ORIGINAL

Distribution of the antibiotic resistance genes amongst methicillin-resistant *Staphylococcus aureus* bacteria isolated from human clinical infections

Distribución de los genes de resistencia a los antibióticos en las bacterias Staphylococcus aureus resistentes a la meticilina aisladas de infecciones clínicas humanas

Atiyeh Ziyaei Chamgordami[1] ⓘ, Hassan Momtaz[1] ⓘ, Noosha Zia-Jahromi[2] ⓘ

1. Department of Microbiology   2. Department of Biology
Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Corresponding author
Prof. Hassan Momtaz (Ph.D)
Full Professor of the Department of Microbiology, Shahrekord Branch,
Islamic Azad University, Shahrekord, Iran
E-mail: hamomtaz@yahoo.com

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are important causes of human clinical infections in the hospital environment. The present survey was performed to assess the distribution of antibiotic resistance genes amongst the MRSA strains isolated from human clinical infections.

Methods: Thirty-five MRSA strains were isolated from human clinical infection samples. All isolates were confirmed using culture, biochemical tests and cefoxitin (30 μg) and oxacillin (1 μg) susceptibility testing. DNA was extracted from isolates and subjected to PCR for detection of antibiotic resistance genes.

Results: Prevalence of MRSA in isolates collected from sputum, urine, pus, and blood samples was 23.68%, 26.31%, 31.57%, and 10.52%, respectively. MRSA bacteria harbored the highest distribution of *blaZ* (100%), *msrA* (68.42%), and *tetK* (57.89%). Distribution of *ermB* (31.57%) and *aacA-D* (42.10%) antibiotic resistance genes were lower than others. Statistically significant differences were obtained between the distribution of *emrA* and *ermB* (*P* value <0.05), and *msrA* and *msrB* (*P* value <0.05). Distribution of ermA and msrB antibiotic resistance genes were 55.26% and 39.47%, respectively.

Conclusion: Regarding the high prevalence of MRSA and antibiotic resistance encoding genes among isolates in the hospitals, special health-based measures should be taken to reduce the use of antibiotics and control infections in the hospital.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA), antibiotic resistance genes, hospital infections, distribution.

Resumen

Antecedentes: Las cepas de *Staphylococcus aureus* resistentes a la meticilina (SARM) son causa importante de infecciones clínicas humanas en el entorno hospitalario. El presente estudio se realizó para evaluar la distribución de los genes de resistencia a los antibióticos entre las cepas de SARM aisladas de infecciones clínicas humanas.

Métodos: Se aislaron 35 cepas de SARM de muestras de infecciones clínicas humanas. Todos los aislados se confirmaron mediante cultivo, pruebas bioquímicas y pruebas de susceptibilidad a la cefoxitina (30 μg) y la oxacilina (1 μg). Se extrajo el ADN de los aislados y se sometió a la PCR para detectar los genes de resistencia a los antibióticos.

Resultados: La prevalencia de SARM en los aislados recogidos de muestras de esputo, orina, pus y sangre fue del 23,68%, 26,31%, 31,57% y 10,52%, respectivamente. Las bacterias MRSA albergaban la mayor distribución de *blaZ* (100%), msrA (68,42%) y *tetK* (57,89%). La distribución de los genes de resistencia a los antibióticos *ermB* (31,57%) y *aacA-D* (42,10%) fue menor que la de los demás. Se obtuvieron diferencias estadísticamente significativas entre la distribución de *emrA* y *ermB* (valor *P* <0,05), y *msrA* y *msrB* (valor *P* <0,05). La distribución de los genes de resistencia a los antibióticos *ermA* y *msrB* fue del 55,26% y del 39,47%, respectivamente.

Conclusiones: En relación con la alta prevalencia de SARM y de genes codificadores de resistencia a los antibióticos entre los aislados en los hospitales, deben tomarse medidas especiales de carácter sanitario para reducir el uso de antibióticos y controlar las infecciones en el hospital.

