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COLLEGE OF MEDICINE AND HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

METHICILLIN-RESISTANT AND VANCOMYCIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND ASSOCIATED FACTORS AMONG HEMODIALYSIS PATIENTS AND THEIR CONTACTS AT HEMODIALYSIS CLINICS IN BAHIR DAR, NORTHWEST ETHIOPIA

BY: ABRHAM GEBREMEDHIN (MSc candidate)

DECEMBER, 2023

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A RESEARCH THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, COLLEGE OF MEDICINE AND HEALTH SCIENCE, FOR PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL MICROBIOLOGY.

DECEMBER, 2023

BAHIR DAR, ETHIOPIA

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ABSTRACT

BACKGROUND: Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Staphylococcus aureus* (VRSA) are the critical pathogens for research and discovery of new antibiotics due to their multidrug resistance. Hemodialysis patients appear to have a greater risk of *S. aureus* colonization, and infection is the second leading cause of death in hemodialysis.

OBJECTIVES: This study aimed at assessing the MRSA and VRSA carriage among hemodialysis patients, HCWs, food handlers and cleaners at hemodialysis clinics in Bahir Dar. It also identified factors associated for the overall MRSA carriage.

METHODS: A cross-sectional study was carried out from 27 April 2023 to 24 June 2023, at Felege Hiwot Compressive Specialized Hospital and Gamby General Teaching Hospital. A total of 382 study participants were included by convenient sampling technique. The demographic data of study participants were collected by self-administered and interviewer administered interviews using questionnaires and observational checklists. Kirby Bauer susceptibility test using cefoxitin (30μg) discs and vancomycin E-test were performed to detect MRSA and VRSA, respectively. Chi-square and independent sample T tests were computed to see the association between variables. Regression analysis was computed to identify the factors associated with the overall MRSA carriage and a p-value < 0.05 at 95% CI was taken as cut off for statistical significance

RESULTS: The overall rate of culture-confirmed MRSA and VRSA was 14% (n = 53), and 2% (n = 6), respectively. The proportion of MRSA was 12% among hemodialysis patients (11/90), 20% among healthcare workers (14/70), 15% among cleaners (18/124) and 10% among food handlers (10/98). The *S. aureus* isolates had 94% sensitivity to clindamycin. Sex of the study participants, diabetes mellitus, and improper use of antibiotics were found to be significantly associated with MRSA.

CONCLUSION: Carriage of MRSA is common among hemodialysis patients' staffs working in the hemodialysis clinics. Its prevalent occurrence in the nasal cavities of HDPs, working clothes of HCWs, cleaners and food handlers is a major concern for the study area. Clindamycin had a higher sensitivity rate for *S. aureus* and MRSA. Being females, having diabetes mellitus, and improper use of antibiotics are factors associated with MRSA carriage in hemodialysis clinics. Therefore, educate on MRSA, especially regarding to its transmissions, cause and prevention should be considered.

Keywords: MRSA, VRSA, hemodialysis patients, HCWs, Food handlers, cleaners, FHCSH, GGTH, Ethiopia

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ABBREVIATIONS AND ACRONYMS

APHI: Amhara Public Health Institute

ARHB: Amhara Regional Health Bureau

AST: Antimicrobial susceptibility testing

ATCC: American Type Culture Collection

CDC: Centers for Disease Control and Prevention

CLSI: Clinical and laboratory standard institution

FHCSH: Felege Hiwot Comprehensive Referral Hospital

GGTH: Gamby Generalized Teaching Hospital

HDPs: Hemodialysis patients

HAIs: Hospital acquires infections

HCWs: Health care workers

IPC: Infection prevention and control

MIC: Minimum inhibition concentrations

MHA: Mueller- Hinton agar

MRSA: Methicillin-resistant *S. aureus*

MSA: Mannitol salt agar

MSSA: Methicillin-sensitive *S. aureus*

SCCmec: *Staphylococcal* Cassette Chromosome mec

SPSS: Statistical Package for the Social Sciences

VISA : Vancomycin intermediate *S. aureus*

VRSA : Vancomycin résistance *S. aureus*

1. INTRODUCTION

1.1 Background

Staphylococcus aureus (*S. aureus*) species belong to the family *Staphylococcaceae* and is Gram-positive cocci may also appear as single cells, pairs, or short chains, large, 0.5 to 1.5 μm in diameter that can potentially produce disease. It grows in facultative anaerobic conditions, in the presence of a high concentration of salt and at temperatures ranging from 18° C to 40° C (1). *S. aureus* colonizes the skin and nasopharyngeal membranes of about 25–30% of healthy people, where it coexists with other members of the normal microbiota (2). However, *S. aureus* can cause varieties of urinary tract infections, respiratory diseases, gastroenteritis, meningitis, surgical site, and other soft tissue infections (3).

The *S. aureus* are irregular clusters of non-motile, non-spore-forming cocci that resemble grapes-like structures by Gram stain and are DNase-positive. They grow well aerobically on blood agar with high salt content, such as Mannitol Salt Agar (MSA), and after overnight incubation, they produce small, round, smooth, and yellow colonies that are 1-2 mm in diameter. It can be distinguished from Streptococci by being catalase positive, as well as from coagulase-negative Staphylococci such as *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* primarily by the clotting and coagulation of plasma that is coagulase positive (4).

Methicillin-resistant *S. aureus* (MRSA) is *S. aureus* that is resistant to all beta -lactam (methicillin, oxacillin) antibiotics currently on the market, except ceftaroline. Vancomycin resistance *S. aureus* (VRSA) and Vancomycin intermediate *S. aureus* (VISA) are *S. aureus* that have reduced susceptibility to vancomycin antimicrobial agent, with minimum inhibition concentrations (MIC) ≥ 16 μg/ml and 4-8 μg/ml, respectively (5). The *MecA, MecB, VanA*, and *VanB* are a few of the resistance genes found in *S. aureus* that allow it to resist a variety of ß-lactam antibiotics (6).

The *S. aureus* is a common pathogen in healthy and immune-compromised hosts, including hemodialysis patients (7). Hemodialysis patients are frequently exposed to *S. aureus* because of their regular and frequent hospital stays, contact with arteriovenous fistulas, and use of central venous catheters (8). Hemodialysis patients are most affected by bacteremia, infective endocarditis, and osteoarticular, skin, and soft tissue infections. Among them, bacteremia is the most known clinical manifestation and infection for hemodialysis patients (9).

The hands of Health Care workers (HCWs), food handlers, and cleaners are good representatives or vectors for the transmission of *S. aureus* to hemodialysis patients during providing care for patients, improper food processing and handling, or touching the contaminated environmental surface and improper waste disposal, respectively (10, 11). Hemodialysis patients are most affected by bacteremia, infective endocarditis, and osteoarticular, skin, and soft tissue infections. Among them, bacteremia is the most known clinical manifestation and infection for hemodialysis patients (9).

The virulence factors' role in disease pathogenesis, tissue damage, and treatment failure has led to their recognition as potential therapeutic targets (12). For methicillin-sensitive *S. aureus* (MSSA) strains, penicillin continues to be the first-line treatment; for MRSA strains, linezolid, daptomycin, tigecycline, and vancomycin are all effective (13). Direct patient-to-patient contact, patient-to-healthcare provider contact, or even the reverse is the mode of transmission (14).

Antimicrobial resistance can occur by altered drug targets, enzymatic drug inactivation, increased efflux of antimicrobial compounds, and altered drug accessibility (15). This resistance mechanism is a serious issue that affects the entire world and is linked to increasing economic costs, mortality, and morbidity risks in developing nations (16). In the study conducted by Abera et al., 2008 methicillin-resistant Staphylococci (MRSA and MRCoNS) were high with prevalence rate of be 55% and 78% respectively (17). MRSA infection poses a concern to public health on a global scale, and in recent years, *S. aureus* isolates that are completely resistant to vancomycin have developed (18). Therefore, continuing research is essential to monitor the burden of MRSA and VRSA and this study aimed to determining the prevalence of MRSA and VRSA among hemodialysis patients and their contacts at hemodialysis clinics in Bahir Dar, Northwest Ethiopia.

1.2 Statement of the Problem

Globally in 2019, more than 100,000 deaths and 3·5 million disabilities were attributed to MRSA (19). The frequency of vancomycin-resistant *S. aureus* and vancomycin-intermediate *S. aureus* has increased by 2% and 3.6%, respectively (20). According to the 2017 Centers for Disease Control and Prevention (CDC) report in United States, at least 119,247 people had serious infections with MRSA and MSSA, resulting in 19,832 deaths annually" (21). Nosocomial infections due to MRSA are 6.2% per 10,000 patients (22). Different studies conducted in Felege Hiwot Compressive Specialized Hospital showed that the prevalence of MRSA is high. It ranges from 16.8% up to 73.6% (23, 24).

Antimicrobial resistance bacteria like MRSA are a prior global and public health concerns. They are highly associated with morbidity and mortality, longer patients stay in hospitals, increased cost for treatment and use of combination therapy than monotherapy(25). Increasing the consumption of vancomycin and genetic material transferred from Enterococci to *S. aureus* increased the occurrence of VRSA (26, 27). A study in Japan found that patients with MRSA bacteremia spent more money and stayed in the hospital longer than those with MSSA (28).

The *S. aureus* is one of the major pathogens involved in Hospital Acquired Infections (HAIs). It is transmitted through HCWs contaminated hands, healthcare attires and uniforms in the healthcare settings (29). HCWs attire is assumed to be the most frequently colonized with MRSA and VRSA (30). Thus, MRSA continues to represent a significant burden to healthcare systems and patients and is considered as the World Health Organization's list of high-priority pathogens and the development of new antibiotics due to inappropriate use of antibiotics and infection prevention practices (31).

Hemodialysis patients appear to have a greater risk of *S. aureus* colonization, and infection is the second leading cause of death in hemodialysis patients (32). This patient is exposed for different drug resistance microorganisms due to low immunity, longer hospital stay, longer duration on hemodialysis, multiple catheter sites, longer catheterization, and chronic diseases like diabetes (33). Moreover, *S. aureus* is powerful to colonize patients on hemodialysis and result serious infections such as skin and soft tissues, pneumonia, endocarditis, bacteremia and sepsis due to its ability to secrete antigens (capsule, adhesins), enzymes (coagulase, lipase, hyaluronidase and nuclease), and toxins (β-Toxin, δ-Toxin, P-V Leukocidins, Enterotoxin, Exfoliative toxin, Toxic Shock Syndrome Toxin) (34). Antibiotic resistance makes it difficult to treat *S. aureus* infections, (35, 36).

Hemodialysis patients are susceptible to various drug-resistant bacteria that lead to bacteremia and eventually death. Additionally, colonization in these patients increases the risk of the spread of the microorganisms both in health institutions and the community because they can act as asymptomatic reservoirs and transporters of these bacteria for long periods, and the burden of these patients is not well known(37).

Within Felege Hiwot Comprehensive Specialized Hospital (FHCSH) and Gamby General Teaching Hospital (GGTH) patients attending to get hemodialysis services are frequent. In the hemodialysis clinics, there is close contact and interaction among patients, healthcare workers, cleaners and food handlers.

As a result, we hypothesized that, there is a high level of contamination of the clinic with MRSA and VRSA and these superbugs can easily have transmitted from staffs and other contacts in the clinic to patients attending for hemodialysis. However, there is no published data on MRSA and VRSA colonization among patients attending for hemodialysis and their contacts in the hemodialysis clinics in Ethiopia and in the current study area (FHCSH and GGTH).

Therefore, this study aimed to determine the carriage of MRSA and VRSA and identify factors associated with MRSA and VRSA among hemodialysis patients and their contacts body and attires in the hemodialysis clinics of the above-mentioned hospitals.

1.3 Significance of the study

The results of this study had provided measurable evidence on the magnitude of MRSA and VRSA at hemodialysis clinics and the potential of working clothing of health care workers, food handlers, and hospital cleaners to carry VRSA and MRSA and used as source of infection for patients on hemodialysis at the hemodialysis clinics at FHCSH and GGTH. It may contribute to creating awareness about infection prevention and controls at healthcare provision sites. Findings from this study also used to improve the proper way of using personal protective equipment (working clothes) and help to develop worksite or local regulations which are used for properly using and handling working clothes and practicing regular hand hygiene. The result of this study could be used as a base-line data for further studies.

2. LITERATURE REVIEW

2.1 Bacteriology and pathogenesis of MRSA and VRSA

As a result of Methicillin-sensitive *S. aureus* (MSSA) strains to acquire the mecA gene-containing *Staphylococcal* Cassette Chromosome mec (SCCmec) and *S. aureus* strains with decreased vancomycin sensitivity, MRSA and VRSA were created respectively (38). Methicillin and vancomycin abuse, clinical illnesses like tumor chemotherapy, preoperative antibiotic prevention, and organ transplantation are all factors in the development of MRSA and VRSA, which are *S. aureus* subtypes that have evolved into these two strains. Drug resistance can arise when the drug target is changed in a way that prevents the inhibitor from binding (15).

A study by Quyet et al, in Northern Vietnam, shows that the resistance genes of MRSA mainly include mecA, femA, and femB (39). Different types of MRSA can be differentiated or identified by according to the type of *staphylococcal* cassette chromosome mec (SCCmec) gene. The MecA gene is responsible for methicillin resistance and is located on SCCmec(40). Vancomycin resistance is mediated by several gene clusters including *vanA, vanB, vanD, vanF, vanI, vanM, vanC, vanE, vanG, vanL, and vanN. The vanA, vanB, vanD, van F, vanI, and vanM* genes encode D-Ala: D-Lac ligases mediating high-level vancomycin resistance (27).

