

References

1. Xinyi W, Huilin Zh, Bingjie W, Ying Zh, Yanlei X, Lulin R, et al. Identification of methicillin-resistant *Staphylococcus aureus* ST8 isolates in China with potential high virulence. *Emerg Microbes Infect.* 2022;11(1):507-18.
2. Zhang Z, Chen Y, Li XP, Wang XR, Li HS. Detection of antibiotic resistance, virulence gene, and drug resistance gene of *Staphylococcus aureus* isolates from bovine Mastitis. *Microb Spect.* 2022.
3. Abdelazeem MA, Helal H, Amr E, Dalal HH, Wael H, Gaber Band, 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;13:3255-65.

Rahimkhani M | Volume 3; Issue 3 (2022) | JCIM-3(3)-057 | Research Article

Citation: Rahimkhani M, et al. A Survey of Methicillin-Resistance *Staphylococcus aureus* Isolated from Patients Hospitalized in the ICU, NICU and Surgical Departments of Tehran University of Medical Sciences Hospitals. *J Clin Immunol Microbiol.* 2022;3(3):1-10.

DOI: <http://dx.doi.org/10.46889/JCIM.2022.3303>

4. Nezhad RR, Meybodi SM, Rezaee R, Goudarzi M, Fazeli M. Molecular characterization and resistance profile of methicillin resistant *Staphylococcus aureus* strains isolated from hospitalized patients in intensive care unit, Tehran-Iran. J J M. 2017;10(3):e41666.
5. Yanguang C, Sijin Y, Xiancai R. Vancomycin resistant *Staphylococcus aureus* infections: A review of case updating and clinical features. J Adv Res. 2020;21(2020):169-76.
6. Turner N, Sharma B, Stacey M A, Eichenberger E, Shah P, a Carugati M, et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. Nature Rev Microbiol. 2019;17:203-18.
7. Performance standards for antimicrobial susceptibility testing, clinical and laboratory standards institute antimicrobial susceptibility testing standards M02, M07 and M11, 31Ed, Pennsylvania 19087 USA. 2021.
8. Bühlmann M, Bögli-Stuber K, Droz S, Mühlemann K. Rapid screening for carriage of methicillin-resistant *Staphylococcus aureus* by PCR and associated costs. J Clin Microbiol. 2008;46(7):2151-4.
9. Azimian A, Havaei SA, Fazeli H, Naderi M, Ghazvini K, Samiee SM and et al. Genetic characterization of a vancomycin-resistant *Staphylococcus aureus* isolate from the respiratory tract of a patient in a university hospital in northeastern Iran. J Clin Microbiol. 2012;50(11):3581-5.
10. Darboe S, Dobreniecki S, Jarju S, Jallow M, Mohammed NI, Wathuo M. Prevalence of Panton-Valentine Leukocidin (PVL) and antimicrobial resistance in community-acquired clinical *Staphylococcus aureus* in an urban Gambian hospital: A 11-year period retrospective pilot study. Front Cell Infect Microbiol. 2019;9(170):1-7.
11. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nature Rev Microb. 2009;7(9):629-41.
12. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA. 2007;298(15):1763-7.
13. Besharati R, Ghafouri M, Safamanesh S, Khosrojerdi M, Ghazvini K, Nojumi S, et al. Molecular epidemiology of panton-valentine leukocidin harboring hospital-associated methicillin-resistant *Staphylococcus aureus* in septicemic children, Northeastern Iran, Bojnurd. JJM. 2019;12(2):1-6.
14. Sasan M, Donyadide N, Askari E, Naderi-Nasab M. Invasive community-acquired *Staphylococcus aureus* among pediatric population of Eastern Iran. Iran J Microbiol. 2014;6(2):84-6.
15. Garoy E, Yacob B, Gebreab YB, Achila OO, Tekeste D, Kesete R, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA): prevalence and antimicrobial sensitivity pattern among patients-A multicenter study in Asmara, Eritrea. Canadian J Inf Dis Med Microbiol. 2019.
16. Park DC, Lee SK, Cha CI, Lee SO, Lee MS, Yeo SG. Antimicrobial resistance of *Staphylococcus* from otorrhea in chronic suppurative otitis media and comparison with results of all isolated Staphylococci. Eur J Clin Microbiol Infect Dis. 2008;27(7):571-7.
17. Gopal Rao G, Michalczyk P, Nayeem N, Walker G, Wigmore L. Prevalence and risk factors for methicillin resistant *Staphylococcus aureus* in adult emergency admissions a case for screening all patients? J Hosp Infect. 2007; 66(1):15-21.
18. Razin B, Shabani M, Nabavi M, Taqvi N, Haghighi M, Farumand M. Determining the frequency of methicillin-resistant *Staphylococcus aureus* infection in Imam Hossein Hospital during 2016-2018). Res J SBUM. 2018;14(5):263-7.
19. Sabouni FM, Bahador S, Pourakbari A, Sadeghi B, Hosseinpour, An R, Taghi Haghi M, et al. Virulence factors of *Staphylococcus aureus* isolates in an Iranian referral children's hospital. Osong. 2014;5(2):96-100.
20. Iyamba JM, Wambale JM, Lukukula CM, Balega T. High prevalence of methicillin resistant staphylococci strains isolated from surgical site infections in Kinshasa. Pan. Afr Med J. 2014;18(1):322-32.
21. Rodrigues M, Aniélén L, Camille D, Silvana G, Santos O, Hörner R. Prevalence of methicillin-resistant *Staphylococcus aureus* in a University Hospital in the South of Brazil. Braz J Pharm Sci. 2015;51(1),35-42.
22. Rahimkhani M, Mordadi A, Varmazyar S, Tavakoli A. Evaluation of urinary Interleukin-8 levels in patients with spinal cord injury. Recent Patents on Anti-Infective Drug Discovery. 2014;9(2):144-9.
23. Rahimkhani M, Saberian M, Mordadi A, Varmazyar S, Tavakoli A. Urinary tract infection with *Candida glabrata* in a patient with spinal cord injury. Acta Medica Iranica. 2015:516-7.