Palabras clave: *Staphylococcus aureus* resistente a la meticilina (SARM), Genes de resistencia a los antibióticos, Infecciones hospitalarias.

Introduction

Despite all advances created in the medical sciences, infectious diseases become an important life-threating issue among people of developed and developing countries[1-8]. Among them, the methicillin-resistant Staphylococcus aureus (MRSA) accounted for severe cases of infections in the hospitals and healthcare units[9]. MRSA strains are Gram-positive, catalase positive, and cocci-shaped bacteria originate from both hospital and the community[10]. They are mostly responsible for plain nosocomial and community-acquired infections, particularly Respiratory Tract Infections (RTIs), soft tissue infections, Urinary Tract Infection (UTIs), wound and burn infections, endocarditis, blood infections, and etc[11].

MRSA strains are mainly resist toward all types of penicillins and cephalosporins, simultaneously[12]. Treatment of infections caused by these strains are mostly difficult, because they are resist toward some other types of antimicrobial agents[13]. MRSA bacteria are the chief causes of around 100,000 morbidity with 20% mortality yearly in the United States[14]. MRSA bacteria harbored significant resistance toward penicillins, cephalosporins, tetracyclines, aminoglycosides, phenicols, fluoroquinolones, lincosamides, macrolides, and glycopeptides[15].

According to the high important of antibiotic resistant-MRSA strains, it is essential to signify the main source for the occurrence of resistance among MRSA strains. Some researches showed the role of antibiotic resistance genes for the occurrence of antibiotic resistance among bacterial strains[16]. In this regard, tet (*K* and *M*), *aacA-D*, *blaZ, erm* (*A, B,* and *C*), and *msr* (*A* and *B*) are the genes that responsible for the occurrence of resistance against tetracyclines, aminoglycosides, penicillins, macrolides, and macrolides antimicrobial agents, respectively[17].

MRSA strains have been tested in hospital infections to assess their role as a definitive cause of nosocomial infections. High pathogenicity of MRSA strains and general weakness of hospitalized patients make it necessary to assess their antimicrobial resistance in human clinical infections. Thus, the present survey was done to assess the prevalence rate and distribution of antibiotic resistance genes amongst the MRSA strains isolated from human clinical infections.

Materials and methods

Bacterial strains and confirmation
Thirty-five MRSA strains were isolated from different human clinical specimens. Strains were isolated from Al-Zahra and Shariati Hospitals, Isfahan, Iran. For confirmation, all isolates were cultured in blood agar (BA, Merck, Darmstadt, Germany) and incubated aerobically

at 37°C for 48 h. Additionally, colonies were subjected to morphological analysis including Gram-staining, microscopical morphology, catalase and coagulase production tests. Furthermore, pigment production, glucose O/F test, resistance to bacitracin (0.04 U) and novobiocin, oxidase test, mannitol fermentation on Mannitol Salt Agar (MSA, Merck, Darmstadt, Germany), nitrate reduction, phosphatase, urease activity, deoxyribonuclease (DNase) test and carbohydrate (sucrose, xylose, maltose, trehalose, mannose, lactose, and fructose) fermentation tests were applied to confirm isolates (18). For MRSA identification, cefoxitin (30 μg) and oxacillin (1 μg) susceptibility testing was performed[18].

DNA extraction and quality assessment
MRSA isolates were sub-cultured on TSB media and further incubated for 48 h at 37ºC. Genomic DNA was extracted from bacterial colonies using the DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany) according to manufacturer's instruction. Purity (A260/A280) and concentration of extracted DNA were then checked (NanoDrop, Thermo Scientific, Waltham, MA, USA). The quality of the DNA was assessed on a 2% agarose gel stained with ethidium bromide (0.5 μg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany)[19-25].

