ORIGINAL ARTICLE

Prevalence and Susceptibility of *Staphylococcus aureus* Nasal Carriage Strains Isolated from Haemodialysis Patients

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ABSTRACT

Introduction: *Staphylococcus aureus* is part of the normal human flora that can commonly be found on the skin and mucous membranes of the nasal area. However, in immunosuppressed patients such as those with kidney failures, colonization can potentially lead to infection. There is a concern of increasing antibiotic resistance in *S. aureus*. This study aimed to determine the prevalence of *S. aureus* nasal colonization and its antimicrobial susceptibility among haemodialysis-dependent populations. **Methods:** A cross-sectional study at the Nephrology Unit, Hospital Canselori Tuanku Mukhriz (HCTM) was conducted among haemodialysis-dependent patients between February 2017 to February 2018. Nasal swabs were obtained and cultured on mannitol salt agar. *S. aureus* isolates were identified by gram staining, tube coagulase and Deoxyribonuclease (DNase). Cefoxitin disc (30 µg) were used to identified the presence of MRSA (methicillin-resistance *S. aureus*). The *S. aureus* colonies were further tested against six antibiotics using Kirby Bauer disc diffusion**. Result:** A total of 134 patients were recruited. *S. aureus* isolates were detected from 27 patients (20.1%). All *S. aureus* were phenotypically identified as methicillin-sensitive *S. aureus* (MSSA) based on the cefoxitin disc. Teicoplanin and linezolid were the most effective with 100% susceptibility. *S. aureus* exhibited a high resistance rate towards erythromycin (29.6%). No MRSA was isolated in this study. **Conclusion:** This study highlighted the high prevalence of *S. aureus* nasal colonization in haemodialysis patients. Teicoplanin and linezolid were found to be the most effective antibiotics against isolated *S. aureus*.
Malaysian Journal of Medicine and Health Sciences (2023) 19(1):181-187. doi:10.47836/mjmhs19.1.25

Keywords: Antimicrobial susceptibility, Haemodialysis (HD), Methicillin-resistance *Staphylococcus aureus* (MRSA); Nasal carriage, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus nasal carriage (SANC) can be detected in up to 30% of healthy people in the absence of symptoms of illness (1). The lower and upper epidermal layers of the nasal epithelium were the most common niches for *S. aureus*, with majority of them found in intranasal squamous epithelium stratum corneum (2). The presence of a sugar polymer, teichoic acid on *S. aureus* cell wall was thought to be a factor for colonization by assisting its adhesion to cellular nasal epithelial surfaces of the host (3). *S. aureus* also have multiple other strategies to modulate cellular function aiding colonization, such as tissue destruction, bacterial dissemination, and immune evasion, making it a highly versatile pathogen (3). This host-microbe interaction has led to a vast spectrum of diseases, from minor to potentially fatal conditions (4). It is postulated that *S. aureus* capable to attack the immune system cells directly by generating pore-forming toxin. These toxins, such as Panton-Valentine leukocidin (PVL), gamma (γ)-haemolysin (HIgACB), leukotoxin AB/GH (LukAB/GH), and leukotoxin ED (LukED), can lead to cell death via osmotic dysregulation of the plasma membrane (5). Combining these traits, *S. aureus* is able to enter the bloodstream and disseminate into extravascular tissues; a septicaemic condition that carries up to 60% mortality rates (6). Depending on organs, some of the said complications are endocarditis, pneumonia, septicemia and toxic shock syndrome (4).

Eradication of *S. aureus* is challenging. Together, colonies produce biofilms that further contribute antibiotic resistance through phenotypic adaptation that down regulates rate of cell division upon antimicrobial exposure. This biofilm also promotes clusters of persisted cells that underwent a metabolically dormant

