ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Staphylococcus aureus and *MRSA* Contamination level of Working Clothe, Hand Carriage, and Knowledge, Attitude and Practice Among Healthcare Workers at Federal Police Hospital; Addis Ababa, Ethiopia.

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A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology).

November, 2021, Addis Ababa, Ethiopia

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

This is to certify that the thesis prepared by Zewditu Kaba entitled: ***Staphylococcus aureus*** **and** ***MRSA*** **Contamination level of Working Clothe, Hand Carriage and Knowledge, Attitude and Practice Among Healthcare Workers at Federal Police Hospital; Addis Ababa, Ethiopia**. Submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Acknowledgments

Primarily praise is to the Almighty God for all my achievements. I like to express sincere appreciation and gratitude to my advisors Kassu Desta (MSs, Ph.D. fellow, Associate Professor of Medical Microbiology) for patiently inspiring and encouraging advice and Asegedech Asmamaw (MSc Medical microbiology) for constructive suggestions. I would like to acknowledge the Addis Ababa University College of Health Sciences, Department of Medical Laboratory Sciences, all dutiful instructors, teaching staff, and administrators. Great thanks to all Federal Police Hospital healthcare workers for sincere co-operation, appreciation, and encouragement. Special thanks to all my friends (Tesfaye Seboka, Bruktawit, Demem, Ethiopia, Wegayehu …), all laboratory staff who offer me time, material, ideas, and moral supports, EPHI microbiology department, and staff for all co-operation and supply. I'm great full for my beloved families, my husband Deresse Admasu, my kids Flagot, Carmel, and Naume for their incredible support and motivation.

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Abbreviations

CoNS	Coagulase Negative Staphylococcus
EPHI	Ethiopia Public Health Institute
FPH	Federal Police Hospital
HCW	Healthcare worker
KAP	Knowledge, attitude and practice
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSA	Mannitol Salt Agar
MSSA	Methicillin Sensitive *Staphylococcus aureus*
PPE	Personal Protective Equipment
S aureus	*Staphylococcus aureus*
SOP	Standard Operational procedure
WC	Working clothe

Abstract

Background: Contaminated workings clothe and hand of health care workers can cause nosocomial infections. *Staphylococcus aureus (S aureus)* is the main causative agent of nosocomial infection and it can be transmitted through working clothe of health care workers from patient to health care workers and from health care workers to patients at hospitals. Poor hand hygiene is the main way of spreading infectious organisms.

Objective: To assess *Staphylococcus aureus* and *MRSA* contamination level of Working Clothes, Hand carriage, and knowledge, attitude, and practice (KAP) among Health Care workers (HCWs) at Federal Police Hospital.

Methods: Cross-sectional study was conducted at the Federal police hospital (FPH) from November 2020-March 2021 by inclusion of 222 healthcare workers. 222 swab samples were collected from the working clothing of all participants and 53 hand swab samples were randomly collected from 53 HCWs. Culturing, identification, and antimicrobial sensitivity tests were performed at the Federal Police Hospital microbiology laboratory using conventional standard microbiology tests. The positive and negative control organisms' *S aureus* and *S epidermidis* were obtained from Ethiopian Public Health Institute (EPHI) microbiology department. The demographic data, knowledge, attitude, and practice of study participants were collected by providing self-administered questioner and observational checklists. The data entry was done by Microsoft Excel and chi-square data analysis done by using SPSS version 21 software.

Results: Out of 222 study participants, 39(17.6%) were contaminated with staphylococcus bacteria. A total of 41 staphylococci were isolated from 35 working clothes,2 hands, and 2 were isolated from both clothes and hands. From a total of 41 isolates, 18(43.9 %) *S aureus*, and 23(56.1%) *coagulase-negative Staphylococcus (CoNS)* were identified. 12(67%) *Methicillin sensitive S aureus (MSSA)* and 6(33%) *Methicillin-resistant S aureus (MRSA)* were identified from a total of 18 *S aureus* isolates. About 86.9% of study participants have good knowledge about the use and handling of working clothes related to bacterial contamination. 60% of them show a good attitude and 70.1% were practicing good on using and handling of working clothes related to the prevention of bacterial contamination.