Detection of antibiotic resistance genes
Table I represents the list of primers and PCR conditions used for amplification of antibiotic resistance genes in the MRSA strains. A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. Ten microliters of PCR product were exposed to electrophoresis in a 2% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with SYBR Green. The UVI doc gel documentation systems (Grade GB004, Jencons PLC, London, UK) was applied for analysis of images[26].

Data analysis
Statistical analysis was done using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between data obtained from the present study. P value <0.05 was considered as statistical significant level[27,28].

Results

Among all 38 isolates, sputum, urine, pus, and blood samples were harbored 9 (23.68%), 10 (26.31%), 12 (31.57%), and 4 (10.52%) MRSA isolates, respectively.

Table II shows the distribution of antibiotic resistance genes amongst the MRSA strains isolated from human clinical infections. According to obtained data, MRSA bacteria harbored the highest distribution of *blaZ* (100%), *msrA* (68.42%), and *tetK* (57.89%). Distribution of *ermB*

(31.57%) and *aacA-D* (42.10%) antibiotic resistance genes were lower than others. Statistically significant differences were obtained between the distribution of *emrA* and *ermB* (P value <0.05), and *msrA* and *msrB* (P value <0.05). Distribution of *ermA* and *msrB* antibiotic resistance genes were 55.26% and 39.47%, respectively.

Discussion

S. aureus is considered as an important bacterium responsible for hospital infections and food poisoning[29-32]. The most important issue regarding infections caused by MRSA strains is the occurrence of antibiotic resistance in bacterial strains. High resistance rate of the MRSA strains harden the procedure of treatment and faced patients with high economic burden. Thus, it is essential to found all epidemiological aspects of MRSA infections in the hospital environment.

One of the most important epidemiological aspects of the MRSA strains is presence of antibiotic resistance genes amongst the bacteria. The present survey showed that human clinical infections harbored the high distribution of MRSA strains harbored antibiotic resistance genes. Widespread and unauthorized administration of antimicrobials and excessive using of disinfectant solutions in hospital environment have been considered to be a major factor in the emergence of antibiotic resistance amongst MRSA strains.

High prevalence of resistance of MRSA bacteria isolated from diverse kinds of clinical infectious

Table I: Target genes, oligonucleotide primers and PCR conditions used for detection of antibiotic resistance genes amongst MRSA strains.

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR volume (50µL)
AacA-D	F: TAATCCAAGAGCAATAAGGGC R: GCCACACTATCATAACCACTA	227	1 cycle: 94 ^{0C} ----------- 5 min.	5 µL PCR buffer 10X
ermA	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190	25 cycles: 94 ^{0C} ----------- 60 s	1.5 mM Mgcl$_2$
tetK	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	360	55 ^{0C} ----------- 70 s 72 ^{0C} ----------- 60 s	200 µM dNTP 0.5 µM of each primers F & R
ermB	F: CCGTTTACGAAATTGGAACAGGTAAAGGGC R: GAATCGAGACTTGAGTGTGC	359	1 cycle: 72 ^{0C} ----------- 10 min	1.25 U Taq DNA polymerase 2.5 µL DNA template
msrA	F: GGCACAATAAGAGTGTTTAAAGG R: AAGTTATATCATGAATAGATTGTCCTGTT	940	1 cycle: 94 ^{0C} ----------- 6 min.	5 µL PCR buffer 10X
msrB	F: TATGATATCCATAATAATTATCCAATC R: AAGTTATATCATGAATAGATTGTCCTGTT	595	34 cycles: 95 ^{0C} ----------- 60 s 50 ^{0C} ----------- 70 s 72 ^{0C} ----------- 70 s 1 cycle: 72 ^{0C} ----------- 8 min	2 mM Mgcl$_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
blaZ	F: TGAACCGTATGTTAGTGC R: GTCGTGTTAGCGTTGATA	681	1 cycle: 94 ^{0C} ----------- 6 min. 30 cycles: 95 ^{0C} ----------- 60 s 59 ^{0C} ----------- 60 s 72 ^{0C} ----------- 60 s 1 cycle: 72 ^{0C} ----------- 10 min	5 µL PCR buffer 10X 2 mM Mgcl$_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template

Table II: Distribution of antibiotic resistance genes amongst the MRSA strains isolated from human clinical infections.