state, thus evading even the bactericidal properties of antibiotics (7). This led to a term coined small colony variant (SCV) phenotype of *S. aureus* that flourished via its biofilm (8). The United States Centers for Disease Control and Prevention (CDC) reported 119,247 invasive *Staphylococcus aureus* infections leading to approximately 19,832 deaths through the year 2017 (9). Meanwhile the prevalence rate of methicillin-resistance *S. aureus* (MRSA) in Europe was 16.4% in 2018 (10). In 2018, the Ministry of Health Malaysia estimated the prevalence of *S. aureus* and MRSA isolated from blood was 23.5% and 19.4% amongst 41 hospitals (11). At the same time, the number of haemodialysis patients in Malaysia in ten years between 2007 to 2016 has risen by double to 35781 (12). These enormous numbers of increment have caused a huge concern towards medical sectors. End stage renal failure patients whose undergo treatment of haemodialysis had been detected of high exposure of MRSA colonization prevalence rates (13). This is thought due to the increased intensive care-related admission, invasive procedures, and the use of artificial devices (14). Haemodialysis patients require longer and more frequent hospital visits, subjected to a higher dose or prolonged antibiotic courses and underwent more procedures compared to standard hospital patients (15). This has led to a higher rate of mortality associated with *S. aureus* colonization (14). *S. aureus* colonization has longed thought to lead into MRSA colonization, which in turn is a risk factor of MRSA infection (16). MRSA infection has burdened a large economic strain on the health system in addition to affecting mortality and morbidity (17). It's alarming to learn that *S. aureus* can be transferred through the nose via a hand-nose pathway (18). As *S. aureus* and MRSA nasal colonization are important highlights in haemodialysis patient care, we found that there was lack of prevalent study in Malaysia. Apart from identifying the rate, properties of antibiotics resistance towards isolated *S. aureus* strain could also be examined.

MATERIALS AND METHODS

Design and Population

A cross-sectional study was conducted in the Nephrology Clinic at Hospital Canselor Tuanku Muhriz (HCTM), a tertiary medical referral centre located in Kuala Lumpur, Malaysia, from February 2017 to February 2018. Haemodialysis-dependent patients who attended the HCTM Nephrology Clinic represented the sampling frame. The number of haemodialysis-dependent patients attending the Nephrology Clinic ranged from eight to 20 from week to week, hence a convenience sampling method was applied. A 95% Confidence Level study on the prevalence of MRSA (9.48%) among haemodialysis patients in Taiwan (16) was used to determine the sample size. The sample size was calculated using the Open Epi software (http//: www.openepi.com). Accordingly, the sample size requirement for this study was 132. Nasal swabs were collected at the clinic after a briefing and consent obtained from the patients. The inclusion criteria for sampling were age 18-80 years old, haemodialysis dependent patients, did not receive a course of antibiotics during the past two weeks and no history of hospitalization for any reasons other than haemodialysis during the past two months. The exclusion criteria were a history of acute respiratory infections within the past three months prior to enrolment, received a course of antibiotics within the past two weeks prior to enrolment, history of hospitalization for any reasons other than HD during the past two months, history of malignancy, or other immunocompromised state, including human immunodeficiency virus (HIV) infection or on immunosuppressant, age less than 18 years or more than 80 years old.

Microbiological Study

For isolation and identification of nasal *S. aureus* carriage, a sterile swab was rotated in the anterior 1.5 cm of the nasal vestibule of both 134 patients' nares and placed into Stuart's transport medium. All swabs were kept at 4°C using an ice box, transported to the microbiology laboratory and processed within four hours. The nasal swabs were streaked on blood agar and mannitol salt agar (MSA), and incubated at 37°C for 24-48 hours. β-hemolytic colonies on blood agar and golden yellow pigmentation colonies on MSA were identified as *S. aureus*. The colonies were further confirmed by gram stain, tube coagulase and DNase tests. The reference strain (ATCC 25923 *S. aureus*) was used as the positive quality control strain for comparison with the sample.

All *S. aureus* isolates were subjected to cefoxitin susceptibility testing for identification of MRSA. Antimicrobial susceptibility testing was also performed using the Kirby Bauer disc diffusion method in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines for six antibiotics teicoplanin (30 µg), linezolid (30 µg), rifampicin (5 µg), fusidic acids (10 µg), tetracycline (30 µg), and erythromycin (15 µg). Preparation of the growth suspension was made in 0.5ml of Mueller Hinton broth medium and adjusted to the turbidity to matched the 0.5 McFarland standards, yielding approximately $1x10^6$ colony forming units (CFU) per ml. A sterile swab was dipped into the suspension and pressed against the tube's sidewalls to remove any excess inoculum. The swab was placed in the middle of the Mueller Hinton agar plate and spread equally across the medium. After 15 minutes of inoculation on Mueller Hinton agar seeded with each isolate, antibiotic discs were placed and incubated at 35-37°C for 24 hours. The diameter of the inhibitory zone surrounding the disc was measured and interpreted using the Clinical Laboratory Standard Institute's 2017 recommendations (19).