Conclusion: In this study the contamination rate of working clothe and hand carriage of *S aoreus* was 6.8% and 5.6% respectively and 33% of the isolates were *MRSA*. Even though study participants have good knowledge, attitude, and practice about working clothe and hand hygiene continuous awerness of staff is paramount to prevent *MRSA* in the hospital.

Keywords: Clothe contamination, *S aureus* , *MRSA* , Healthcare worker, hand-carriage, Nosocomial.

1. Introduction

1.1. Background

S aureus is a significant organism for causing nosocomial and community-acquired infection. It causes serious superficial skin infections, localized abscesses, and food poisoning in humans. In hospitals, it is the main cause of infections associated with surgical wounds and indwelling medical devices (1). The identification characteristic of *S aureus* is it's a gram-positive cluster-forming coccus, non-motile, non-spore-forming, and facultative anaerobe organism (2).

S aureus is a pathogen to humans and also found on nasal passages, skin, and mucous membranes as normal flora (3).When cultured on mannitol salt agar it ferments glucose and produces a golden yellow colony which distinguished it from other *staphs*. The other identifying characteristics of this bacteria are catalase-positive, coagulase-positive. It can grow at temperature ranges from 15ºc to 45ºc and at NaCl concentrations as high as15% (2). It causes a wide range of pus-producing infections, food poisoning, and toxic shock syndrome. The human major combatting mechanism for *Staphylococcus* is phagocytosis. Antibodies neutralized toxins and promote opsonization (3). *Methicillin-resistant S aureus (MRSA)* causes diseases ranged from simple skin infection to bloodstream infection (sepsis) which may result in death (4). *S aureus* food poisoning caused by consumption of food contaminated by bacterial enterotoxin and the release of a bacterial super antigen in the bloodstream causes septic shock syndrome in human beings (2). Since the most spreading way of *S aureus* is skin contact, it spreads in hospitals to the patient, healthcare workers, and even to visitors by touching persons who are infected by staphs and contaminated objects (5).

This organism can cause a variety of simple self-limiting to serious life-threatening diseases in humans. *Staphylococcal* food intoxication involves the rapid onset of nausea, vomiting, abdominal pain, cramps, and diarrhea (6). The bacteria produce surface protein to colonize host tissue and overcome phagocytosis by its capsule and by producing immunoglobulin binding protein A (1). The skin infection appears red swollen or crusty skin. Early recognizing of sign helps to receive treatments before the infection become severe (6). The pathogenicity of *S aureus* is supported by its ability of producing different enzymes such as protease, lipase, and hyaluronidase those which destroy host tissue (7). The often causes of hospital-acquired *Staphylococcus* infection is the anti-microbial resistant strains (1). The *Staphylococcus aureus*

bacteria that is resistant to a certain antibiotic is called *Methicillin-Resistant Staphylococcus aureus/MRSA/*. *MRSA* causes severe infections among patients in the healthcare setting. The spread of *MRSA* can be prevented by good general hygiene, proper cleaning and covering of wounds, and with no sharing of personal items (6). Anyone can prevent staph infection at the hospital by washing hands before and after they touch every patient (5).The implementation of surface cleaning, antimicrobial surface, and hand hygiene are potentially important *MRSA* control measures in health settings (8).

Hands can be cleaned by washing thoroughly and by using alcohol-based gel. Wearing gloves and protective clothes at the time of treating wounds, and handling and processing body fluids prevent the spread of *S aureus*(5).

1.2 Statement of the Problem

According to CDC 2012 in the USA, in years 2005-2011, 80,461 severe infections and 11,285 deaths from *MRSA* were annually estimated (4). In a systemic review of 23 articles published in 2000-2020 and studied by Lean p *et a*l., the *MRSA* isolation rate from attires of HCWs was ranged from 1.3% to 79% (8). In a healthcare setting, the potential transmission source for pathogenic bacteria is the attire of healthcare providers (9). *S aureus* was the most frequently isolated organism from the uniforms of nurses and other medical staff (10). About one in three (33%) people carry *S aureus* bacteria in their nose without any illness. About two in every 100 people carry *methicillin-resistant Staphylococcus aureus (MRSA*).