Clinical specimens) (N. MRSA	N. isolates harbored each gene (%)						
	aacA-D	*tetK*	*balZ*	*ermA*	*ermB*	*msrA*	*msrB*
Sputum (9)	4 (44.44)	5 (55.55)	9 (100)	5 (55.55)	2 (22.22)	6 (66.66)	3 (33.33)
Urine (10)	5 (50)	6 (60)	10 (100)	6 (60)	4 (40)	7 (70)	4 (40)
Pus (12)	6 (50)	9 (75)	12 (100)	8 (66.66)	5 (41.66)	10 (83.33)	6 (50)
Blood (4)	1 (25)	2 (50)	4 (100)	2 (50)	1 (25)	3 (75)	2 (50)
Total (38)	16 (42.10)	22 (57.89)	38 (100)	21 (55.26)	12 (31.57)	26 (68.42)	15 (39.47)

specimens have been described toward penicillins (33-38), cephalosporins[33-35,39], tetracyclines[33-35,40], macrolides[33-35,41], and aminoglycosides[33-35,42]. Abdolmaleki et al. (2019)[43] reported that *BlaZ, aacA-D, tetK, msrA, dfrA, ermA, gyrA, grlA* and *rpoB* were the most commonly detected antibiotic resistance genes amongst the MRSA strains with a distribution rate between 11 to 100%. Rahi et al. (2020)[44] reported that the most frequently distinguished antibiotic resistance markers were *blaZ* (100%), *tetK* (85.71%), *dfrA1* (71.42%), *aacA-D* (67.85%), *ermA* (50%) and *gyrA* (42.85%). Akanbi et al. (2017)[45] reported that *blaZ, mecA, rpoB, ermB* and *tetM* were the most generally identified antibiotic resistance genes amongst the *S. aureus* bacteria recovered from food samples in South Africa which was relatively similar to our findings. In this regard, Momtaz and Hafezi (2014)[46] reported that the distribution of *mecA, tetK, ermA, ermC, tetM, aacA-D, linA, msrA, vatA, vatC* and *vatB* antibiotic resistance genes in the *S. aureus* strains isolated from human clinical infections were 80.30%, 34.84%, 30.30%, 25.75%, 24.24%, 19.69%, 7.57%, 7.57%, 6.06%, 3.03% and 1.51%, respectively.

Conclusion

Sum it up, we recognized boost incidence of MRSA bacteria in human clinical infections on top of boost incidence of the genes encode resistance toward antibiotic agents. High prevalence of MRSA bacteria and high distribution of *blaZ, tetK, aacA-D ermA* and *msrA* antibiotic resistant genes may pose a possible menace regarding the MRSA human infections in hospitals.

Interests conflict
The authors declare no conflict of interest.

References

1. Ranjbar R, Mahmoodzadeh Hosseini H, Safarpoor Dehkordi F. A review on biochemical and immunological biomarkers used for laboratory diagnosis of SARS-CoV-2 (COVID-19). The Open Microbiology Journal. 2020 Dec 15;14(1).

2. Sheikhshahrokh A, Ranjbar R, Saeidi E, Dehkordi FS, Heiat M, Ghasemi-Dehkordi P, Goodarzi H. Frontier therapeutics and vaccine strategies for sars-cov-2 (COVID-19): A review. Iranian Journal of Public Health. 2020 Oct;49(Suppl 1):18.

3. Mirzaie A, Halaji M, Dehkordi FS, Ranjbar R, Noorbazargan H. A narrative literature review on traditional medicine options for treatment of corona virus disease 2019 (COVID-19). Complementary therapies in clinical practice. 2020 Aug 1;40:101214.