RESULTS

Sociodemographic Data

The sociodemographic characteristics of participants are

described in Table I. A total of 134 patients enrolled in this study. The age ranged was between 23 to 83 years old with mean of 59.63 with standard deviation (SD) of 14.10.

Table I: Sociodemographic Characteristics of The Respondents (N=134)

Variable	n	(%)
Age (mean ± SD) in years	59. 63 ± 14.10	
Gender		
Male	69	51.5
Female	65	48.5
Race		
Malay	76	56.7
Chinese	49	36.6
Indian	7	5.2
Others	2	1.5
Education		
Primary	36	26.9
Secondary	67	50.0
Tertiary	31	23.1
Household Income		
< RM 2999	116	86.6
> RM 3000	18	13.4

SD= Standard Deviation

Prevalence rate of *S. aureus* and MRSA

Table II presents the result of nasal swab. *S. aureus* was present in 20.1% of the participants, whereas *Staphylococcus* spp. was isolated from the remaining 79.9%. All *S. aureus* were phenotypically identified as MSSA based on the cefoxitin disc. No MRSA isolates were found.

Table II: The Prevalence Rate of S. aureus in Haemodialysis Patients

Prevalence (N=134)	n	(%)
S. aureus	27	20.1
Staphylococcus spp.	107	79.9
MRSA	0	0
Total	134	100

S. aureus = Staphylococcus aureus
MRSA = Methicillin-Resistance *S. aureus*

Antibiotic susceptibility of *S. aureus* isolates

S. aureus exhibited a high resistance rate towards erythromycin (29.6%), whereas teicoplanin and linezolid showed highest (100%) susceptibility to the isolated *S. aureus* (Table III).

Table III: Antibiotic Susceptibility Testing

Antibiotic (N=27)	Resistance n (%)	Susceptible n (%)
Erythromycin	8 (29.6)	19 (70.4)
Tetracycline	5 (18.5)	22 (81.5)
Fusidic acid	2 (7.4)	25 (92.6)
Rifampicin	1 (3.7)	26 (96.3)
Teicoplanin	0 (0)	27 (100)
Linezolid	0 (0)	27 (100)
Cefoxitin*	0 (0)	27 (100)

* A screening disc was used for the differentiation of Methicillin Susceptible *Staphylococcus aureus* (MSSA) and Methicillin Resistance *Staphylococcus aureus* (MRSA) strains

DISCUSSION

This study showed that the prevalence of *S. aureus* nasal carriage among patients receiving haemodialysis was 20.1% higher than a previous study from Taiwan, which was 15% (16). Nevertheless, other countries showed higher prevalence, Columbia 45.5% (20). Meanwhile, the prevalence of MRSA among haemodialysis patients were ranging from 3.3% to 13.3% (16, 20, 21). The highest prevalence rate of MRSA was from Ghasemian et al (22) study, with 27.4% in Iran. Previous studies showed haemodialysis patients were at high risk of MRSA acquisition. However, no MRSA was isolated in this present study.

A study by Sapri et al (23), found as many as 880 methicillin-susceptible *S. aureus* (MSSA) cases infections were isolates from wound and tissue swabs discovered in various wards at Hospital Canselor Tuanku Muhriz UKM (HCTM) the same centre where this study was carried out. Collagen adhesin-encoding gene (cna), staphylococcal enterotoxin H (seh), Panton-Valentine leukocidin (PVL) and Toxic Shock Syndrome Toxin-1 (TSST-1) were the virulence genes of the MSSA isolates determined in the study. The cna gene was the most predominant in the MSSA strains in HCTM, with 51.59%, followed by seh (21.82%), PVL (10.23%) and TSST-1 (6.82%). The presence of 880 MSSA strains scattered throughout HCTM necessitates MSSA study. As 880 cases is a large number, more studies need to be done as there is a lack of research regarding MSSA in HCTM. While MSSA is susceptible to most antibiotics, its high prevalence may contribute to increased morbidity and mortality if not closely monitored. It's worth noting that some MSSA strains harbour virulence genes (24).