2.2 Nasal, catheter insertion sites, working clothes and hand carriage rate of MRSA and VRSA

Globally, MRSA accounts for almost 40% of all *S. aureus* isolates, while the prevalence and distribution of VRSA was 2.4% (20). A cross-sectional study was done at Phramongkutklao Hospital and Ananda Mahidol Hospital, Thailand to determine the nasal MRSA carriage, on hemodialysis patients. No MRSA was found (41). Another study was made in Taiwan to detect MRSA carriage among patients receiving hemodialysis; only 3.8% of MRSA were identified (42).

A study conducted at tertiary care hospitals in northeastern Brazil to determine MRSA and VRSA contamination level of hand and laboratory coats on HCWs from November 2012 to March 2013. The total contamination level of MRSA and VRSA from hand was 4.5% and 0.4%, respectively, and from the laboratory coats 3.75% and non-identified, in that order (43). Another study was made in Medellín, Colombia, on hemodialysis patients; 12.5% of MRSA were identified (44).

A cross-sectional study was performed in Tabriz, Iran, from July 6, 2018, to August 6, 2018, to detect MRSA colonization on HDPs. Among a total nasal swab samples, 30.7% were MRSA. While 28.7% of

MRSA were detected on their skin around catheter insertion (45). In the other study conducted in India, to investigate skin colonization and catheter-associated infection on HDPs, 24.1% of MRSA were identified (46).

The study was made in Northeast India on the hands of HCWs for the detection of MRSA, it was reported that 13% hand swabs were positive for MRSA (47). Another study made on the hand of HCWs to detect MRSA, only 4.46% were confirmed as MRSA (48). The other research was carried out at Gandaki Medical College and Teaching Hospital, Nepal, from April 2017 to December 2017 to assess MRSA contamination in the hands of healthcare workers. Among a total samples, 7% were MRSA, and no VRSA was observed (49).

A cross-sectional study was conducted in Menoufia University, Egypt to determine the hand carriage rates of VRSA on HCWs. The study reported that VRSA was 26.1% (50). Another study was carried out to determine the bacterial contamination of healthcare workers' white coats and hands on 154 swab samples. The study indicated that bacterial contamination of MRSA healthcare workers' hands and white coats was 22% and 24.7%, respectively (51). Another study conducted in Fez City, Morocco, from May 2015 to June 2016 among food handlers, 17.33% of them had MRSA (52).

A study was conducted in Al Jazirah State, Sudan, on 372 hand swab specimens collected from food handlers working in kitchens or cafeterias to determine the prevalence of methicillin-resistant *S. aureus*. The study indicated that PCR confirmed MRSA was 10.5% (53).

A cross-sectional study was conducted on people who handled food in hotels and restaurants in Jimma town, Ethiopia. The study reported that MRSA carriage was 2% (54). Another study was conducted in Addis Ababa Black Lion Hospital from May to August 2012 to detect MRSA and VRSA from the hands of participants. The study indicated that 46% of participants were contaminated with MRSA and 24% were VRSA (55). Another study was made at TASH, Addis Ababa, to detect MRSA gowns and uniforms carriage among healthcare workers; only 2.9% of MRSA were identified (56).

2.3 Antimicrobial Susceptibility Profile of MRSA and VRSA

A longitudinal study was conducted in Southern Italy's hospital on hands of HCWs from June 2017 to May 2018. Isolated *S. aureus* species, showed a high resistance pattern for cefoxitin (50%) while none resistant patter for vancomycin (57).

An antimicrobial susceptibility test was performed in the blood bank of the Brazilian Amazon. The total isolated MRSA, showed 100% resistance pattern for penicillin and cefoxitin. However, the resistance pattern of 44% was observed for erythromycin. Resistance to vancomycin was not detected (58). Another study was made in Mandalay Myanmar, isolated *S. aureus* showed a low resistance pattern for clindamycin (90.2%) and vancomycin (85.2%). On the other hand, the resistance pattern of 51.22% was observed for oxacillin, which is MRSA (59).

A study was conducted in north-west of Iran from July 6, 2018 to August 6, 2018 to assess nasal and extra nasal MRSA colonization in hemodialysis patients. Among isolated *S. aureus*, the resistance pattern of 63%, 25%, 22% and 20% was observed for cefoxitin, clindamycin, Trimethoprim /sulfamethoxazole and erythromycin, respectively.

A study was conducted in Menoufia University, Egypt from May 2020 to May 2021 to assess the nasal and hand carriage rates of methicillin-resistant *S. aureus* species. Isolated *S. aureus* was highly resistant to cefoxitin (97.8%), gentamycin (52.2%), ciprofloxacilin (60.9%), erythromycin (73.9%), and vancomycin (26.1%) (50).

A community-based cross-sectional study was conducted on the hands of food handlers working in hotels and restaurants from February to May 2017 in Jimma town. Isolated *S. aureus* showed a high resistance pattern for penicillin while a low resistance pattern for Ciprofloxacin (96.5%), Cefoxitin (95.3%), and Vancomycin (80%) (54).

2.4 Factors Associated with MRSA and VRSA

A systematic review and meta-analysis was conducted on USA from patients to identify risk factors for MRSA colonization. Hospitalization within the previous 12 months and chronic lung disease were associated with MRSA colonization. There is no statistically significant relationship between MRSA colonization and hypertension, antibiotic use within the previous six months, and coronary artery disease (CAD) (60).

The study which was conducted at a tertiary care hospital in northeastern Brazil from November 2012 to March 2013. Nursing technicians (P-value=0.001) were associated with MRSA carriage (61). Another study conducted on hemodialysis patients in Khoo Teck Puat Hospital, Singapore, diabetes mellitus and shorter hemodialysis duration were associated with MRSA colonization ($P < 0.05$) (62).

A cross-sectional study was conducted from May 2020 to May 2021 at the National Liver Institute, Menoufia University in Egypt, with 79 medical staff members. There is no statistically significant relationship between MRSA colonization and age, sex and working experience (50).

The study which was conducted in Debre Markos Referral Hospital to detect methicillin and vancomycin resistant *staphylococcus aureus* and associated factors from February-April 2020. Use of antibiotics were significant association with MRSA.

2.5 Conceptual framework

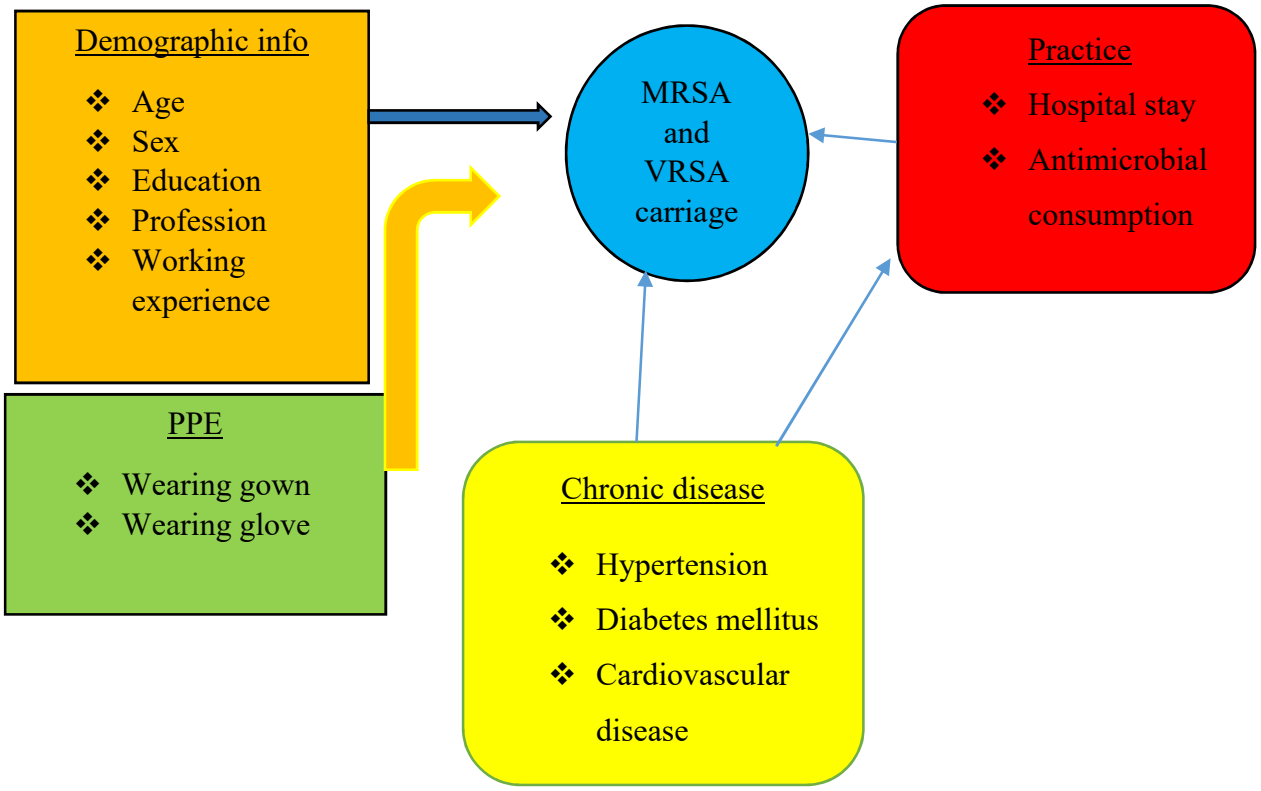

Figure :1 ***Conceptual framework for the study***

3. OBJECTIVES

3.1 General objective

- To assess MRSA and VRSA carriage, antimicrobial susceptibility pattern and associated factors among hemodialysis patients, health care workers, food handlers and cleaners at hemodialysis clinics of Bahir Dar, Northwest Ethiopia from 27 April 2023 to 24 June, 2023.

3.2 Specific objectives

1) To determine methicillin-resistant and vancomycin- resistant *Staphylococcus aureus* carriage among hemodialysis patients at hemodialysis clinics in Bahir Dar.
2) To determine methicillin-resistant and vancomycin- resistant *Staphylococcus aureus* carriage among healthcare workers, food handlers, and cleaners at hemodialysis clinics in Bahir Dar.
3) To determine the antimicrobial susceptibility pattern of *Staphylococcus aureus* and MRSA strain isolated from hemodialysis patients, healthcare workers, food handlers, and cleaners at hemodialysis clinics in Bahir Dar.
4) To identify factors associated with the overall MRSA carriage.

4. MATERIALS AND METHODS

4.1 Study design, period, and area

A cross-sectional study was carried out at hemodialysis clinics of FHCSH and GGTH from 27 April 2023 to 24 June, 2023. These two hospitals are found in Bahir Dar city and are the only hospitals which have hemodialysis clinics. According to the Bahir Dar city administration plan and service officer annual report, the city has an estimated population size of 422,580 in 2022. Of which, 222,064 are males and 215,516 are females. At the time of sampling, FHCSH and GGTH hemodialysis clinics have 12 and 8 beds, respectively as well as 73 healthcare workers, 102 food handlers, 131 cleaners, and 94 hemodialysis patients.

4.2 Population

4.2.1 Study population

Hemodialysis patients, healthcare workers, food handlers and cleaners from hemodialysis clinics of FHCSH and GGTH were the studied population

4.2.2 Study participant

Hemodialysis patients, healthcare workers, food handlers, and cleaners from the hemodialysis clinics of FHCSH and GGTH that fulfilled the inclusion criteria and actually participated in this study were the study participants.

4.3 Inclusion and Exclusion criteria

4.3.1 Inclusion criteria

Hemodialysis patients, HCWs, food handlers and cleaners from hemodialysis clinics of FHCSH and GGTH were included.

4.3.2 Exclusion Criteria

Any guests or healthcare providers who hadn’t contact with the hemodialysis patient, and those who had hand antiseptic cream were excluded from the study. Hemodialysis patients who had nasal bleeding and received antibiotics therapy 2 weeks prior to the study were also excluded from the study.

4.4 Study variables

4.4.1 Dependent variables

Methicillin- resistant *S. aureus* and vancomycin- resistant *S. aureus*

4.4.2 Independent variables

Demographic variables such as age, sex, year of experience and antimicrobial consumption, hospital stay duration of hemodialysis patients, infection prevention training, chronic disease such as, hypertension, cardiovascular disease, and diabetes mellitus of the study participants.

4.5 Sample size determination and Sampling technique

The sample size was calculated using a single population proportion formula ($n = \frac{(Za/2)2\ P(1-P)}{d2}$) based on the assumption of a 95 % confidence interval (Zα/2 = 1.96), 5% margin of error, and prevalence of 46% hand swabs of MRSA from a previous study in Addis Ababa Black Lion Hospital, Ethiopia (55). The calculated sample size was 382. The sample size was allocated proportionally according to the total number of participants (Figure 2). A convenient sampling technique was employed to include the study participants. The hemodialysis patients and other study participants from both hospitals were included consecutively until the required sample size was achieved.