Staphylococcus is mostly spread by skin-to-skin direct contact or by touching. Medical professionals and any healthcare providers, or even visitors may have *staph* germs on their body and then transmitted them to the patient and other HCW (5,6). Working clothes of HCW are known to be potential transmitting agents of multidrug-resistant organisms at different contamination rates (11, 12). The male medical professionals' white coats have more contamination rate than those of female physicians since males have the habit of keeping items in their pockets. Most females used other alternatives (handbag, purse) to keep their own belongs (13). Different types of healthcare workers' attires could be contaminated with *MRSA* at different rates of contamination. The contamination rate depends on exposing status of health care activities being provided.

Studies on the prevalence of bacterial contaminations in health care workers through personal items and working equipment were done in different university hospitals in Ethiopia (14-16) and showed that *S. aureus* was the most frequent isolate over other pathogens. In Addis Ababa city nine government hospitals' operating rooms were studied and it showed *S aureus* was the secondary predominant isolate of operating rooms. Even though the Addis Ababa federal police hospital participated in this study and the resulting outcome was not specifically disclosed in the article (17). Personal protective equipment (PPE) is an important component in infection control strategies to protect HCW against infections and the spread of infectious agents to others. By preventing contamination of HCWs' hands and garments, microbial infections in the individual HCP (healthcare provider), as well as secondary spread to other HCP and patients, will be

prevented. Knowing the contamination occurrence status is important for improving PPE and infection control practices for both routine and specialized patient care (18).

In Ethiopia different researches were conducted on the prevalence of microorganisms in health care workers through PPE, personal equipment of health care workers, and medical equipment. But still, there is document scarcity about bacterial isolation from personal protective working clothes of the health care worker in Addis Ababa health care institutions and especially rearmost at Ethiopia federal police health institutions.

1.3. Significance of the study

This study will provide practical based evidence upon the potential of working clothe of health care workers to carry *Staphylococcus* pathogens. It may contribute to create awareness to design and upgrade the infection prevention tools at health care provision sites. It is also used to improve the proper way of using PPE (working clothe) and helps to develop worksite or local regulations which used for properly using and handling of working clothe and practicing regular hand hygiene. It provides information about the prevalence of *S aureus* through working clothes and the hands of health care providers. This study may use as supplemental information for further studies.

2. Literature review

2.1. Contamination level of HCWs working clothes and hand carriage.

Staphylococcus aureus has become resistant to several antibiotics and caused major antibiotic-resistance problems (6). It remains a wide spread and dangerous human pathogen for more than 100 years (7). The systematic review which was done by Shrey Goyal *et al*. to identify studies of bacterial contamination or dissemination by clothe of health care workers focuses on white coats and surgical scrubs. Twenty-two publications between 1990 and 2018 were included in the study and the findings suggest that the healthcare worker clothe (attire) is a potential source of pathogenic bacterial transmission in healthcare settings. *S aureus* was the most studied organism in reviewed publications (9).

The study done by Nestor L in Lima, Peru shown that among 73 healthcare worker participants, 43% of clothing was contaminated with different bacteria and 4% of the contaminant was *S aureus* (10). The study which was done on 50 white coats of undergraduate students, (32 males' and 18 females') in Iran, medical science university indicates that 30 (72.2%) of whole white coats were contaminated with microorganisms. Among the contaminated coats, 20(62.5 %) were males' and 13(72.2%) were females. According to the finding, 64.7% isolate was *S. aureus* and four coats were contaminated with *methicillin-resistant S. aureus (MRSA)*. The study indicated that *S aureus* was the major isolated organism (11).

The other study carried by Shermil Says *et al*. to determine the micro-organisms present on the white apron of health care workers at Kannur Dental College, India, in 2018, 54 samples were collected from three different parts (lapel, pocket, and sleeve) of 18 white aprons of health care workers and 68.5% of the aprons were contaminated with different micro-organisms. Overall contaminated aprons, *S aureus* was isolated from 48% of the aprons. Among the isolated *S aureus,*28.9% were *MRSA*. The pocket of the aprons was the most contaminated region and more *MRSA* isolated from sleeves when compared to those isolated from lapel and cuffs (12).