4. Halaji M, Farahani A, Ranjbar R, Heiat M, Dehkordi FS. Emerging coronaviruses: first SARS, second MERS and third SARS-CoV-2: epidemiological updates of COVID-19. Infez Med. 2020;28(suppl):6-17.

5. Mashak Z, Jafariaskari S, Alavi I, Shahreza MS, Dehkordi FS. Phenotypic and genotypic assessment of antibiotic resistance and genotyping of vacA, cagA, iceA, oipA, cagE, and babA2 alleles of Helicobacter pylori bacteria isolated from raw meat. Infection and drug resistance. 2020;13:257.

6. Dehkordi FS. Prevalence study of Bovine viral diarrhea virus by evaluation of antigen capture ELISA and RT-PCR assay in Bovine, Ovine, Caprine, Buffalo and Camel aborted fetuses in Iran. AMB express. 2011 Dec;1(1):1-6.

7. Rahimi E, Yazdanpour S, Dehkordi FS. Detection of Toxoplasma gondii antibodies in various poultry meat samples using enzyme linked immuno sorbent assay and its confirmation by polymerase chain reaction. J Pure Appl Microbiol. 2014;8(1):421-7.

8. Dehkordi FS, Saberian S, Momtaz H. Detection and segregation of Brucella abortus and Brucella melitensis in Aborted Bovine, Ovine, Caprine, Buffaloes and Camelid Fetuses by application of conventional and real-time polymerase chain reaction. The Thai Journal of Veterinary Medicine. 2012 Mar 1;42(1):13.

9. Martí M, Tuñón-Molina A, Aachmann FL, Muramoto Y, Noda T, Takayama K, Serrano-Aroca Á. Protective face mask filter capable of inactivating SARS-CoV-2, and methicillin-resistant Staphylococcus aureus

and Staphylococcus epidermidis. Polymers. 2021 Jan;13(2):207.

10. Mitevska E, Wong B, Surewaard BG, Jenne CN. The Prevalence, Risk, and Management of Methicillin-Resistant Staphylococcus aureus Infection in Diverse Populations across Canada: A Systematic Review. Pathogens. 2021 Apr;10(4):393.

11. Jaradat ZW, Ababneh QO, Sha'aban ST, Alkofahi AA, Assaleh D, Al Shara A. Methicillin Resistant Staphylococcus aureus and public fomites: a review. Pathogens and Global Health. 2020 Nov 16;114(8):426-50.

12. Grema HA, Geidam YA, Gadzama GB, Ameh JA, Suleiman A. Methicillin resistant Staphylococcus aureus (MRSA): a review. Adv Anim Vet Sci. 2015;3(2):79-98.

13. Purrello SM, Garau J, Giamarellos E, Mazzei T, Pea F, Soriano A, Stefani S. Methicillin-resistant Staphylococcus aureus infections: a review of the currently available treatment options. Journal of Global Antimicrobial Resistance. 2016 Dec 1;7:178-86.

14. 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;298:1763-71.

15. Hasanpour Dehkordi A, Khaji L, Sakhaei Shahreza MH, Mashak Z, Safarpoor Dehkordi F, Safaee Y, Hosseinzadeh A, Alavi I, Ghasemi E, Rabiei-Faradonbeh M. One-year prevalence of antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus recovered from raw meat. Trop Biomed. 2017;34(2):396-404.

16. Otarigho B, Falade MO. Analysis of antibiotics resistant genes in different strains of Staphylococcus aureus. Bioinformation. 2018;14(3):113.

17. Li SM, Zhou YF, Li L, Fang LX, Duan JH, Liu FR, et al. Characterization of the multi-drug resistance gene cfr in methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from animals and humans in China. Frontiers in microbiology. 2018 Nov 27;9:2925.

18. Dehkordi FS, Gandomi H, Basti AA, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrobial Resistance & Infection Control. 2017 Dec;6(1):1-1.