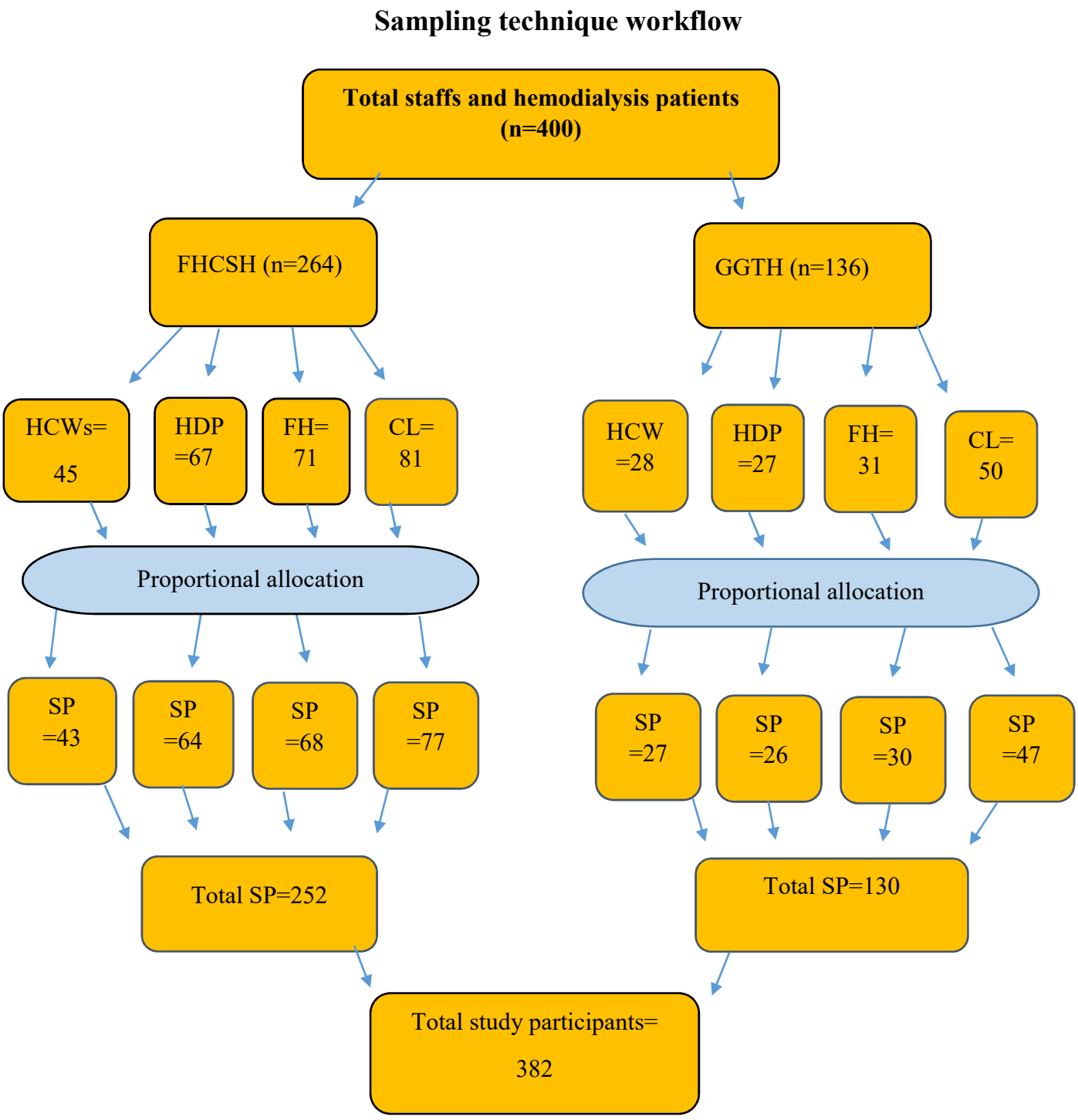

Figure: 2 Sampling technique workflow

Key: HCW= Healthcare worker CL = Cleaners, FH= Food handler, HDP= Hemodialysis patient SP, Study participants.

4.6 Operational Definitions

The following operational definitions were used for the interpretations of findings from this study

- **Contacts;** Healthcare workers, food handlers and cleaners at hemodialysis clinics of FHCSH and GGTH.
- **Staff: -** Health care workers, food handlers and cleaners, who are working in the hemodialysis clinics of FHCSH and Gamby Teaching General Specialized Hospital.
- **Workings clothe꞉ -** Gowns and uniforms were worn by staff at FHCSH and GGTH

4.7 Data collection

The socio-demographic data of participants related to MRSA and VRSA were collected by face-to-face interviews, self-administered interview, and direct observation using a standard data collection tool. Clinical data such as history of hypertension, hospital duration time, and history of diabetes mellitus were collected from patients' registration books. In addition, data on profession of the HCWs and the job of other staffs were collected by face-to-face interviews.

4.8 Specimen Collection

A total of 764 samples from 382 study participants were swabbed aseptically from hands (n = 292), working clothes (n = 292), nares (n = 90), and catheter insertion sites (n = 90), via moist sterile cotton swabs following standard procedures (63). Samples from hands were collected by a sterilized cotton swabs moistened with normal saline was rotated onto the overall surface area of both hands' palms and between the fingers of HCWs, food handlers, and cleaners whereas, sample from the working clothes were collected by rotating the sterile moistened cotton buds over the surface of sleeve ends, the collar and the lower part of the pocket. On the other hand, a sterile cotton swab moistened with normal saline was passed into the anterior nares of both nostrils of hemodialysis patients and rotated in both directions to obtain nasal swabs while, the skin of the hand around the catheter insertion were swabbed with a sterile swab moistened in a sterile saline. The sample from HCWs was collected during ready-to-health care provision time, while sample collection from food handlers, and cleaners were made, during preparation of food, and handling and cleaning of wastes, respectively. Samples from hemodialysis patients were collected before the central venous catheter was inserted into the skin. The collected swabs were quickly transported to the microbiology laboratory for additional processing after being promptly placed into a sterilized screw-capped tube containing Amies transport media.

4.9 Sample processing and identification

Swab samples were collected and inoculated onto 5% sheep blood agar (Oxoid, Ltd., UK), which was then incubated aerobically for 24 hours at 37°C (64). The culture examined and smooth white or yellow hemolysis colonies were sub- cultured on Mannitol Salt Agar (Oxoid, Ltd., UK) and incubated aerobically at 37ºc for 24 hours. After 24 hours of incubation, the culture was examined, and *S. aureus* produces a golden yellow colony on Mannitol Salt Agar. Then catalase tests were done direct from MSA, but Gram stain and coagulase tests were performed after subculture on 5% blood agar. *S. aureus* produced Gram-positive cocci that form clusters, catalase-positive, and coagulase production by converting fibrinogen to fibrin in plasma (65).

4.10 Antibiotic susceptibility testing

Antibiotic susceptibility testing was done for all *S. aureus* isolates using the Kirby Bauer disk diffusion susceptibility testing. Using a sterile loop, 4 colonies were picked and emulsified in 2 ml of sterile physiological water, and the turbidity of the suspended cells were standardized by naked eye with the 0.5 McFarland standard. By using sterile cotton tip swabs, the suspension was streaked on the entire surface of Mueller- Hinton (Oxoid, Ltd., UK) agar. Accordingly, CLSI guideline and local prescription protocol, 10 antibiotic discs namely Penicillin (10 µg), doxycycline (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), cefoxitin (30 µg), Clindamycin (2µg), Erythromycin (5 µg), Gentamicin (10 µg), Vancomycin (30 µg) and Trimethoprim /sulfamethoxazole (23.5/1.5 µg) were placed evenly (about 24 mm apart) and incubated aerobically at 35 ^{o}c for 18 hours. Following incubation, the inhibitory zone's diameter was measured with a clear ruler and expressed in millimeters. Finally, the results were interpreted as susceptible, intermediate, and resistant according to Clinical and Laboratory Standard Institute 2022 (CLSI, 2022) guidelines (66).

4.11 Detection of Methicillin Resistant *Staphylococcus aureus*

The MRSA was detected by Kirby Bauer susceptibility test using cefoxitin discs. Cefoxitin (30µg) discs were placed on *S. aureus* isolates streaked on Muller Hinton Agar (MHA) plates by considering the 24 mm distance between each disc and 15 mm from the border according to the CLSI 2022(66). Then, the medium was incubated aerobically at 35 ℃ for 18 hours. The result examined and interpreted by looking the zone of inhibition, and inhibition zone of ≥22mm for cefoxitin was interpreted as *S. aureus* sensitive to methicillin, while inhibition zone of ≤ 21mm was interpreted as methicillin resistant *S. aureus* (66) (Figure 3

4.12 Determination of vancomycin resistant *Staphylococcus aureus*

The *S. aureus* isolates which showed a vancomycin zone of inhibition size of <17mm in the above disc diffusion method and/or MRSA were further tested for the detection of vancomycin resistant *S. aureus* (VRSA) and vancomycin intermediate *S. aureus* (VISA) by commercially available Vancomycin E-test strips (E-test) (66).Hence, a pure colony of MRSA or Vancomycin zone size <17mm isolates were taken from subcultured blood agar and emulsified with saline and the turbidity was compared to the turbidity standard (0.5 McFarland standards). The suspensions of isolates were inoculated on the MHA (Gradient diffusion technique) plates. Vancomycin E- test strip was applied over the MHA plate with the minimum inhibitory concentration (MIC) scale facing the opening of the plate. Plates were incubated aerobically at 35 ℃ for 18 h and a teardrop zone of inhibition was observed. The zone edge intersecting the graded strip at the minimum concentration of the antibiotic was interpreted as the MIC. Based on CLSI 2022 (66), isolates MIC breakpoints such as ≤2 µg/mL, 4–8 µg/mL and ≥16 µg/ml were considered as sensitive, intermediate, and resistant to vancomycin, respectively (Figure 3).

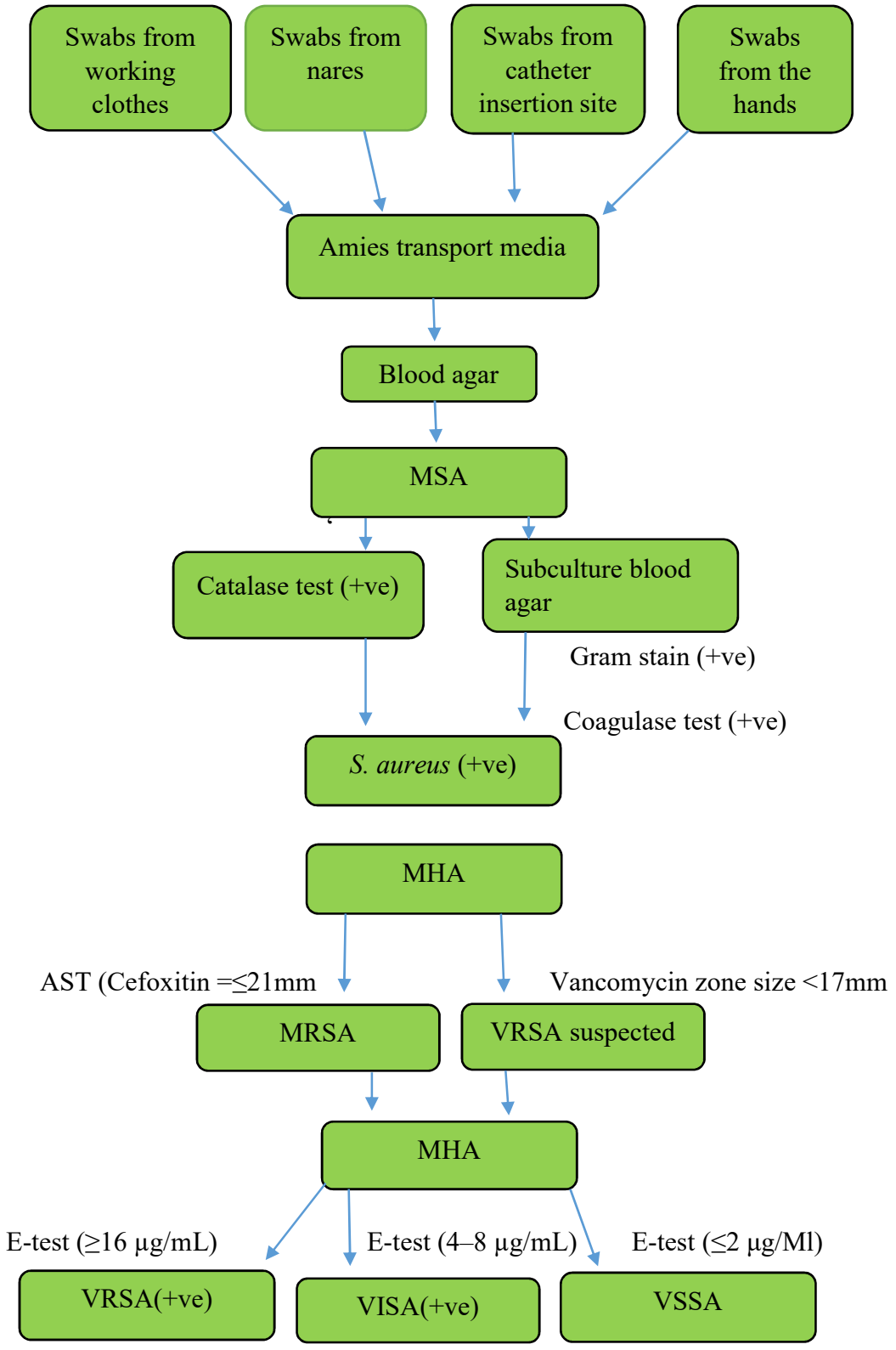

Figure: 3 Workflow of MRSA and VRSA identification

4.13 Quality Control

The questionnaires were prepared in English, and translated into the local language (Amharic) for better understanding of the respondents, and finally, the collected data was translated into English for analysis and presentation. The complete filling of socio-demographic data and questionnaires were checked at the site during collection from the individual participant. Bacteriological sample collection procedures were strictly followed. All culture media were prepared according to the manufacturer's instructions. A sterility test of the culture media was done by randomly picking 2% of the prepared culture media, incubating it aerobically at 37℃ for 24 hours, and observed for any growth. *S. aureus* American Type Culture Collection (ATCC) 25923) was used as a positive control to check the performance of the media and antibiotic susceptibility testing. Normal saline was tested for sterility by inoculating on Mannitol Salt Agar and observed for any growth after incubating for 24 hours at 37ºc. Guidelines from the Clinical and Laboratory Standards Institute (CLSI, 2022) were utilized to interpret the findings. The results were recorded carefully and checked before data entry and analysis.