In Austria, at Medical University Hospital Innsbruck, Michael Berktold *et al*. when studied the microbial contamination on long sleeves of health workers' clothes, two hundred samples were collected from each sleeve of one hundred doctors' long sleeve coats and different micro-organisms isolated from one hundred and ninety-seven samples. Only nine coats were contaminated with pathogenic bacteria. *S aureus* isolated from samples of four sleeves (2% of

sleeves and 3% of coats). The isolation rate of pathogens is low in this study. The studied coats were provided by the vending machine and also it was possible to have three coats at the same time. These can reduce the pathogenic contamination of coats. The main *MRSA* transmission way in hospitals thought as through fomites by direct contact with infected wounds or from contaminated hands(19). Wearing duration, the fabric of the coat, the micro flora surrounding hospital and adequate hand hygiene measures are valued in reducing contamination of pathogenic bacteria (20). Radwan MA *et al.* studied 115 uniforms of HCWs at the ICU of a large military hospital in Jordan for microorganisms. The contamination was see in 85% of physicians' and 79.3% of nurses' uniforms(21).

One hundred and twenty-two sampled medical uniforms of one hundred and twenty medical person participants were studied by Zl Yan Du *et a*l. for bacterial contamination in China Second Affiliated Hospital of Soochow University. 336 samples had been collected from three different parts (neckline, cuff, back of uniform parallel to the lower pocket) of each uniform and tested for bacterial contamination. 294(80.3%) of test samples were contaminated by different types of bacteria. A high contamination rate (61.5%) was seen at the cuff part of the uniform. The contamination was high (96.2%) in HCWs who dried their hands at the back of the uniform. Gram-positive bacteria were isolated from the neckline of uniform. The most overall isolated were gram-negative bacteria and S*taphylococci* was 21.4%. The study indicates that fitting enough uniform cuff and avoiding wiping hands on the back of uniform can reduce bacterial contamination of medical uniforms (22).

In AB Shetty Memorial Institute of Dental Sciences, Mangalore, Karnataka, India in 2016, Tony Saj *et al.* made a research to determine the types of microbial flora present on the white coats of dental doctors in a Dental Institution and Hospital a total of 100 white coats of dental doctors were studied. The swab samples were taken from sleeves and pockets of the doctors' white coats and *S aureus* was more (75%) found on sleeves (23).

At Shaare Zedek Medical center Israel, by Yonatan Ilibman Arzi *et al*. one hundred thirty-three surgeon scrubs were tested for detection of bacterial contamination of surgical scrubs in the operating theatre and the detection rate of pathogens was low. Only seventeen scrubs were contaminated with pathogenic bacteria. *S aureus* detected low and *MRSA* was not detected. The

scrubs provided by a vending machine, and changing frequently in the median of four hours. Wearing scrubs *outside* the hospital facility is strictly forbidden by local regulation (24).

In hand carriage study done by Kanayama AK *et al*. in Tokyo Japan, swab samples were collected from 221 HCWs' hand and mobile phones, 11(5%) *S aureus*, and 2(0.9%) *MRSA* was isolated from both mobile phone and hands of user HCWs. The total contamination level of HCWs hand with *S aureus* and *MRSA* was 55/221 and 13/221 respectively (25).

A study done in Northeast India by Gogoi M *et al*. on 200 swab samples from100 HCWs (100 nasals and 100 from hands) for detection of *S aureus* presence,36(36%) nasal samples, and 18(18%) hand swabs were positive for *S aureus*. Among 18 *S aureus* from hand sample, was13 *MRSA* and 5 *MSSA* (*Methicillin sensitive S aureus)* were identified (26). The other research in India by Mundada SG et al. on 112 HCWs nasal and hand swab to detect *MERSA*, it detected from hands of 5(4.46%) HCW participants. HCWs who be colonized by *MRSA* are the main source for transmission of *MRSA* to patients and other HCWS (27). A study conducted in Pokhara Nepal by Sharman BK.et al. from 100 HCWs hand and mobile phone swab samples were collected and 107 microorganisms were isolated from hand swab samples. Among hand contaminants,39.6% were *CoNS* and 26.42% were *S aureus*. All isolated *CoNS* and *S aureus* showed 100% resistance to penicillin (28).