In three months from January to March 2000 at Hospital Canselor Tuanku Muhriz University Kebangsaan Malaysia (HCTM), 71 MRSA strains were circulating around intensive care unit (ICU), surgical wards and medical wards (25). The highest MRSA infections were recorded in 2009 with 318 cases where most isolated strains found in medical and surgical wards in HCTM (26). Recent study regarding MRSA in HCTM in 2015 showed four different sequence types (STs) ST30-IV (surgical 7 ward), ST239-II (orthopaedic clinic), ST239-III (medical 3 ward, general intensive care unit and

high dependency ward), ST1178-IV (paediatric 1 ward, paediatric intensive care unit and surgery 7 ward,) and ST772-V (endocrine clinic and orthopaedic ward) were found along with their corresponding SCCmec types (27). All these previous studies showed that there were MSSA and MRSA presented in different units in Hospital Canselor Tuanku Muhriz UKM (HCTM). However, in the Nephrology unit, where the current study being conducted, no MRSA was detected. This result might due to the sample size, as only 134 of patients were enrolled in this study, plus an extra two from the sample size calculation of 132. The sample size should be in a larger quantity as newer haemodialysis patients increase every year. Although the sample size was based on previous study (16), adding more numbers for the sample population between the range of an additional 200 to 300 might contribute to new findings or more accurate results that represent the whole populations. However, too many large samples might contribute to statistically significant difference even though they are clinically insignificant (28).

Varying degree of susceptibility of 27 *S. aureus* isolates were tested against six types of antimicrobials that were teicoplanin (30 µg), linezolid (30 µg), fucidic acids (10 µg), tetracycline (30 µg), erythromycin (15 µg) and rifampicin (5 µg). The most effective antibiotics to *S. aureus* were teicoplanin and linezolid with all recorded 100% susceptibility. This was in agreement with other studies that showed both linezolid and teicoplanin were the most effective drugs choice for *S. aureus* strains (29, 30). Meanwhile, resistance towards rifampicin, fusidic acid, tetracycline, and erythromycin were also observed. Rifampicin was the least resistance of the *S. aureus* isolates, with 3.7% resistance rate. Another study found a similar range of resistance to rifampicin, with 2.4% (31) and 6.2% (32). In this study, the rate of resistance to fusidic acid was 7.4%. This is slightly higher to Liu et al in 2016 study (31), which 1.4% *S. aureus* were found resistant to fusidic acid but lower than Yilmaz and Aslantaş's reported 12.4% (32). Tetracycline, with 18.5% resistance, was the second most resistant antibiotic. This is consistency with Yilmaz and Aslantaş (32), 16.5%, however, comparatively low to 42% tetracycline resistance on *S. aureus* (31). Our *S. aureus* isolates were mostly resistant to erythromycin (29.6%). Yilmaz and Aslantaş (32) highlighted 63.9%, while to Liu et al (31), contradicted with our result highest resistance observed 97.3% of *S. aureus* resistant towards erythromycin. Another suggestion is that a D-test among study isolates will be deemed beneficial in detecting macrolide inducible clindamycin resistance. Clindamycin, a member of the macrolide-lincosamide-streptogramin type B (MLSB) antibiotic family, can be used to treat *S. aureus*. However, due to excessive clindamycin exposure, many *S. aureus* strains have developed resistance, most commonly via the *erm* genes' target site modification mechanism. These *erm* genes produce an enzyme called methylase, which prevents antibiotic binding via ribosomal target site modification, resulting in constitutive and inducible resistance. Inducible resistance develops when erythromycin, a strong inducer of the methylase enzyme, is present. This isolate is erythromycin resistant but clindamycin susceptible. The D-test is achieved by seeding a Mueller-Hinton agar with erythromycin (15 µg) and clindamycin (2 µg) disk 15 mm apart. The presence of a clindamycin inhibition zone ≥21 mm (D-shape) around the erythromycin disk suggests that erythromycin has caused clindamycin resistance (33). Yilmaz and Aslantas (32) and Liu et al (31) also included clindamycin in their antibiotic sensitivity testing, but neither report addressed the D-test. These studies showed that the degree of resistance and susceptibility of *S. aureus* strains varied across the globe. A study by Ventola CL, (34) elaborated the reason bacteria become crucially resistant due to misuse, overuse and common antibiotic being overprescribing which some were unnecessary and inappropriate in outpatient care. The study also highlighted the bacteria which exposes to the antibiotic may die, but some become resistant and multiply which spread rapidly worldwide. This is one of the reasons why erythromycin become more resistant which could be the most common or longer antibiotics that being used in such community. However, linezolid and teicoplanin shown the most susceptible antibiotics in all studies (30, 31).