4.14 Data analysis and interpretation

Data was entered into Statistical Package for the Social Sciences (SPSS) version 25 for statistical analysis. Descriptive statistics were presented using text, tables, figures, and percentages. The normality of data distribution in the case of age of the study participants were checked by histogram distribution curve. Chi-square and independent samples T test were computed to see the association between variables. Before regression analysis, frequencies, mean, standard deviation were used to describe the study population in relation to socio-demographic and other related variables. Bivariable and multiple-variable logistic regression analyses were performed to determine the association between outcome and independent variables. Variables with p-value of < 0.25 in the bivariable analyses were considered in the multiple logistic regression model and variables with a p-value < 0.05 at 95% CI in the final mode of the multivariable logistic regression model were considered statistically significant.

4.15 Ethical considerations

Ethical approval was obtained from the Institutional Review Board of College of Medicine and Health Sciences of Bahir Dar University and a support letter was written from APHI to FHCSH and GGTH. Permission letter was obtained from FHCSH and GGTH administrations before data and sample collection. The purpose of the study was clearly explained to study participants and informed written consent was obtained before sampling. Furthermore, the name and any identification of the respondents were not recorded to ensure confidentiality and a code was used to manage the data. Laboratory results were reported to physicians for the management of MRSA and VRSA in the hemodialysis clinics.

4.16 Dissemination of the results

The finding of this study will be disseminated to Bahir Dar University College of Medicine and Health Sciences, Department of Medical Laboratory Sciences, Amhara Public Health Institute, Amhara Regional Health Bureau, Gamby General Teaching Hospital, and Felege Hiwot comprehensive Specialized Hospital FHCSH. Finally, a manuscript will be prepared and submitted to peer- reviewed journals for possible publication.

5. RESULTS

5.1 Socio-demographic and clinical characteristics of study participants

A total of 764 samples from 382 study participants (90 from the nose, and 90 from skin around the catheter of 90 HDPs, 292 from working clothes and 292 from the hands of 70 HCWs, 98 food handlers and 124 cleaners) were collected and examined (Table 1). Of the 382 study participants, 257 (67%) were females. The mean age of study participants was 43 ± 11.8 for HDPs, 31 ± 4.2 for HCWS, 25 ± 4.3 for food handlers and 23 ± 4 years for cleaners. The majority (58%) of staffs served for less than two years. In terms of profession of HCWs, the majority (86%) were nurses (Table 3).

5.2 MRSA and VRSA carriage rates per sample sources

Overall, 15.4% (n = 59) of the study participants had either MRSA or VRSA carriage. The proportion of MRSA carriage was 13.9% (n = 53). The overall proportion of VRSA carriage was 1.6% (n = 6) (Table 1).

From 90 HDPs screened, 5 (5.5%) had MRSA contamination both in their nasal and catheter insertion site at the same time. The proportion of MRSA contamination on both the working clothes and hands of HCWs was 5.7%. In 8.6% of HCWs, only the working clothes were contaminated by MRSA. From 124 cleaners, 14.5% had MRSA either on their hands or working clothes or both sample sources. The proportion of carriage was 5.8% in both samples and 6.2% only from the working clothes. Of the 98 food handlers, 10.2% had MRSA contamination and the rate of contamination on both hands and working clothes was 4.1%. The rate of VRSA contamination was 2.4% among cleaners (Table 1).

Table 1. MRSA and VRSA carriage rates per clinical samples of the different study participants in the hemodialysis clinics of Bahir Dar, 2023

Sample sources	MRSA	VRSA	Total
	N (%)	N (%)	N (%)
From HDPs nasal only (n = 90)	5 (5.5)	0 (0)	5 (5.5)
From HDPs catheter insertion site only (n = 90)	1 (1.1)	0 (0)	1 (1.1)
From HDPs nasal and catheter insertion samples (n = 90)	5 (5.5)	1 (1.1)	6 (6.7)
Total HDPs(n=90)	**11 (12.2)**	**1 (1.1)**	**12 (13.3)**
From HCWs hand samples only (n = 70)	4 (5.7)	0 (0)	4 (5.7)
From HCWs Working clothes only (n=70)	6 (8.6)	0 (0)	6 (8.6)
From HCWs hand and WC samples (n=70)	4 (5.7)	1 (1.4)	5 (7.1)
Total HCWs (n=70)	**14 (20)**	**1 (1.4)**	**15 (21.4)**
From cleaners hand samples only (n=124)	1 (0.8)	0 (0)	1 (0.8)
From cleaner WC samples only (n=124)	8 (6.5)	2 (1.6)	10 (8.1)
From cleaner hand and WC samples (n=124)	9 (7.3)	1 (0.8)	10 (8.1)
Total cleaners (n=124)	**18 (14.5)**	**3 (2.4)**	**21 (16.9)**
From FHs hands samples only (n=98)	2 (2)	1 (1)	3 (3.1)
From FHs WC samples only (n=98)	4 (4.1)	0 (0)	4 (4.1)
From FHs hand and WC samples (n=98)	4 (4.1)	0 (0)	4 (4.1)
Total food handlers (98)	**10 (10.2)**	**1 (1)**	**11 (11.2)**
Total study participants (n=382)	**53 (13.9)**	**6 (1.6)**	**59 (15.4)**

Key: HDPs; Hemodialysis patients, HCWs; Health Care Workers, FHs; Food Handlers, CI; Catheter Insertion sites, WC; Working Clothes.

5.3 MRSA and VRSA carriage differences between study participants and sample sources

A total of 241 *S. aureus* were isolated from 764 samples, of which 75 were MRSA. From 382 study participants, 161 carried *S. aureus*, of which 32.9% (53/161) were MRSA. The overall rate of MRSA carriage was 13.9% (53/382), (95% CI: 10–17%). The proportion of VRSA among MRSA and vancomycin discs' zone of inhibition <17mm was 6.7% (6/90), (95% CI: 0–3%).

As shown in Table 2, the proportion of MRSA was 20% among HCWs, 14.5% among cleaners, 12.2% among HDPs, and 10.2% among food handlers. The difference was statistically significant (p= 0.008). The proportion of MRSA carriage was significantly higher in the anterior nares (11.1%) than catheter insertion sites (6.7%) of HDPs (p<0.001). The proportion of MRSA was significantly higher in the working clothes (14.3%) than the hands (10%) of HCWs (p = 0.025). Similarly, carriage of MRSA was significantly higher in the working clothes (14.5%) than the hands (8.1%) of cleaners (p<0.001)

Table 2. MRSA and VRSA carriage differences among study participants and their respective sample sources

Type of study participants	**MRSA status**		
	MRSA Pos	MRSA Neg	*P-value*
HDPs (n=90)	11 (12.2)	79 (87.8)	0.008
HCWs (n=70)	14 (20)	56 (80)	
FH (n=98)	10 (10.2)	88 (89.8)	
Cleaners (n=124)	18 (14.5)	106 (85.5)	
Sample source per study participants			
Nasal swab from HDPs (n=90)	10 (11.1)	80 (88.9)	< 0.001
Catheter insertion samples from HDPs (n=90)	6 (6.7)	84 (93.3)	
Swab from the hands of HCWs (n=70)	7 (10)	63 (90)	
Swab from working clothes of HCWs (n=70)	10 (14.3)	60 (85.7)	0.025
Swab from the hands of cleaners (n=124)	10 (8.1)	114 (91.9)	
Swab from working clothes of cleaners (n=124)	18 (14.5)	107 (85.5)	<0.001
Swab from the hands of food handlers (n=98)	6 (6.1)	92 (93.9)	
Swab from working clothes of food handlers (n=98)	8 (8.2)	90 (91.8)	0.125

5.4 MRSA carriage and demographic, and other explanatory variables

As shown in Table 3, overall, the proportion of MRSA carriage was higher (17.9%) in females (n=46) compared to males (n=7, 5.6%) ($p < 0.001$). Similarly, the rate of MRSA contamination was significantly higher (27.3%) among female HDPS compared to males (3.4%) (p=0.007). The proportion of MRSA contamination was also higher among females HCWs (37.1%) compared to males (2.9%) (p=0.018). While, the proportion of MRSA contamination was 75% among male cleaners compared to females (13%) (p=0.045). In terms of profession, the proportion of MRSA carriage was higher among nurses (21.7%) compared to other health care workers (12.5%). However, the difference was not statistical significant (p=0.470) (Table 3).

Overall, the proportion of MRSA carriage was significantly higher among study participants with diabetes mellitus (n = 18, 23.4%) than those without (n = 35, 11.5%) (p = 0.002). Similarly, the proportion of the total MRSA carriage was higher among HDPs with diabetes mellitus (18.4%) compared to those without (4.9%) (p= 0.043). The mean age was significantly lower in HCWs with MRSA (30.6+than those with MRSA negative (33+ 5.1) (p=0.012). Similarly, the mean age was significantly lower in overall study participants with MRSA (29.8+than those with MRSA negative (31.5+9.9) (p=0.034).

Table 3 : Association between MRSA carriage status of HDP, HCWs, food handlers and cleaners with different variables of interest , 2023.

Variables	MRSA in HDPS		MRSA in HCW		MRSA in Cleaners		MRSA in food handlers		MRSA Neg Total	MRSA total
	Pos **N(%)**	Neg **N(%)**	Pos **N(%)**	Neg **N(%)**	Pos **N(%)**	Neg **N(%)**	Pos **N(%)**	Neg **N(%)**	Pos N(%)	Pos **N(%)**
Sex										
Male	2(3.4)	56(96.6)	1 (2.9)	34(98.1)	3 (75)	1(25)	1(3.6)	27(96.4)	118(94.4)	7 (5.6)
Female	9(27.3)	23(72.7)	13(37.1)	22(62.9)	15(12.5)	105(87.5)	9 (12.9)	61(87.1)	211(82.1)	46(17.9)
p-value	**0.007**		**0.018**		**0.045**		**0.025**		**<0.001**	

Profession for HCWs										
Medical doctors	-	-	1(12.5)	7(87.5)	-	-	-	-	7 (87.5)	1(12.5)
Anesthetists	-	-	0(0)	2(100)	-	-	-	-	2 (100)	0(0)
Nursing	-	-	13(21.7)	47(78.3)	-	-	-	-	47 (78.3)	13(21.7)
p-value	-		**0.470**		-		**-**		**0.470**	
Mean age	41.7± 11.4	44.5 ± 12.3	30.6± 3.2	31 ± 5.1	22±4.6	24.1±3.3	24±5.1	26+4.1	29.57± 7.4	31.5±9.9
p-value	0.474		0.012		0.079		0.365		0.034	
Work Experiences in years										
< 2	-	-	1(100)	0 (0)	12(12.6)	83(88.4)	7(9.5)	67(90.5)	150(88.2)	20(11.8)
2 - 4	-	-	0 (0)	1(100)	5 (20.8)	19(79.2)	3(13.6)	19(86.4)	39(82.9)	8 (17.1)
5 - 7	-	-	2(25)	6(75)	1 (20)	4(80)	0(0)	2(100)	12(80)	3 (20)
8 - 10	-	-	7(23.3)	23(76.7)	0 (0)	0(0)	0(0)	0(0)	23(76.7)	7 (23.3)
➢ >10	-	-	4(13.3)	26(86.7)	0 (0)	0(0)		0(0)	26(86.7)	4 (13.3)
p-value	**-**		**0.325**		**0.25**		**0.263**		**0.325**	
History of Hospitalization										

Yes	2(15.4)	11(84.6)	0 (0)	5(100)	2 (40)	3(60)	0 (0)	6(100)	25(86.2)	4 (13.8)
No	9(11.7)	68(88.3)	14(21.5)	51(78.5)	16(13.4)	103(86.6)	10(10.9)	82(89.1)	304(86.1)	49 (13.9)
p-value	**0.708**		**0.59**		**0.25**		**0.697**		**0.492**	
Have DM										
Yes	9(18.4)	40(81.6)	4(33.3)	8(66.7)	3 (30)	7(70)	2 (33.3)	4(66.7)	59 (76.6)	18 (23.4)
No	2 (4.9)	39(95.1)	10(17.2)	48(82.8)	15(13.2)	99(86.8)	8 (8.7)	84(91.3)	270(88.5)	35(11.5)
p-value	**0.043**		**0.208**		**0.145**		**0.055**		**0.002**	
Hypertension										
Yes	0 (0)	29(100)	1(16.7)	5(83.3)	0 (0)	0 (0)	0(0)	6(100)	40 (97.6)	1 (2.4)
No	11(18)	50(82)	13(20.3)	51(79.7)	18(14.5)	106(85.5)	10(10.9)	82(89.1)	289 (84.8)	52(15.2)
p-value	**0.253**		**0.832**		**NA**		**0.397**		**0.215**	
Antibiotics prescribed										
Yes	1 (4)	24(96)	3(25)	9(75)	-	-	-	-	33 (89.2)	4(10.8)
No	10(15.4)	55(84.6)	11(19)	47(81)	-	-	-	-	102 (82.9)	21(17.1)
p-value	**0.342**		**0.637**		**-**		-			**0.369**

Key: HDPs; Hemodialysis patients, HCWs; Health care workers, CVD; Cardiovascular disease, DM; Diabetes mellitus

5.4 Antibiotic susceptibility profile of the *S. aureus*

Susceptibility testing for 10 antibiotics was determined on 241 *S. aureus* isolates by Kirby Bauer disk diffusion susceptibility testing. *S. aureus* isolates from HDPs' anterior nares, HCWs working clothes, and the hands of HCWs and food handlers' were 100% resistant to penicillin. Overall, S. *aureus* isolates revealed resistance 31%, 24%,18%, and 17% resistance to cefoxitin, erythromycin, gentamicin, and cotrimoxazole, respectively. Isolates from the anterior nares, and catheter-inserted sites of HDPs, and hands of cleaners showed 33%, 33% and 24% resistant to methicillin, erythromycin and gentamicin, respectively. *S. aureus* isolates from all swab samples, showed high rates of susceptibility to clindamycin (94%), chloramphenicol (88%), doxycycline (86%), and ciprofloxacin (85%) (Table-4).