19. Dehkordi FS, Khamesipour F, Momeni M. Brucella abortus and Brucella melitensis in Iranian bovine and buffalo semen samples: The first clinical trial on seasonal, Senile and geographical distribution using culture, conventional and real-time polymerase chain reaction assays. Kafkas Univ Vet Fak Dergisi. 2014;20(6):821-.

20. Dehkordi FS, Valizadeh Y, Birgani TA, Dehkordi KG. Prevalence study of Brucella melitensis and Brucella abortus in cow's milk using dot enzyme linked immuno sorbent assay and duplex polymerase chain reaction. J Pure Appl Microbiol. 2014;8(2):1065-9.

21. Ghorbani F, Gheisari E, Dehkordi FS. Genotyping of vacA alleles of Helicobacter pylori strains recovered from some Iranian food items. Tropical Journal of Pharmaceutical Research. 2016 Sep 5;15(8):1631-6.

22. Ranjbar R, Seif A, Dehkordi FS. Prevalence of antibiotic resistance and distribution of virulence factors in the shiga toxigenic Escherichia coli recovered from hospital food. Jundishapur Journal of Microbiology. 2019;12(5):8.

23. Ranjbar R, Yadollahi Farsani F, Safarpoor Dehkordi F. Antimicrobial resistance and genotyping of vacA, cagA, and iceA alleles of the Helicobacter pylori strains isolated from traditional dairy products. Journal of Food Safety. 2019 Apr;39(2):e12594.

24. Dehkordi FS. Prevalence study of Coxiella burnetii in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. American Journal of Animal and Veterinary Sciences. 2011.

25. Dehkordi FS, Momtaz H, Doosti A. Application of Real-Time PCR for detection of Aspergillus species in aborted ruminant foetuses. Bulg. J. Vet. Med. 2012 Mar 1;15(1):30-6.

26. Abdolmaleki Z, Mashak Z, Safarpoor Dehkordi F. Molecular and virulence characteristics of methicillin-resistant Staphylococcus aureus bacteria recovered from hospital cockroaches. Jundishapur Journal of Microbiology. 2019 Dec 31;12(12).

27. Dehkordi FS, Tirgir F, Valizadeh Y. Effects of Guajol® ointment synthesized from medicinal smoke condensate of jennet feces on burn wound healing on Wistar rat. InVeterinary Research Forum 2017 (Vol. 8, No. 3, p. 215). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

28. Ranjbar R, Dehkordi FS, Heiat M. The frequency of resistance genes in Salmonella enteritidis strains isolated from cattle. Iranian Journal of Public Health. 2020 May;49(5):968.

29. Momtaz H, Dehkordi FS, Rahimi E, Asgarifar A, Momeni M. Virulence genes and antimicrobial resistance profiles of Staphylococcus aureus isolated from chicken meat in Isfahan province, Iran. Journal of Applied Poultry Research. 2013 Dec 1;22(4):913-21.

30. Madahi H, Rostami F, Rahimi E, Dehkordi FS. Prevalence of enterotoxigenic Staphylococcus aureus isolated from chicken nugget in Iran. Jundishapur journal of microbiology. 2014 Aug;7(8).

31. Momeni Shahraki M, Shakerian A, Rahimi E, Safarpoor Dehkordi F. Study the frequency of enterotoxin encoding genes and antibiotic resistance pattern of methicillin-resistant Staphylococcus aureus isolated from vegetable and salad samples in Chaharmahal Va Bakhtiari province. Journal of Food Microbiology. 2020 Jun 21;7(2):55-69.

32. Madahi H, Rostami F, Rahimi E, Safarpoor Dehkordi F, Jalali M. Detection of classical enterotoxins of Staphylococcus aureus isolates from chicken nugget and ready to eat foods in Esfahan province by ELISA technique. Food Hygiene. 2013;3(11):1-10.