24. García-Álvarez L, Holden MT, Lindsay H. Methicillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infect Dis*. 2011;11(8):595-603.
25. Breves A, Miranda CA, Flores C, Filippis ID, Clementino MM. Methicillin- and vancomycin-resistant *Staphylococcus aureus* in health care workers and medical devices. *J Bras Patol Med Lab*. 2015;51(3):143-52.
26. Bokaian M, Shahraki Z S, Shahrakipour M, Tahmasabi H, Adabi J. A study of the antibiotic resistance pattern of MRSA strains isolated from patients referred to Ali-Ibn abi talib Zahedan hospital in 2014. *Med J TAUMS* 2016;39(6):12-20.
27. Foloum AK, Founo LL, Karang S, Maled Y, Tsayem C, Keute M, et al . Methicillin resistant staphylococci isolated in clinical samples: a 3-year retrospective study analysis. *Future Sci*. 2021;7(4):1-10.
28. Rocchettia TT, Martinsa B K, Faccioli PY, Oliverac NA, Mondellid NA , Castelo NMC, et al. Detection of the mecA gene and identification of *Staphylococcus* directly from blood culture bottles by multiplex polymerase chain reaction. *Braz J Inf Dis*. 2018;22(2):99-105.
29. Dilnessa T, Bitew A. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. *BMC Inf Dis*. 2016;16(398):1-11.
30. Soju Ch, Dawn S, Jeffrey H, Matthew B, Fred T, Frances PD, et al. Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. *N Engl J Med*. 2003;348:1342-7.
31. Koosha RZ, Fooladi AA, Hosseini HM, Aghdam EM. Prevalence of exfoliative toxin A and B genes in *Staphylococcus aureus* isolated from clinical specimens. *J Infection and Public Health*. 2014;7(3):177-85.
32. Kavousi M, Nehmati F, Mehdiyon SM. Investigating the prevalence of antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) and determining the aminoglycoside ac(6')-Ie/aph(2'') resistance gene isolated from patients hospitalized in Tehran's Imam Hossein, Luqman hakim and Pars hospitals by PCR method . *Sci J ILUMS*. 2010;27(1):1-10.
33. Ting G, Gang S, Hualin F, Qigui Y, Wei Z, Huaqiao T, et al. Antimicrobial resistance and genotyping of *Staphylococcus aureus* obtained from food animals in Sichuan Province, China. *BMC VET Res*. 2021;17(177):1-10.
34. Dhungel S, Rijal KR, Yadav B, Dhungel B, Adhikari N, Shrestha UT, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence, antimicrobial susceptibility pattern, and detection of mec a gene among cardiac patients from a tertiary care heart center in Kathmandu, Nepal. *Infect Dis (Auckl)*. 2021;14:1-10.