Table 4: Antimicrobial susceptibility test profiles of the *S. aureus* from working clothes, hand, nares and catheter inserted site of study participants 2023.

Antibiotics		***S. aureus* isolates from different samples and participants**								
		HDPs nares N=30	HDPs CI N=24	HCWs WC N=29	HCWs hand N=23	FH WC N=31	FH hand N=21	Cleaner WC N=45	Cleaner hand N=38	Total N=241
FOX	S	20 (67)	18 (75)	19 (65)	16 (70)	23 (74)	14 (67)	28 (62)	28 (74)	166 (69)
	R	10 (33)	6 (25)	10 (35)	7 (30)	8 (26)	7 (33)	17 (38)	10 (26)	75 (31)
EM	S	19 (63)	15 (63)	20 (69)	15 (66)	23 (74)	13 (62)	28 (62)	27 (71)	160 (66)
	I	3 (10)	1 (4)	4 (14)	4 (17)	3 (10)	3 (14)	6 (13)	0 (0)	24 (10)
	R	8 (27)	8 (33)	5 (17)	4 (17)	5 (16)	5 (24)	11 (25)	11 (29)	57 (24)
CL	S	27 (90)	21 (88)	28 (97)	21 (91)	30 (97)	20 (95)	42 (93)	37 (97)	226 (94)
	I	0 (0)	2 (8)	1 (3)	0 (0)	0 (0)	1 (5)	1 (2)	0 (0)	5 (2)
	R	3 (10)	1 (4)	0 (0)	2 (9)	1 (3)	0 (0)	2 (5)	1 (3)	10 (4)
CN	S	17 (57)	20 (83)	23 (79)	19 (82)	27 (87)	17 (81)	36 (80)	29 (76)	188 (78)
	I	0 (0)	0 (0)	2 (7)	2 (9)	0 (0)	1 (5)	4 (9)	0 (0)	9 (4)
	R	13 (43)	4 (17)	4 (14)	2 (9)	4 (13)	3 (14)	5 (11)	9 (24)	44 (18)
PEN	S	0 (0)	2 (8)	0 (0)	0 (0)	1 (3)	0 (0)	4 (9)	3 (8)	10 (4)
	R	30(100)	22 (92)	29(100)	23(100)	30 (97)	21(100)	42 (91)	35 (92)	231 (96)
SXT	S	16 (53)	19 (79)	24 (83)	18 (78)	26 (84)	17 (81)	39 (87)	30 (79)	189 (78)

	I	4 (14)	0 (0)	0 (0)	2 (9)	2 (6)	1 (5)	0 (0)	3 (8)	12 (5)
	R	10 (33)	5 (21)	5 (17)	3 (13)	3 (10)	3 (14)	6 (13)	5 (13)	40 (17)
CI	S	21 (70)	19 (79)	26 (90)	20 (87)	28 (90)	18 (86)	40 (89)	33 (87)	205 (85)
	I	4 (13)	2 (8)	1 (3)	1 (4)	0 (0)	1 (5)	1 (2)	2 (5)	12 (5)
	R	5 (17)	3 (13)	2 (7)	2 (9)	3 (10)	2 (9)	4 (9)	3 (8)	24 (10)
CHR	S	23 (77)	21 (88)	26 (90)	21 (92)	29 (94)	19 (90)	41 (91)	33 (87)	213 (88)
	I	3 (10)	1 (4)	1 (3)	1 (4)	1(3)	0 (0)	0 (0)	3 (8)	10 (4)
	R	4 (13)	2 (8)	2 (7)	1 (4)	1 (3)	2 (10)	4 (9)	2 (5)	18 (8)
VAN	S	17 (57)	14 (58)	16 (55)	12 (52)	20 (65)	11 (52)	20 (44)	25 (66)	135 (56)
	I	1 (3)	2 (8)	3 (10)	5 (22)	4 (13)	4 (19)	7 (16)	6 (16)	32 (13)
	R	12 (40)	8 (34)	10 (35)	6 (26)	7 (22)	6 (29)	18 (40)	7 (18)	74 (31)
E-test	S	13 (93)	11 (92)	14 (93)	10 (91)	14 (93)	12 (100)	22(88)	16(94)	112 (93)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	1 (7)	1 (8)	1 (7)	1 (9)	1 (7)	0 (0)	3 (12)	1 (6)	9 (7)
DC	S	22 (73)	20 (83)	25 (86)	20 (87)	27(87)	18 (86)	42 (93)	33 (87)	207 (86)
	I	1 (4)	0 (0)	1 (3)	1 (4)	1 (3)	0 (0)	0 (0)	2 (5)	6 (2)
	R	7 (23)	4 (17)	3 (11)	2 (9)	3 (10)	3 (14)	3 (7)	3 (8)	28 (12)

PEN: Penicillin; CHR: Chloramphenicol; CI: Ciprofloxacin; CN: Gentamicin; FOX: Cefoxitin; SXT: trimethoprim-sulfamethoxazole; CL: Clindamycin; EM: Erythromycin; DC: Doxycycline; VAN: Vancomycin; HDPs: Hemodialysis patients; HCWs: Healthcare workers; FH: Food handlers.

5.6 Factors associated with MRSA

From sociodemographic characteristics of study participants, females had a significantly higher number of MRSA isolates compared to males (AOR: 5.69, 95% CI: 2.266–14.303, P<0.001). Diabetes mellitus (AOR = 3.38, 95% CI: 1.548–7.377, p=0.002) and improper use of antibiotics (AOR = 12.650, 95% CI: 6.135-26.087, p < 0.001) exhibited a significant association with the overall MRSA carriage. On the other hand, trained infection prevention (P=0.045, AOR = 3.746, 95% CI: 1.029–13.642) for healthcare workers, sharing towels (p=0.002, AOR = 4.189, 95% CI: 1.679–10.452) for food handlers and cleaners were significantly associated with MRSA colonization. (Table 8).

Table 5. Bivariable and multivariable analysis to identify factor associated with the overall MRSA carriage 2023.

Variables	Category	MRSA		COR (95% CI)	P-value	AOR (95% CI)	P-value
		Pos	Neg				
Sex	Male	7 (5.6)	118(94.4)	1		1	
	Female	46 (17.9)	211(82.1)	3.68(1.61-8.39)	0.002	5.69 (2.27-14.3)	<0.001*
Improper use of antibiotic	Yes	41 (35)	76 (65)	11.37(5.69-22.73)	0.000	12.65(6.14-26.09)	<0.001*
	No	12 (4.5)	253(95.5)	1		1	
Diabetes mellitus	Yes	18 (23.4)	59 (76.6)	2.35(1.25-4.44)	0.008	3.38 (1.55-7.38)	0.002*
	No	35 (11.5)	270(88.5)	1		1	
Share towels of FHCs	Yes	14 (25.5)	41 (74.5)	3.73(1.65-8.45)	0.002	4.19 (1.68-10.5)	0.002*
	No	14 (8.4)	153(91.6)	1		1	
Trained IP of HCWs	Yes	5 (11.6)	38 (88.4)	1	0.033	1	
	No	9 (33.3)	18 (66.7)	3.8(1.11-12.98)		3.75(1.03-13.6)	0.045*

Key: HDPs; Hemodialysis patients, FHC; Food handlers and Cleaners IP; infection prevention.

6. DISCUSSION

The emergency and distribution of MRSA and VRSA are a challenge or threat to humans, and WHO has put them on their priority list (22). Therefore, identifying MRSA and VRSA carriage that are common contaminants of nares and catheter insertion sites for HDPs, working clothes and hands for HCWs, food handlers and cleaners, are important to intervene nosocomial infection and the spread of antimicrobial resistance. This study presented the carriage of MRSA and VRSA among hemodialysis patients, HCWs, cleaners and food handlers in the hemodialysis clinics of Bahir Dar which is the first of its type in Ethiopia.

In the present study, the overall proportion of culture-confirmed MRSA carriage was 14%. This finding is lower compared to previous study in Black Lion Hospital, Ethiopia (46%) (55), and Egypt (22%) (51). In contrast, it was higher than other studies done in northeastern Brazil (43) and Nepal (49) that reported 5% and 7%, respectively. Furthermore, the overall proportion of VRSA carriage (2%)in the present study is consistent with reports from northeastern Brazil (2%) (43) and Nepal (0%) (49). However, our finding was much lower than a study done in Egypt (26.1%) (50). This variation might be associated with differences in terms of professions, infection prevention & control practices, and use of antimicrobials across studied hospitals. Lack of hand washing before and after each activity, and easy access to antimicrobial drugs might contribute to a considerable MRSA carriage (67). So, strict use of antiseptics, hand washing before and after each activity, proper use of antibiotics, and proper handling of waste are mandatory to prevent and reduce MRSA and VRSA carriage.

In the present study, the proportion of MRSA carriage in the nares of HDPs' was 11%, which is consistent with a study conducted in Medellin, Colombia, (12.5%) among HDPs (44). However, it was higher than a study in Thailand (0%) and Taiwan (3.8%) (41, 42). In contrast, it was found to be much lower than other studies done in Iran (30.7%) (45). Similarly, from the total nasal swabs isolate, 1% was VRSA in the present study. This figure was lower than a report from Menoufia University in Egypt (26.1%) (50). This might be associated with variations in fingering of the nasal, exposed to the healthcare environment, use of nasal mupirocin, receive prolonged courses of antibiotics, immunity, use of personal protective equipment, especially masks, and accessibility of antimicrobial susceptibility testing facilities (68).

In this study, the proportion of MRSA and VRSA carriage among HDP catheter-insertion site was 7% and 1%, respectively. This is lower compared to a study done in India, in which the prevalence of MRSA was

24.1% (46) and in Iran (28.7%) (45). The low MRSA carriage among HDPs in the present study might be attributed to the presence of trained dialysis personnel, use of sterile techniques during catheter insertion, proper use of antiseptics and the hands of health care workers who do comply with infection control precautions(69). Similarly, health care workers wearing gloves, surgical masks, or face shields when removing the catheter cap and dressing changes, meticulous care of hemodialysis catheters, use of skin cream and good bathing habits might be contribute decrease of MRSA(70).

The rate of MRSA contamination on working clothes of HCWs' in this study (14%) is higher than previous studies done in Addis Ababa (2.9%) (56) and Northeastern Brazil (3.8%) (43). However, higher rate of contamination has been reported in Mansoura University Children's Hospital (24.7%), Egypt (51). This could be due to variations in: in infection prevention training, following standard guidelines concerning the management of attire, sampling methods and methods of detection of MRSA, laundering practices, poor perceptions about contamination of white coats, and a poor waste management system(71).

The proportion of HCWs hands MRSA carriage (10%) was in line with previous reports in northeastern Brazil (4.5%) (43), northeastern India (13%) (47), and Nepal (7%) (49). On the other hand, it was lower than earlier studies in Addis Ababa (46%) (55) and Egypt (22%) (51). Likewise, the proportion of HCWs hand-carried with VRSA (1%) was lower compared to reports in Addis Ababa (13%) (55) and Egypt (26.1%) (50). These variations might be due to poor practice habits, close contact with sick people, frequent contact with contaminated medical devices and environmental surfaces, and awareness of the transmission of resistant organisms.

The proportion of food handlers and cleaners working clothes contaminated with MRSA in this study was 11%, which is in agreement with similar study findings in Al Jazirah State, Sudan, which reported 10.9% MRSA (53). On the other hand, our finding was higher than a report from Jimma town (2%), Ethiopia (54). In contrast, it was found to be lower than other studies done in Morocco (17.3%) (52). This variation could be due to differences in disinfection preparation during working clothes washed, personal cleanliness habits of the workers, sample size, laboratory method, use of antimicrobial drugs, and the availability of antimicrobial susceptibility tests for selecting the most effective drugs.

The proportion of food handlers and cleaners' hands contaminated with MRSA in this study was 8%, whereas the prevalence of VRSA was 1%. This finding of MRSA was supported by a study done in Al Jazirah State (10.9%), Sudan (53). However, it was higher compared to a study in Jimma town (2%), Ethiopia (54). The high prevalence of MRSA in the present study might be associated with a lack of

screening and treating of workers, self-medication, a lack of awareness and training about antimicrobial resistance microorganisms, level of education, and poor infection control practices.

In our study, the rate of MRSA carriage is significantly higher in HCWs (20%) and cleaners (15%) as compared to HDPs and food handlers. This may be due to frequently contact with different patients, asymptomatic carrier, hospitals toilet seats, contaminated medical devices, environmental surfaces, patients' wastages and working highly risk full rooms for a long time. In addition, in hospital busy working environment in the hemodialysis clinic set up, and insufficient time(30, 72).