The other study conducted in Tanzania on 180 white coats of medical doctors and students at Kilimanjaro Christian Medical University College, 132(73.33%) of white coats were contaminated with different pathogens. The most dominant isolated pathogen 120(91.6%) was *S aureus* and the most contaminated coats (66.67%) were from students. 84.44% of this study participants stored their working clothes at home/hostel and 4.44% worn it outside the working area (29).

In Egypt Mansoura university children's Hospital, the study was done by Nawal S. Gouda *et al*. on the role of healthcare workers' white coats in transmitting hospital-associated infections and to determine the association between bacterial contamination of healthcare workers' hands and white coats. One hundred fifty-four healthcare workers participated in the study and only twenty-five participants were negative for hands and white coat tests. 92(59.2%) of healthcare workers' white coats were contaminated with pathogenic micro-organisms. 42(27.3%) white coats were

contaminated with *S aureus* and 38(24.7) with *MRSA*. The hands of 100(64.9%) healthcare workers were contaminated with different bacteria and 29.2% was *S aureus. MRSA* was isolated from 22.1% HCWs The study indicated that healthcare workers' coats may be an important vector for transmission of nosocomial pathogens and the importance of hand hygiene in health care settings (30).

The study to assess the nosocomial risk at University Teaching Hospital in Lusaka, Zambia, by Susan Mwamungule *et al*. showed that white coats of health care workers have contributed to the transmission of pathogenic micro-organisms in a hospital environment. One hundred seven white coats of health care workers were sampled for detection of contamination with micro-organisms and 94(72.8%) of white coats were contaminated. The white coats worn by laboratory persons and those worn for a long time have a high contamination rate. *S aureus* was the highest frequency (17.8%) isolated pathogen. White coats worn at pediatrics and medical units were more contaminated than those worn at surgical wards (31).

The cross-sectional study done by AlzhowAltayeb I. *et al*. at Sudan Science and Technology University indicates the moderate rate of bacterial contamination of physicians' white coats. Swab samples were collected from white coats of one hundred physicians who were working at different three governmental hospitals of Khartoum, Sudan from A 43, B 33, and C 24. Among tested white coats,29(29%) of them were contaminated with different types of bacteria. The isolation rate of *S aureus* was 4(13.8%) (32).

In North-West Ethiopia, Felege Hiwot Referral Hospital, Worku A. *et al.* in 2017, studied a total of 422 healthcare workers' fomites for bacterial contamination and anti-bio gram of bacteria isolated. Overall studied fomites 247(57.6%) of them were contaminated by bacteria. Among the studied fomites, 194 white coats of HCWs were included and the contamination rate was 39(20.1%). *S aureus* and *K pneumonia* were the most frequent isolated organism. High rate (27%) *S aureus* was isolated from fomites of midwives. In the study, *S aureus* showed a high rate of (82.7%) penicillin and 88.9% multidrug resistance. The study showed that the fomites of HCWs were contaminated by bacteria at a high rate and they are a major health care problem in the study area (15).

Since most health care workers are keeping their statoscopes in the pocket of working clothes, cross-contamination will have occurred. In the study done at Addis Ababa Black Lion Hospital by Chaka ET *et al*. on bacteria isolated from cell phones and hands of health care workers, swab samples were collected from cell phones and hands of 100 participants. 78% of participant's hands were contaminated with different bacteria and *S aureus* was the most dominant isolate. 56.4% *S aureus* and 62% *CoNS* were isolated from the hand swab of participants. The hand isolated *S aureus* were showed high (71%) resistance to penicillin. Among the hand swab isolated *S aureus*,40% were *methicillin-resistant (MRSA*) (16).