There are two clinical laboratory tests for detecting MRSA, according to Clinical and Laboratory Standards Institute M100 (19): a cefoxitin disk diffusing test or a plate containing 6 µg/ml of oxacillin in Mueller-Hinton agar supplement with 4% Sodium Chloride NaCl. However, CLSI recommended cefoxitin testing as it is more accurate and more reproducible as cefoxitin is a far better inducer and more sensitive towards mecA gene compared to oxacillin.

Vancomycin had been the most effective first-line antibiotic against *S. aureus* for decades (35). However, vancomycin dosing and administration requires based on consideration of pathogen, severity and type of infection, patient weight and kidney function. The reason vancomycin was not included in this study is due to optimal vancomycin dosing which very challenging in patient with unstable kidney function, as fluctuations occurred due to distribution and elimination of the drug (36). Various study had highlighted the association of vancomycin with nephrotoxicity: toxicity in the kidney. The nephrotoxicity is more common in higher doses (≥4 g/d) in treatment for *S. aureus* (37). This kidney toxicity is presented as proximal tubular cells injury with or without necrosis and as acute interstitial nephritis. This is also mentioned in a case reported by Barcely et al (38) that exposure to vancomycin at high level for a long time in patient had led to kidney damaged.

The limitations of this study include that this research

used convenience sampling method and this may not be accurate to represent the whole population of haemodialysis patients. Another limitation was the lack of genetic analysis test which to differentiate classes or variant strain of *S. aureus* such as s*ea, sed, seb, tst, etb, eta, LuKS/F-PV, hld, hla* and distribution of *Sccmec* types strain in our sample might be one of the limitations in this study. The finding of this result could be useful in evaluating the major strain circulating among our haemodialysis patients in this country.

CONCLUSION

Our results showed that prevalence of *S. aureus* among haemodialysis patients was quite high (20.1%) but no MRSA carriage was detected. Susceptibility testing showed highest sensitivity to teicoplanin and linezolid to the isolated *S. aureus* and least sensitivity to erythromycin. Due to the risk of severe infection in haemodialysis patients, proper infection control practice should be in place. The knowledge about basic infection control should not only given to the staff but the most importantly to the patients and their relatives. Future research is to raise awareness and educational value in creating and spreading a simple infographic printed material of *S. aureus* colonization, risk factors group, transmission and prevention of infections among haemodialysis patients.

ACKNOWLEDGEMENTS

The team would like to thank the Department of Nephrology and its Head, Prof. Dr. Abdul Halim Abdul Gafor (2017; currently Deputy Director HCTM), and his entire staff for their full support throughout the research duration. This research was funded by the Universiti Sains Islam Malaysia (USIM/PPP/USG-0114/PSK/30/11314).