The concurrent occurrence of MRSA in both the nasal cavity and catheter insertion sites of HDPs was 6%, whereas on both the working clothes and hands of HCWs, cleaners and food handlers was 6%, 7% and 5%, respectively. This might be due to dry hands by own gown/working clothes, air born contamination, fingering nose by hands. Hand contamination of study participants are a major problem. The rate is high as they are the source of spread everywhere, whether catheter insertion, nasal cavity or white coats(73). Therefore, strengthening of infection prevention and control is mandatory to reduce the above type of infection or source of transmission.

The *S. aureus* isolates tested in our study exhibited a range of antimicrobial sensitivity profiles to the various antibiotics. *S. aureus* exhibited 100% penicillin resistance on HDPs nares. On the other hand, it showed the least resistance to clindamycin, chloramphenicol, and ciprofloxacin at 4%, 8%, and 13% on HDPs catheter insertion site, respectively. This finding was coherent with other studies done in Brazilian that reported 100% resistance of *S. aureus* to penicillin (58). On the other hand, our finding was lower than a report from Iran (45) that reported 62% and 25% resistance of *S. aureus* to cefoxitin and clindamycin, respectively.

S. aureus showed 100%, 35% and 33% resistance to penicillin, cefoxitin and erythromycin on HCWS working clothe, respectively. However, it showed the least resistance to clindamycin (0%) followed by ciprofloxacin (7%), chloramphenicol (7%) and doxycycline (10%). This finding was lower than a similar study done in Egypt (50), that reported 97.8%, 52.2% and 73.9% resistance of *S. aureus* to cefoxitin, ciprofloxacin and erythromycin, respectively.

S. aureus isolates from food handlers of the present study showed 100% resistance to Penicillin. However, most of *S. aureus* isolates from food handlers were sensitive to clindamycin, ciprofloxacin, chloramphenicol and doxycycline with sensitivity rates of 97%, 90%, 94% and 87%, respectively. The

present finding was in line with a similar study done in Jimma town that reported 90.7% resistance of *S. aureus* to penicillin and 96.5% sensitive to ciprofloxacin (54). Likewise, similar finding was also reported in Myanmar where 90.2% of the isolates were sensitive to clindamycin (59). So in general, the predominance of *S. aureus* was resistant to penicillin. While, it is susceptible to clindamycin, ciprofloxacin, chloramphenicol, and doxycycline, this provide clue used as a treatment of choice.

From the total study participants, *S. aureus* isolates showed higher level of resistance to Penicillin at 96%. This may be due to marked the first natural antibiotic that leading to over-prescription, frequently used and overuse of penicillin(74). On the other hand, it showed the least resistance to clindamycin (94%) followed by chloramphenicol (88%), doxycycline (86%) and ciprofloxacin (85%).The reported an increased susceptibility attributed by a number of factors including, it does not confer cross resistant with ß- lactam antibiotics, used as second line treatment or after fall of ß- lactam antibiotics and reserved for resistance strains(74).

In this study, the rate of MRSA carriage was significantly higher in females than males ($p < 0.001$), which might be due to the enrolment of more female's in food handler and cleaner programs. In addition, female food handlers and cleaners, did not trim their fingernails as compared to males. Therefore, strict trained females to infection prevention protocol, transmission and prevention of resistance microbes, and regular hand washing practice is required to reduce health care-associated infection, proper management of patients and monitor the spread of drug-resistant pathogens.

In the current study, study participants who use antibiotics inappropriately were more contaminated with MRSA carriage than others ($p < 0.001$). This finding could be explained by the fact that study participants with self-medication, doses that not meet the requirements, not taken for the recommended time period and frequency leading to antibiotics drug resistance when the bacteria will not die or stop multiply in the presence of an antibiotic to which they are susceptible (71). A study by Matthew J. Arduino and the Centers for Disease Control and Prevention(69) supported our finding in that received prolonged courses of antimicrobial agents were found predictors of MRSA and VRSA carriage among patients in hemodialysis centers.

Participants with diabetes mellitus were 3.38 times more likely to have MRSA than participants free from MRSA at the time of sampling. This finding could be explained by the fact that patients with diabetes mellitus are more likely to have obese, which lowers antibiotic blood concentrations, impaired immune

systems, and visit healthcare facilities frequently to manage complications on a regular basis. Similar finding, was reported in Singapore's Khoo Teck Puat Hospital.(62).

MRSA carriage was significantly associated with HCWs who were not trained in infection prevention control and food handles and cleaners who shared towels. Unlike our study, systematic review and meta-analysis done in USA, hospitalization within the previous 12 months and chronic lung disease were found an associated with MRSA colonization(60).

7. CONCLUSION

Carriage of MRSA and VRSA are common in hemodialysis patients and health care workers, food handlers and cleaners working in the hemodialysis clinics. The concurrent carriage of MRSA in both the nose and catheter insertion sites of HDPs, on both the working clothes and hands of health care workers, food handlers, and cleaners is a major concern. Based on the findings, use antibiotics inappropriately, diabetes mellitus and being females were found significantly associated with MRSA and MRSA was significantly prevalent in nasal of HDPs, working clothes of HCWs and cleaners. Both S. aureus and MRSA isolates are susceptible to clindamycin.

8. RECOMMENDATIONS

Based on the findings of these studies,

- HCWs who work at FHCSH and GGTH hemodialysis clinics create awareness of the transmission of MRSA and inappropriate uses of antibiotics to HDP study participants to reduce the spreading of MRSA and VRSA infection.
- ARHB, FHCSH and GGTH administrations should be periodically updated to HCWs study participants on the rational use of antibiotics, standard treatment guidelines, and the practice of good infection prevention and control (IPC) at the hemodialysis clinic.
- Additionally, participants in MRSA and VRSA-colonized studies should be treated with mupirocin nasal disinfection or chlorhexidine skin disinfection to lower the risk of transmission.
- FHCSH and GGTH administrations, HCWs, should train to cleaners and food handlers on proper disinfectant preparation and follow SOPs for disinfectant preparation to reduce the spreading and carriage of MRSA and VRSA.

9. LIMITATION

The limitation of this study is that rabbit plasma could not be used for coagulase test identification of *S. aureus.* Further, daptomycin, linezolid, and ceftaroline antibiotic disks are not used for VRSA, and automated commercial phenotypic methods such as VITEK are not used for MRSA and VRSA isolations.

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11. ANNEXES

I. Research Participant Information Sheet

Study title: MRSA and VRSA carriage and associated factors among hemodialysis patients, health care workers, food handlers and cleaners at hemodialysis clinics of Bahir Dar, Northwest Ethiopia from 27 April 2023 to 24 June, 2023.

Investigator: Abrham Gebremedhin Gebremeskel

Organization: College of Medicine and Health Sciences, Bahir Dar University

Healthcare workers, hemodialysis patients, food handlers, and hospital waste handlers working cloth, hand, nasal, and catheter insertion swabs are invited to be involved in research that provides practical evidence in the study area. It is better to understand the information in this form before deciding to participate in the research since it outlines your rights and our responsibilities to you. This information and consent form consists of the purpose, examinations, possible advantages, risks, and inconveniences related to this study. Your right to resign from taking part in the research is also explained below. You have the right to ask any question at any time regarding the research. Your participation is voluntary. You may talk to anyone you feel joyful with, and you may take time to decide whether to participate or not.

Purpose of the Research Project: - This study aims to assess MRSA and VRSA carriage and associated factors among hemodialysis patients and their contacts at hemodialysis clinics in Bahir Dar City hospitals, Northwest Ethiopia, in 2022. In line with this, the findings also provided baseline information for health sector administrators, concerned bodies, and the study participants themselves in planning and managing nosocomial infections that reduce the transmission associated with drug resistance by *S. aureus* species.

Procedure:

If you are willing to participate in the study and are eligible, you need to understand the purpose of the study and give your consent. You were interviewed about socio-demographics, risk factors, hand hygiene, and using working clothes with a structured questionnaire for not more than 20 minutes. The required sample was collected from the desired part of your hands, working clothes, nasal area, and around catheter insertion with a sterile swab moistened in normal saline by the principal investigator.

The risk associated with the study:

There is no physical or psychological risk and no damage resulting from the research procedures.

Benefits of the study:

This study had a crucial value for the prevention of nosocomial or hospital-acquired infections and community-acquired infections. The results of this study are important for providing indirect additional information for effective treatment of *S. aureus* and MRSA. It is also important for government officials and healthcare professionals to take appropriate corrective actions.

Compensation: You did not receive any payment for your participation in this study.

Confidentiality of your information:

All information gathered from the study participants was kept confidential. Your participation in this study is strictly anonymous, and only a code was used to identify your information. Personal information was treated confidentially, and it was not transmitted to any person or organization under any circumstances. The results of this study were evaluated and summarized, and feedback on the results was given to the study participants by the principal investigator. Your name did not appear in any database, report, or publication resulting from this study.

Right to Refusal or Withdraw:

Your participation in this study is purely voluntary, and you have the right to cease participation at any time, or you may refuse to answer some of the questions if you feel uncomfortable. You are free to refuse to participate in the study, or you can withdraw your consent at any time without giving reasons, and this does not involve any penalty or loss of benefits to which you are entitled. If you are not comfortable, please feel free to stop it at any level of the study.

Ethical considerations:

A formal letter of ethical clearance was obtained from the Ethical Committee of the College of Medicine and Health Sciences, Bahir Dar University, and a permission letter was obtained from APHI before starting the task. A support letter from the FHCSH administrator was also obtained and shown to the concerned body. Before starting data collection, the purpose of the study was explained to all respondents. Furthermore, the names and any identification of the respondents were not recorded to ensure confidentiality.

Person to Contacts:

If you want to know more information, you can contact the following individuals, and you may ask at any time.

Sr. No	Name	Phone no.	Email address

1	Abrham Gebremedhin	+251942951578	abrhamgebremedhin38@gmail.com

II. የተሳታፊ መረጃ

የጥናት ርዕስ፡- በባህር ዳር ከተማ በሚገኙ በሄሞዳያሊስስ ክሊኒኮች ዉስጥ ያሉ ሄሞዳያሊስስ ታማሚዎች እንዲሁም ከታካሚዎች ጋር ግንኙነት ያላቸዉ ሰዎች ሜታሲሊን እና ቫንኮሚሲን የሚቋቋም ስታፊሎኮከስ አውሬስ እና ተዛማጅ ሁኔታዎች ምን ይመስላል።

መርማሪ፡- አብርሀም ገብረመድህን ገብረመስቀል

ድርጅት፡- ባህር ዳር ዩኒቨርሲቲ ህክምናና ጤና ሳይንስ ኮሌጅ

ስፖንሰር፡ የአማራ ክልላዊ መንግስት ጤና ቢሮ

የሄሞዳያሊስስ ታማሚዎች፣ የጤና ባለሙያ ሰራተኞች፣ ምግብ አብሳዮች እና የጽዳት ሰራተኞች ጥናቱ ላይ እንዲሳተፉ ተጋብዘዋል። በጥናቱ ውስጥ ለመሳተፍ ከመወሰንዎ በፊት በዚህ ቅጽ ውስጥ ያለውን መረጃ መረዳት የተሻለ ነው ምክንያቱም መብቶችዎን እና ለእርስዎ ያሉትን ሀላፊነቶች ስለሚገልጽ። ጥናቱን በሚመለከት በማንኛውም ጊዜ ማንኛውንም ጥያቄ የመጠየቅ መብት አልዎት። የእርስዎ ተሳትፎ በፈቃደኝነት ላይ የተመሰረተ ነው። ለርስዎ ደስታ የሚሰጠዎትን ማንኛውንም ሰው ማነጋገር ይችላሉ እና ለመሳተፍ ወይም ላለመሳተፍ ለመወሰን ጊዜ ሊወስዱ ይችላሉ።

የምርምር ፕሮጄክቱ ዓላማ፡- ይህ ጥናት ሜታሲሊን እና ቫንኮሚሲን የሚቋቋም እስታፊሎኮከስ አውረስ ባክቴሪያ በሄሞዳያሊስስ ታማሚዎች እና ግንኙነት ካላቸዉ ሰራተኞች ላይ በፈለገሂወት እና በጋምቢ አጠቃላይ ሆስፒታል ምን እንደሚመስል ለመገምገም ያለመ ነው። የጥናቱ ተሳታፊዎች ራሳቸውን እና ሆስፒታሉን ከዚህ ባክቴሪያ የሚከላከሉበትን እና የሚቀንሱበትን ሁኔታ የመነሻ መረጃዎችን ይሰጣል።

ሂደት፡- በጥናቱ ለመሳተፍ ፍቃደኛ ከሆናችሁ እና ብቁ ከሆናችሁ የጥናቱ አላማ ተረድታችሁ ፍቃዳችሁን መስጠት አለባችሁ። ስለ ማህበራዊ፣ ተያያዥ ምክንያቶች፣ የእጅ ንፅህና ፣ የስራ ልብስዎ አጠቃቀም እና ሌላ ጥያቄዎችን ከተዋቀረ መጠይቅ ጋር ከ20 ደቂቃ ላነሰ ጊዜ ቃለ መጠይቅ ይደረግልዎታል። የሚፈለገውን ናሙና ከሚፈለገው የእጅዎ ክፍል፣ የስራ ልብስ፣አፍንጫ እና ካቲተር ባለበት ዙሪያ በዋና መርማሪው በኩል እርጥብ በሆነ እና በጸዳ እቃ ናሙና ይሰበሰባል።