33. Dormanesh B, Siroosbakhat S, Darian EK, Afsharkhas L. Methicillin-resistant Staphylococcus aureus isolated from various types of hospital infections in pediatrics: Panton-Valentine leukocidin, staphylococcal chromosomal cassette mec SCCmec phenotypes and antibiotic resistance properties. Jundishapur journal of microbiology. 2015 Nov;8(11).

34. Abubakar U, Sulaiman SA. Prevalence, trend and antimicrobial susceptibility of Methicillin Resistant Staphylococcus aureus in Nigeria: a systematic review. Journal of infection and public health. 2018 Nov 1;11(6):763-70.

35. Vicetti Miguel CP, Mejias A, Leber A, Sanchez PJ. A decade of antimicrobial resistance in Staphylococcus aureus: A single center experience. PLoS One. 2019 Feb 12;14(2):e0212029.

36. Liang Y, Tu C, Tan C, El-Sayed MA. Antimicrobial resistance, virulence genes profiling and molecular relatedness of methicillin-resistant Staphylococcus aureus strains isolated from hospitalized patients in Guangdong Province, China. Infection and drug resistance. 2019;12:447.

37. 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 research notes. 2018 Dec;11(1):1-5.

38. Jafari-Sales A, Farhadi F, Ezdiyadi M, Tarbiat-Nazloo D. Study of antibiotic resistance pattern in methicillin-resistant Staphylococcus aureus isolated from clinical samples of hospitals in Tabriz–Iran. International Journal of Biomedicine and Public Health. 2018 Apr 27;1(2):71-5.

39. Nicolau DP, Silberg BN. Cefazolin potency against methicillin-resistant Staphylococcus aureus: a microbiologic assessment in support of a novel drug delivery system for skin and skin structure infections. Infection and drug resistance. 2017;10:227.

40. Hau SJ, Haan JS, Davies PR, Frana T, Nicholson TL. Antimicrobial resistance distribution differs among methicillin resistant Staphylococcus aureus sequence type (ST) 5 isolates from health care and agricultural sources. Frontiers in microbiology. 2018 Sep 11;9:2102.

41. Saba CK, Amenyona JK, Kpordze SW. Prevalence and pattern of antibiotic resistance of Staphylococcus aureus isolated from door handles and other points of contact in public hospitals in Ghana. Antimicrobial Resistance & Infection Control. 2017 Dec;6(1):1-6.

42. Hauschild T, Sacha P, Wieczorek P, Zalewska M, Kaczyńska K, Tryniszewska E. Aminoglycosides resistance in clinical isolates of Staphylococcus aureus from a University Hospital in Bialystok, Poland. Folia Histochemica et Cytobiologica. 2008;46(2):225-8.

43. Abdolmaleki Z, Mashak Z, Dehkordi FS. Phenotypic and genotypic characterization of antibiotic resistance in the methicillin-resistant Staphylococcus aureus strains isolated from hospital cockroaches. Antimicrobial Resistance & Infection Control. 2019 Dec;8(1):1-4.

44. Rahi A, Kazemeini H, Jafariaskari S, Seif A, Hosseini S, Dehkordi FS. Genotypic and phenotypic-based assessment of antibiotic resistance and profile of staphylococcal cassette chromosome mec in the methicillin-resistant Staphylococcus aureus recovered from raw milk. Infection and drug resistance. 2020;13:273.

45. Akanbi OE, Njom HA, Fri J, Otigbu AC, Clarke AM. Antimicrobial susceptibility of Staphylococcus aureus isolated from Recreational waters and beach sand in Eastern Cape province of south Africa. Int J Environ Res Publ Health. 2017;14(9):1001.

46. Momtaz H, Hafezi L. Meticillin-resistant Staphylococcus aureus isolated from Iranian hospitals: virulence factors and antibiotic resistance properties. Bosnian journal of basic medical sciences. 2014 Nov;14(4):219.