REFERENCES

1. Sakr A, Brégeon F, Mége JL, Rolain JM, Blin O. *Staphylococcus aureus* Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. Front Microbiol. 2018 Oct 8; 9: 2419. doi: 10.3389/fmicb.2018.02419.
2. Hanssen AM, Kindlund B, Stenklev NC, et al. Localization of *Staphylococcus aureus* in tissue from the nasal vestibule in healthy carriers. BMC Microbiol. 2017 April 5;17(1):89. doi:10.1186/s12866-017-0997-3.
3. Baur S, Rautenberg M, Faulstich M, et al. A nasal epithelial receptor for *Staphylococcus aureus* WTA governs adhesion to epithelial cells and modulates nasal colonization. PLoS Patho. 2014;1;10(5): e1004089. doi:10.1371/journal.ppat.1004089.
4. Vivekananda J, Salgado C, Millenbaugh NJ. DNA aptamers as a novel approach to neutralize *Staphylococcus aureus* α-toxin. Biochemical and Biophysical Research Communications. 2014;14;444(3):433-8. https://doi: 10.1016/j.bbrc.2014.01.076.
5. Yoong P, Torres VJ. The effects of *Staphylococcus aureus* leukotoxins on the host: cell lysis and beyond. Current Microbiol. 2013;1;16(1):63-9. doi: 10.1016/j.mib.2013.01.012.
6. Jacobsson G, Nasic S. Long-term outcome of invasive *Staphylococcus aureus* infections. Scandinavian Journal of Infectious Diseases. 2012;1;44(5):350-4. doi: 10.3109/00365548.2011.641504.
7. Lewis K. Persister cells. Annual Review of Microbiology. 2010;13; 64:357-72. doi:org/10.1146/annurev.micro.112408.134306.
8. Kim NH, Kang YM, Han WD, et al. Small-colony variants in persistent and recurrent *Staphylococcus aureus* bacteremia. Microbial Drug Resistance. 2016;1;22(7):538-44. doi: 10.1089/mdr.2015.0262.
9. Kourtis AP, Hatfield K, Baggs J, et al. Vital signs: epidemiology and recent trends in methicillin-resistant and in methicillin-susceptible *Staphylococcus aureus* bloodstream infections United States. Morbidity and Mortality Weekly Report. 2019;8;68(9):214-9. doi: 10.15585/mmwr.mm6809e1.
10. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2018. [Internet]. Stockholm: ECDC. 2019. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe-2018.pdf
11. Ahmad N, Hashim R, Amran F, Zamri H, Liow Y, Abd Rashid F. National Antibiotic Resistance Surveillance Report. [Internet]. Imr.gov.my. 2018. Available from: http://www.imr.gov.my/images/uploads/NSAR/2018/260619-NATIONAL-ANTIBIOTIC-RESISTANCE-SURVEILLANCE-REPORT-2018-1.pdf
12. Goh BL, Wong HS. 24th Report of the Malaysian Dialysis and Transplantation Registry. The National Renal Registry. 2018;30-33.
13. Price A, Sarween N, Gupta I, Baharani J. Meticillin-resistant *Staphylococcus aureus* and meticillin-susceptible *Staphylococcus aureus* screening in a cohort of haemodialysis patients: carriage, demographics and outcomes. Journal of Hospital Infection. 2015;1;90(1):22-7. doi:10.1016/j.jhin.2015.01.001.
14. Grünewald T, Lindner M, Weiß S, et al. Staphylococcus colonization, mortality and morbidity in hemodialysis patients: 10 years of observation. International Journal of Hygiene and Environmental Health. 2013;1;216(6):751-4. doi: 10.1016/j.ijheh.2013.02.005.
15. Parker MG, Doebbeling BN. The challenge of methicillin-resistant *Staphylococcus aureus* prevention in hemodialysis therapy. In Seminars