ከጥናቱ ጋር የተዛመደ ስጋት፡- በምርምር ሂደቱ ምንም አይነት አካላዊ ወይም ስነ-ልቦናዊ አደጋ እና ጉዳት አይደርስበዎትም።

የጥናቱ ጥቅሞች፡- ይህ ጥናት በሆስፒታሉ ወይም በማህበረሰቡ ውስጥ በዚህ ባክቴሪያ ብክለት እንዳይኖር ለመከላከል ወሳኝ ጠቀሜታ ይኖረዋል። የዚህ ጥናት ውጤቶች ሜቲሲሊን እና ቫንኮሚሲን የሚቋቋም ስታፊሎኮከስ አውሬስ ለማከም በተዘዋዋሪ ጥሩ መረጃ ይሰጣሉ። እንዲሁም የመንግስት ባለስልጣናት እና የጤና ባለሙያዎች ተገቢውን የእርምት እርምጃዎችን እንዲወስዱ አስፈላጊ ነው።

ማካካሻ፡ በዚህ ጥናት ውስጥ ለተሳትፎዎ ምንም አይነት ክፍያ አያገኙም።

የመረጃዎ ምስጢራዊነት፡- ከጥናቱ ተሳታፊዎች የተሰበሰቡ ሁሉም መረጃዎች ሚስጥራዊ ሆነው ይቆያሉ። በዚህ ጥናት ውስጥ ያለዎት ተሳትፎ አይታዎቅም ስለሆነም መረጃዎን ለመለየት ኮድ ብቻ ጥቅም ላይ ይውላል። የግል መረጃ በሚስጥር ይያዛል፣ እና በማንኛውም ሁኔታ ለማንኛውም ሰው ወይም ድርጅት አይተላለፍም። በዚህ ጥናት ምክንያት የእርስዎ ስም በማንኛውም መረጃ ፣ ሪፖርት ወይም ህትመት ውስጥ አይታይም።

እምቢ የማለት ወይም የመውጣት መብት፡- በዚህ ጥናት ውስጥ ያለዎት ተሳትፎ በፈቃደኝነት ላይ ብቻ የተመሰረተ ነው፣ እና በማንኛውም ጊዜ ተሳትፎዎን የማቆም መብት አለዎት ወይም ካልተመቸዎት አንዳንድ ጥያቄዎችን አለመመለስ ይችላሉ። ይህ ጥናት ምንም ዓይነት ቅጣት የለዉም እንዲሁም ጥቅማጥቅሞችን አያሳጣም። ካልተመቻችሁ እባክዎን በማንኛውም ጊዜ ለማቆም ነፃነት ይሰማዎት።

ሥነ ምግባራዊ ጉዳዮች፡- መደበኛ የፍቃድ ደብዳቤ ከአማራ ክልል የህብረተሰብ በጤና ኢንስቲቱት ስራውን ከመጀመራችን በፊት ወስደናል። ከፈለገሂወት እና ጋምቢ ሆስፒታል አስተዳዳሪዎች የፈካድ ደብዳቤ ወስደን ለሚመለከተው አካል ሰጠን ፈቃድ አግኝተናል። መረጃ መሰብሰብ ከመጀመሩ በፊት የጥናቱ ዓላማ ለሁሉም ምላሽ ሰጪዎች ተገለጻል።

ማሳሰቢያ፡- ለበለጠ መረጃ ለማወቅ ከፈለጉ የሚከተለዉን ግለሰብ ማነጋገር እና መጠየቅ ይችላሉ።

1. አብርሀም ገብረመድህን ገብረመስቀል +251942951578 abrhamgebremedhin38@gmail.com

III. Consent form

Consent form

I, the undersigned study participant, have been well informed about the objective of the study entitled MRSA and VRSA carriage and associated factors among hemodialysis patients, health care workers, food handlers and cleaners at hemodialysis clinics of Bahir Dar, Northwest Ethiopia from 27 April 2023 to 24 June, 2023. I am also informed that all the information obtained during the course of the study is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of, decline to cooperate, and drop out of the study if I want, and none of my actions have had any bearing at all. I agreed voluntarily to provide the requested information.

Yes ☐ No ☐

Code _____

Signature _____

Date_____

IV. የስምምነት ዉል

እኔ ከዚህ በታች የፈረምኩት የጥናቱ ተሳታፊ ካቲተር ባለበት አካባቢ ፣ በስራ ልብስ፣በአፍንጫ እና እጅ ላይ ያለውን ሜቲሲሊን እና ቫንኮማይሲን የሚቋቋሙ ስታፊሎ ኮከስ አዉረስ የሚባለውን የባክቴሪያ ዝርያዎችን በጋንቢ እና በፈለገሂዎት ስፔሻላይዝድ ሆስፒታል በሄሞዲያሊሲስ ክሊኒክ ዉስጥ በሚታከሙ የኩላሊት ህመምተኞች እና ግንኙነት ካላቸዉ ሰራተኞች ውስጥ መኖራቸውን መለየት እና በምን ያህል መጠን እንደሚገኙ ለማሳወቅ የሚሰራውን ጥናት ፣ አላማ እና ጥቅም በሚገባ ተረድቻለሁ። ጥናቱ ላይ መሳተፍም ሆነ አለመሳተፍ በራሴ ፍቃድ የሚወሰን መሆኑንም ተገልጾልኛል። በተጨማሪም ከጥናቱ ባልሳተፍም ሆነ አቋርጬ ብወጣ ከሆስፒታሉ በማገኘዉ የህክምና አገልግሎት ምንም አይነት ችግር እንደማይደርስብኝ ተነግሮኛል።በመሆኑም አስፈላጊውን መረጃ እና ናሙና ለመስጠት አስፈላጊ መሆኑን ስለተስማማሁበት ለመስጠት ሙሉ ፈቃደኛ መሆኔን በፊርማዬ እገልጻለሁ።

የተሳታፊ ኮድ---------- ፊርማ _____ቀን _____

መረጃ ሰብሳቢው ስም ----------ፊረማ----------ቀን _____

V. Questionnaire English version for HDPs

Participant ID _____

Department _____

Date of the interview in the Gregorian calendar (dd/mm/yy) _____/_____/_____

Section 1. Socio-demographic data

1001. Sex of the respondent 1) Male 2) Female

1002. What is your age in a year? _____

1003. Education 1) Can't read and write 2) Primary 3) Secondary 4) Diploma and above

1004. Occupation 1) Farmer 2) Merchant 3) Civil servant 4) NGO 5) Housewife
6) Other _____(specify)

Section 2. Other clinical characteristics

2001.Did you take medicines below or above length of dates prescribed? 1)Yes 2) No

2002. Do you have history of hospitalization for the last six months? 1)Yes 2) No

2003. Do you have a history antibiotics prescribed in the last 6 months? 1)Yes 2) No

2004. Chronic disease 1) Diabetes mellitus 2) Hypertension 3) Cardiovascular disease 4) Other_____
(Specify)

VI. አማረኛ መጠይቅ ለኩላሊት በሽተኞች

ቀን (ቀን/ወር/ዓ/ም) _____/_____/_____ የተሳታፊ ኮድ _____ክፍል _____

ክፍል አንድ ማህበራዊ ጉዳይ በሚመለከት

1001. ዕድሜ _____

1002. ጾታ _____

1003. የትምህርት ደረጃ 1) የማያነብ 2) የመጀመሪያ ደረጃ 3) ሁለተኛ ደረጃ 4) ዲፕሎማ እና ከዚያ በላይ

1004. ስራ 1) ገበሬ 2) ነጋዴ 3) የመንግስት ሰራተኛ 4) መንግሥታዊ ያልሆነ ድርጅት 5) የቤት እመቤት 6) ሌላ _____ (ይግለጹ)

ክፍል ሁለት የጤና ባህሪያት

2001. ከታዘዙት ቀኖች በታች ወይም ከዚያ በላይ መድሃኒቶችን ወስደዉ ያዉቃሉ? 1) አዎ 2) አይ

2002. ላለፉት ስድስት ወራት ሆስፒታል ተኝተዉ ታክመው ያዉቃሉ? 1) አዎ 2) አይ

2003. ባለፉት 6 ወራት ውስጥ መድሃኒት ወስደዋል? 1) አዎ 2) አይ

2004. ተላላፊ ያልሆነ በሽታ አለብዎ? 1) አዎ 2) አይ

2005. ለጥያቄ ቁጥር 2019 መልስዎ አዎ ከሆነ የትኛዉ ተላላፊ ያልሆነ በሽታ ነው ያለብዎት? ከአንድ በላይ መልስ መምረጥ ይቻላል። 1) የስኪር በሽታ 2) የደም ግፊት 3) የልብ በሽታ 4) ሌላ_____(ይግለጹ)

VII: Questionnaire English version for HCWs

Participant ID _____

Department _____

Date of the interview in the Gregorian calendar (dd/mm/yy) _____/_____/_____

Section 1. Socio-demographic data

1001. Sex of the respondent 1) Male 2) Female

1002. What is your age in a year? _____

1003. Education 1) Diploma 2) Degree 3) MSC and above

1004. Profession 1) MD 2) Anesthetists 3) Clinical Nurses 5) Other_____ (Specify)

1005. Years/months of experience in this occupation_____

Section 2. Other clinical characteristics

2001. Have you trained infection prevention? 1)Yes 2) No

2002. Do you have MRSA or VRSA in the previse months? 1) Yes 2) No

2003. Are you Hospitalization in the previous 12 months? 1) Yes 2) No

2004. Do you have a history antibiotics prescribed in the last 6 months? 1)Yes 2) No

2005. Chronic disease 1) Diabetes mellitus 2) Hypertension 3) Cardiovascular disease 4) Other_____ (Specify)

VIII: Questionnaire English version for food handlers and cleaners (FHC)

Participant ID _____

Department _____

Date of the interview in the Gregorian calendar (dd/mm/yy) _____/_____/_____

Section 1. Socio-demographic data

1001. Sex of the respondent 1) Male 2) Female

1002. What is your age in a year? _____

1003. Education 1) Can't read and write 2) Primary 3) Secondary 4) Diploma & above

1004. Occupation 1) Food handler 2) Cleaner

1005. Years/months of experience in this occupation_____

Section 2. Other clinical characteristics

2001.Do you handle body fluids with bare hands if gloves are not available? 1) Yes 2) No

2002. Do you share towels? 1) Yes 2) No

2003.Do you have history of hospitalization for the last six months? 1)Yes 2) No

2004. Chronic disease 1) Diabetes mellitus 2) Hypertension 3) Cardiovascular disease 4) Other_____
(Specify)

IX. አማረኛ መጠይቅ ለምግብ አብሳይ እና ለጽዳት ሰራትኞች

የተሳታፊ ኮድ _____ ክፍል _____

የቃለ መጠይቅ የተሰተበት ቀን (ቀን/ወር/ዓ/ም) _____/_____/_____

ክፍል አንድ ማህበራዊ ጉዳይ በሚመለከት

1005. ዕድሜ _____

1006. ጾታ _____

1007. የትምህርት ደረጃ 1) 2) የመጀመሪያ ደረጃ 3) ሁለተኛ ደረጃ 4) ዲፕሎማ እና ከዚያ በላይ

1008. ስራ 1) የጽዳት ሰራተኛ 2) ምግብ አብሳይ

1009. የስራ ልምድ _____

ክፍል ሁለት የጤና ባህሪያት

2001. የእጅ ጓንት በሌለ ሰአት የሰውነት ፈሳሾችን ማለትም ደም እና ሌሎችን ቆሻሻዎችን በባዶ እጅዎ ይነካሉ? 1) አዎ 2) አይ

2002. ፎጣዎችን ተዋዉሰዉ ይለብሳሉ? 1) አዎ 2) አይ

2003. ላለፉት ስድስት ወራት ሆስፒታል ተኝተዉ ታክመው ያዉቃሉ? 1) አዎ 2) አይ

2004. ተላላፊ ያልሆኑ በሽታዎች አለብህ/ሽ? 1) አዎ 2) አይ

2005. ለጥያቄ ቁጥር 2009 መልስዎ አዎ ከሆነ የትኛዉ ተላላፊ ያልሆነ በሽታ ነው ያለብዎት? ከአንድ በላይ መልስ መምረጥ ይቻላል። 1) የስኪር በሽታ 2) የደም ግፊት 3) የካርዲዮቫስኩላር በሽታ 4) ሌላ _____ (ይግለጹ)

Annex X: Sample collection, culture media principle, preparation, storage, and interpretation

Necessary materials for sample collection: - Sterilized normal saline (physiological saline), sterilized cotton tips, sterilized test tube with screw cap, marker, and glove.

Sample collection procedure

1. Moisten the sterile cotton tip by rinsing it in sterilized normal saline
2. Roll the wet cotton tip on the sleeve ends, the collar, and mouth tips of both fronts down pockets of sample clothes (for clothing sampling), and roll the backside and palm side of both hands, a sterile cotton swab moistened with normal saline was passed into the anterior nares of both nostrils and skin surround catheter insertion for hemodialysis patients.
3. Immediately put the swab sample in a sterilized screw cap test tube. (Keep clothed sample, skin surround catheter insertion, anterior nares of both nostrils and hand sample in different test tubes)
4. Label with participant code and sample source (clothe (sleeve ends, the collar clothes, pockets), hand, anterior nares of both nostrils or skin surround catheter insertion)
5. Transport to the microbiology laboratory within 30 minutes.

Necessary materials for culture media preparation: - Culture media powder (mannitol salt agar, tryptone soya broth, Blood agar base powder, Mueller Hinton agar) analytical balance, distilled water, measuring cylinder, boiling bottle (flask), stove, autoclave, sterility indicator tape, Ph indicator, Bunsen burner, sterile Petri dish, clean heat resistant test tubes with screw cup, Nunc tube, Refrigerator.

Preparation: - Check for expire date and any formedness of media powder. All fluid and solid culture media are prepared as per manufacturers' instructions.

Storage: - Store the prepared media in the refrigerator at 2-8℃ by labeling it with the media name and preparation date.

Blood agar media preparation

Purpose: - This procedure provides instructions how to prepare Blood Agar Media.

Principle: - Meat extract and Peptone provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids in Blood Agar Base. The medium contains sodium chloride for the osmotic balance. Blood Agar Bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic microorganisms. Sheep blood gives best results for *S. aureus.*

Hemolytic patterns may vary with the source of animal blood or type of base medium used. The low pH helps in stabilization of red blood corpuscles and favors the formation of clear hemolysis zone.

Procedure

1. Weigh and Suspend 40 grams of powder in1littr distilled water

2. Mix thoroughly and heat to boiling to dissolve the medium completely with frequent agitation

3. When cool adjust the ph to 7.3

4. Autoclave at 15 lbs pressure at (121℃) for 15 minute

5. Cool the medium at 48℃

6. Add 50ml of sheep blood and mix with gentle rotation.

7. Dispense 20ml of the solution in to sterile Petri dish

8. Allow the medium to solidify label with date and store at 4 ℃

Identification: - A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms. The medium is often used to observe the forms of hemolysis from pathogenic microorganisms.

Limitations: - Blood agar is not a selective media so we couldn‟t differentiate microorganisms from the agar

Mannitol Salt Agar (MSA): -

PURPOSE: -This procedure provides instructions how to prepare mannitol salt agar.

PRINCIPLE: - Mannitol Salt Agar is a nutritive medium due to its content of peptones and beef extract, which supply essential growth factors, such as nitrogen, carbon, sulfur and trace nutrients. The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator, aids in the differentiation of staphylococcal species.

Procedure

1. Suspend 111grams of MSA powder in1liter distilled water.

2. Heat to dissolve the medium completely.

3. Autoclave at 15 lbs pressure at (121℃)

4. Cool and pour 20ml in to sterile Petri dish within safety cub net

5. Store at 2-8℃ cup to usage

Identification: - The grown organisms were identified by colony character on culture media, gram stain, and different biochemical tests.

Limitation: - Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium

Colony characteristics: - *S. aureus* produces a **yellow(golden)** colony with a yellow background on mannitol salt agar.

Mueller Hinton Agar

Principle of MHA

Mueller Hinton Media contains Beef Extract, Acid Hydrolysate of Casein, Starch, and Agar. Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, amino acids, sulfur, and other essential nutrients. Starch is added to absorb any toxic metabolites produced. **Starch** hydrolysis yields dextrose, which serves as a source of energy. Agar is the solidifying agent.

Preparation of MHA

1. Suspend 38 gm of the medium in one liter of distilled water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Pour cooled Mueller Hinton Agar into sterile Petri dishes on a level, horizontal surface to give uniform depth.
5. Allow cooling to room temperature.
6. Check for the final pH 7.3 ± 0.1 at 25ºC.
7. Store the plates at 2-8 ºC.

Limitations of MHA

1. Numerous factors can affect results: inoculum size, rate of growth, medium formulation, and pH. Strict adherence to protocol is required to ensure reliable results.
2. Drug inactivation may result from the prolonged incubation times required by slow growers. Variation in the concentration of divalent cations, primarily calcium and magnesium affects the result of aminoglycoside, tetracycline, and colistin test with *P. aeruginosa* isolates

XI. **Biochemical test**

Catalase: - This test is used to differentiate those bacteria that produce the enzyme catalase, such as *staphylococci*, from non-catalase-producing bacteria such as *streptococci*.

Principle: - Catalase acts as a catalyst in the breakdown of hydrogen peroxide into oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer.

Necessary materials (Required): -Hydrogen peroxide, (3%H2O2), clean slide, dropper,

Test Procedure

1. Check for the expiry date of 3% H2 O2
2. Place a growth colony on a clean slide and add a drop of hydrogen peroxide solution to it.
3. Look for immediate bubbling.

Interpretation: - Active bubbling = Positive catalase test, no bubbles = Negative catalase test

Coagulase test: - This test is used to identify *S. aureus* which produces the enzyme coagulase.

Test Principle: - Coagulase causes the plasma to clot by converting fibrinogen to fibrin.

Necessary material (Required): - Plasma, clean slide, dropper, distilled water.

Test Procedure

1. Place a drop of distilled water on each end of a slide or two separate slides.
2. Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.
3. Add a loopful (not more) of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds,
4. No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Interpretation: - Clumping within 10 secs = *S. aureus,* no clumping within 10 secs = No bound coagulase

XII. Anti-microbial Susceptibility Test (AST) (Kirby-Bauer disc diffusion technique)

Susceptibility (sensitivity) testing is used to select effective antimicrobial drugs. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organism.

Necessary materials (REQUIRED): - MHA, Antimicrobial discs, McFarland 0.5, Ruler

Procedure

1. Check for expire date of antimicrobial discs and bring to room temperature.
2. Using a sterile wire loop, touch 4 well-isolated colonies of similar appearance to the test
3. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities, it is easier to view against a printed card or sheet of paper.
4. Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions.
5. With the petri dish lid in place, allow 5 minutes (no longer than 15 minutes) for the surface of the agar to dry.
6. Using sterile forceps, a needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated plate. Note: The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.
7. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35°C for 18 h (temperatures over 35°C invalidate results for oxacillin).
8. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate measures the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.

Interpretation of zone sizes: - Using the Interpretative Chart, interpret the zone sizes of each antimicrobial, reporting the organisms Resistant, Intermediate, and Susceptible.

XII. Determination of MIC

Vancomycin MIC by E-test

It is an automated system for measuring the minimum inhibitory concentration of the bacterial isolate. It is very simple to perform MIC of the bacterial isolate as compared to other techniques like broth and agar dilution methods which are technically cumbersome. An elliptical zone of growth inhibition is seen around the strip after incubation. The MIC is read from the scale at the intersection of the zone with the strip. It is easy to interpret the result.

Necessary materials: -

- Muller-Hinton agar
- E-test strips (5X50 mm antibiotic carrier)
- McFarland Densitometer/ O.5 McFarland Std
- Forceps
- Sterile cotton swabs
- Sterile normal saline

Procedure opening an E-test package

1. Remove the package stored at -20 °C or -70°C.
2. Equilibrate at room temperature. This time takes approximately 30 minutes if stored at – 20°C and approximately one hour if stored at-70°C. Ensure all the moisture has evaporated before opening.
3. Inspect the package for holes or crack. Do not use if damaged.
4. Cut along with the broken line at the top of a blister. Do not cut between the blisters.
5. Tip the strip out of the opening slightly and take them out with forceps.
6. If strips stick together, twist them apart with your fingers.
7. Only the handle i.e. the area labeled E.
8. Place the strips to be used into a dry clean Petri dish.

Application of E-strips

- Apply E -test strips with forceps. Ensure the MIC scale is facing upward i.e. towards the opening of the plate.
- Emulsify 3 or 4 individual test strain colonies and transfer them to a tube of saline.
- Compare turbidity to that in the 0.5 McFarland standards. Adjust turbidity of inoculum to match that standard or read with McFarland Std.
- Ensure that the agar surface is dry before swabbing it. Dip a swab in the inoculum, remove excess fluid and swab the entire agar surface evenly in 3 directions (60℃).
- Allow the agar surface to dry for 10-15 minutes.
- Open the E-test package and place the strips in a dry Petri dish
- Apply the strips to the agar surface with forceps. Always apply the strip with the MIC scale facing the opening of the plate. Do not apply it upside down.
- Use the templates to position 1-2 strips on a 90 mm plate.
- Place the handle of the strip closest to the rim of the plate.
- Incubate at 37°C for 18-24 hours.

Result and Interpretation of E-test

- Read MIC at the point where the ellipse intersects the scale. If a MIC value between two twofold dilutions is seen, always round up to the highest value.
- Read the MIC value at complete inhibition of all growth.
- If the intersection differs on either side of the strip, read the MIC as the greater value. Ignore any growth at the edge of the strip.
- MIC values of the bacteria should be interpreted as S (Susceptible), I (Intermediate), or R (Resistant) by comparing the breakpoint values of each antibiotic with the criteria recommended by CLSI.

XIV. McFarland Standards

McFarland Standards are used as the reference to adjust the turbidity of the liquid/ bacterial suspension in the tube in the microbiology laboratory. It helps to maintain that the number of bacteria will be within a given range to standardize microbial testing. The McFarland standard can be prepared of varying concentrations ranging from 0.5 to 4 concentration and depending upon the concentration, the cell count density varies. However, the most commonly used concentration for antimicrobial susceptibility testing and the culture media performance testing is usually done by 0.5 McFarland standard in microbiological laboratories.

Principle of McFarland Standards

A McFarland Standard is a chemical solution of barium chloride and sulfuric acid. The chemical reaction between these two chemicals results in the production of a fine precipitate of barium sulfate. After shaking well, the turbidity of a McFarland Standard is visually comparable to a bacterial suspension of known concentration. McFarland turbidity standards are prepared by mixing various volumes of 1% sulfuric acid and 1% barium chloride to obtain solutions with specific optical densities. By adjusting the volume of these two chemical reagents, McFarland standards of varying degrees of turbidity can be prepared which represent different bacterial densities or cell counts. 0.5 McFarland turbidity standard provides an optical density comparable to the density of a bacterial suspension with 1.5 x 10^8 colony forming units (CFU/ml).

Preparation of 0.5 McFarland Standards

1. Prepare a 1% solution of anhydrous barium chloride (BaCl2) and a 1% solution of sulfuric acid (H2SO4)
2. Combine and completely mix the 0.05ml barium chloride and 9.95ml sulfuric acid solutions to form a turbid suspension.
3. Place the resulting mixture in a foil-covered screw-cap tube.
4. Store the McFarland standard at room temperature (25 °C) when not in use. McFarland standard density solution will precipitate and clump over time, and it needs vigorous
   agitation (vortexing) or shaking before each use. Note: Mark the tube to indicate the level of liquid, and check before use to be sure that evaporation has not occurred.

5. In the presence of good lighting, visually compare the turbidity by holding the bacterial sample and McFarland Standard tubes up against the black and white bars printed on the enclosed card.
6. In case of heavy turbidity, adjust the turbidity of the log growth of the bacterial suspension with the addition of broth or saline via a sterile pipette to match the turbidity to that of a known McFarland Equivalence Standard.
7. If the test suspension is too light, inoculate with additional organisms or incubate tubes until turbidity matches that of the standard.

McFarland Standard	1% BaCl2(ml)	1% H2SO4 (ml)	Approximate Cell Count Density (x10^8 cells)
0.5	0.05	9.95	1.5 x 10^8

Limitations of McFarland Standards

- In the case of the colored media, it may not provide proper contrast with McFarland Equivalence Standards with the occurrence of incorrect results or incorrect densities.
- During the storage time, exposure of the McFarland standard to the light can affect the turbidity measurement.

XVI. Quality control (QC)

Sterility and growth test.

Sterility test: - Incubate 5% of the batch at 37℃ overnight. Contamination by microorganisms capable of overnight growth was shown by turbidity in a fluid medium and growth on solid media. Media in Petri dishes are best examined for contamination immediately before use. All media, even those that have been sterility tested at the time of preparation, should always be checked visually immediately before being inoculated for any change in appearance that could indicate contamination or deterioration. A sterility test of normal saline is done by inoculating it on mannitol salt agar culture media and incubating it at 37℃ for 24 hours and checking for any growth.

Growth test: - The growing ability of the culture was tested by inoculating known microorganisms (*S. aureus*) which was obtained from Amhara Public Health Institute (APHI), incubate at 37℃ for overnight, the growth tested, and compare with the previous result.

Sample processing Before starting sample processing, disinfecting the working area with 5% bleach and performing of all sample processing procedures near to flame of the Bunsen burner is mandatory.

Declaration

This to certify that the thesis entitled "methicillin and vancomycin resistant *Staphylococcus aureus* carriage among hemodialysis patients, and their contacts at hemodialysis clinics in Bahir Dar City hospitals, Northwest Ethiopia", submitted in partial fulfilment of the requirements for the Master of science in medical microbiology of Department of medical laboratory science, Bahir Dar University, is a recode of original work carried out by me and has never been submitted to this or any other institution to get any other degree or certificates. The assistance and help I received during the course of this investigation have been duly acknowledged.

Abrham Gebremedhin (B.Sc.)

Signature: _____

Date of submission: _____

Advisor's approval form

I hereby certify that I have supervised, read, and evaluated this thesis titled methicillin-resistant and vancomycin-resistant *staphylococcus aureus* and associated factors among hemodialysis patients and their contacts at hemodialysis clinics in Bahir Dar, northwest Ethiopia by Abrham Gebremedhin prepared under my guidance. I recommend the thesis be submitted for oral defense.

Advisor's name	Signature	Date
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Co-Advisor's name	Signature	Date
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Department head	Signature	Date
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Examiners' Approval Form

As member of a board of examiners, we examined this dissertation/ thesis entitled methicillin-resistant and vancomycin-resistant *staphylococcus aureus* and associated factors among hemodialysis patients and their contacts at hemodialysis clinics in Bahir Dar, northwest Ethiopia by Abrham Gebremedhin. We hereby certify that the thesis / dissertation is accepted for fulfilling the requirements for the award of the degree of master of science in medical microbiology.

Board of Examiners

External examiners name	Signature	Date
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Internal examiners name	Signature	Date
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Chair person's name	Signature	Date
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