in Dialysis. 2012; Jan 25(1):42-49. Oxford, UK: Blackwell Publishing Ltd. doi: 10.1111/j.1525-139X.2011.00999x.
16. Lai CF, Liao CH, Pai MF, et al. Nasal carriage of methicillin-resistant *Staphylococcus aureus* is associated with higher all-cause mortality in hemodialysis patients. Clinical Journal of the American Society of Nephrology. 2011;1;6(1):167-74. doi: 10.2215/CJN.06270710.
17. Gandra S, Barter DM, Laxminarayan R. Economic burden of antibiotic resistance: how much do we really know?. Clinical Microbiology and Infection. 2014;1;20(10):973-80. doi.org/10.1111/1469-0691.12798
18. Iyer A, Kumosani T, Azhar E, Barbour E, Harakeh S. High incidence rate of methicillin-resistant *Staphylococcus aureus* (MRSA) among healthcare workers in Saudi Arabia. The Journal of Infection in Developing Countries. 2014;13;8(03):372-8. doi: 10.3855/jidc.3589.
19. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CSLI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. 2017.
20. Hidalgo M, Carvajal LP, Rincyn S, et al. Methicillin-resistant *Staphylococcus aureus* USA300 Latin American variant in patients undergoing hemodialysis and HIV infected in a hospital in Bogotá, Colombia. PloS one. 2015;16;10(10): e0140748. doi: 10.1371/journal.pone.0140748.
21. Oumokhtar B, Elazhari M, Timinouni M, et al. *Staphylococcus aureus* nasal carriage in a Moroccan dialysis center and isolates characterization. Hemodialysis International. 2013;17(4):542-7. doi: 10.1111/j.1542-4758.2012.00759.x.
22. Ghasemian R, Najafi N, Makhlough A, Khademloo M. Frequency of Nasal Carriage of *Staphylococcus aureus* and Its Antimicrobial Resistance Pattern in Patients on Hemodialysis. Iranian journal of kidney diseases. 22010;4(3):218-22. PMID: 20622310.
23. Sapri HF, Sani NA, Noordin A, Neoh H, Hussin S. Virulence gene typing of Methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates in Universiti Kebangsaan Malaysia Medical Centre (UKMMC). Asia-Pacific Journal of Molecular Medicine. 2011; 1:4.
24. Horváth, A., Dobay, O., Sahin-Tyth, J. et al. Characterisation of antibiotic resistance, virulence, clonality and mortality in MRSA and MSSA bloodstream infections at a tertiary-level hospital in Hungary: a 6-year retrospective study. Ann Clin Microbiol Antimicrob 19, 17 (2020). doi: 10.1186/s12941-020-00357-z.
25. Alfizah H, Norazah A, Nordiah AJ, Lim VK. DNA fingerprinting of methicillin-resistant *Staphylococcus aureus* (MRSA) by pulsed-field gel electrophoresis (PFGE) in a teaching hospital in Malaysia. Medical Journal of Malaysia. 2002;1;57(3):319-28. PMID: 12440272.
26. Noordin A, Sapri HF, Sani NA, et al. Antimicrobial resistance profiling and molecular typing of methicillin-resistant *Staphylococcus aureus* isolated from a Malaysian teaching hospital. Journal of Medical Microbiology. 2016;16;65(12):1476-81. doi: 10.1099/jmm.0.000387.
27. Muttaqillah NA, Hussin S, Neoh H, Noordin A. Clonal diversity of methicillin-resistant *Staphylococcus aureus* in UKM Medical Centre: characterisation by multilocus sequence typing of different SCCmec type representatives. Sains Malaysiana. 2015;1;44(9):1315-23.
28. Faber J, Fonseca LM. How sample size influences research outcomes. Dental Press Journal of Orthodontics. 2014;19(4), 27-29. doi: 10.1590/2176-9451.19.4.027-029.ebo
29. Chen X, Wang WK, Han LZ, et al. Epidemiological and genetic diversity of *Staphylococcus aureus* causing bloodstream infection in Shanghai, 2009-2011. PLoS One. 2013;9;8(9): e72811. doi.org/10.1371/journal.pone.0072811.
30. Hasani A, Sheikhalizadeh V, Hasani A, Naghili B, Valizadeh V, Nikoonijad AR. Methicillin resistant and susceptible *Staphylococcus aureus*: Appraising therapeutic approaches in the Northwest of Iran. Iranian Journal of Microbiology. 2013;5(1):56-62. PMID: 23467268; PMCID: PMC3577566.
31. Liu Y, Xu Z, Yang Z, Sun J, Ma L. Characterization of community-associated *Staphylococcus aureus* from skin and soft-tissue infections: a multicenter study in China. Emerging Microbes & Infections. 2016;1;5(1):1-1. doi: 10.1038/emi.2016.128.
32. Yilmaz ES, Aslantaş Ll. Antimicrobial resistance and underlying mechanisms in *Staphylococcus aureus* isolates. Asian Pacific Journal of Tropical Medicine. 2017;10(11), 1059-1064. doi:10.1016/j.apjtm.2017.10.003.
33. Timsina R, Shrestha U, Singh A, Timalsina B. Inducible clindamycin resistance and erm genes in *Staphylococcus aureus* in school children in Kathmandu, Nepal. Future Science OA 2020; 20;7(1):FSO361. doi: 10.2144/fsoa-2020-0092.
34. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P & T: a peer-reviewed journal for formulary management. 2015;40(4), 277–283. PMID: 25859123; PMCID: PMC4378521.
35. Van Hal SJ & Fowler VG. Is it time to replace vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* infections. Clinical Infectious Disease. 2013. doi: 10.1093/cid/cit178.
36. Richard HD. Vancomycin: Parental dosing, monitoring, and adverse effects in adults. https://www.uptodate.com/contents/vancomycin-parenteral-dosing-monitoring-and-adverse-effects-in-adults. 2021.
37. Stokes MB. Vancomycin in the kidney-a novel cast nephropathy. Journal of the American Society of Nephrology: JASN, 28(6), 1669-1670. 2017. doi: 10.1681/ASN.2017010091.

38. Barcely-Vidal J, Rodriguez GE, Grau S. Extremely high levels of vancomycin can cause severe renal toxicity. Infect Drug Resist. 11:1027-1030. 2018. doi: 10.2147/IDR.S171669.