2.2. KAP of HCWs towards to use and handling of working clothes

The purpose wearing of working clothes during health care activity is to protect self and others against infection-causing agents. The white coats were also worn by doctors as a symbol of medicine and for the identification of medical professionals (13). In both studies done by Robati R *et al.* in Iran and Sayd S *et al*. in India, showed that HCWs wore white coats to be identified as medical professionals and they used to wear them outside the clinical or the working area and also used to clean the working clothes by washing at home.

In a study by Sayd S *et al.* of India, even though 88.88% of study participants believed that clinical coat carries germs, only 11.11% of them were practicing not wearing clinical working clothes outside the working place and worn them to cover their clothes (11). In a study done by Du ZY *et al*.(22) on bacterial contamination of medical uniforms, 25/26(96.2%) study participants were practicing hand drying by wiping at the backside of their uniform. The HCWs could transfer micro-organisms to others by carrying through working clothes. In a study by Qaday J et al. Tanzania, 84.44% of studied HCWs (Health students) took their working clothes to their home/hostel for storage, and 4.44% worn their working clothes outside the working area(29). In another study in Lusaka Zambia,33/107 study participants worn the working clothes beyond the working area and 62/107 of them were stored the clothe at home/hostel (31).

In north-east Ethiopia, Felegehiwot Hospital, Study of Ayalew W *et al*, 57.2%, 56.2%, studied HCWs had no practicing of regular hand hygiene before and after touching of patients respectively (15). In another study by Chaka ET *et al*. in A. Ababa Black Lion Hospital, from 100 participants were studied and 4% male and 37.5% female participants were practicing frequent hand cleaning during health care activity(16).

3. Objectives

3.1. General objective

- To assess *Staphylococcus aureus* and *MRSA* contamination level of Working Clothe, Hand carriage and KAP among Health Care workers at Federal Police Hospital**.**

3.2. Specific objectives

- To determine the contamination level of *S. aureus* in working clothe of healthcare worker
- To assess *S. aureus* hand carriage of health care workers**.**
- To assess the *MRSA* contamination level among *S aureus* isolated from working clothe and hands of healthcare workers.
- To assess the Knowledge, Attitude, and Practice of HCWs towards infection prevention related to working clothes.

4. Materials and Methods

4.1. Study area

The hospital based cross-sectional study was carried out on health care workers' working clothe contamination level, hand carriage of *S aureus* and KAP at Addis Ababa Federal police hospital from November 2020 – March 2021. Doctors, nurses' health officers and other health care professionals like laboratory, pharmacy, radiology /X-ray/ physiotherapy professionals and also those who handle the medical wastes at the hospital were participated in the study.

The Federal Police hospital is located in Addis Ababa city at Lideta sub-city near west side to Mexico square. It established in 1955 E.C. (1963 G.C) by Emperor Hilesilassie with fund contribution from each police members all over the country. The hospital has 338 medical professional and 307 non-medical staffs with capacity of 300 beds for inpatient service. It provides medical service for police staffs and families' all over the country. And also, it provides the private wing medical services for civilians. 450 - 500 patients being served at the hospital per day.

4.2. Study design and period

Hospital based cross-sectional study was conducted at Federal police hospital from November 2020- March 2021

4.3. Population

4.3.1. Source Population

All healthcare workers who working at Federal Police Hospital.

4.3.2. Study population

Healthcare workers who participate in health care activities, presented at a worksite on data collection time, and fulfill the inclusion criteria.

4.4. Inclusion and Exclusion criteria

4.4.1. Inclusion criteria

- Health care workers who are present on work site by wearing working clothe during data collection time.

- Those who had direct or indirect contact with patient.
- Those who volunteer to participate in the study**.**

4.4.2. Exclusion Criteria

- Health professional students who attached for practical sessions in the hospital during the study time.
- The invited /gust/ health care providers who stayed for hours or few days.

4.5. Study variables

4.5.1. Dependent variables

- Working clothe contamination level of *S. aureus* and *MRSA*.
- *S. aureus* and *MRSA* hand carriage of health care worker.
- Knowledge, Attitude and Practice of healthcare workers

4.5.2. Independent variables

Age, sex, education, profession, working department, work experience.

4.6. Measurement and data collection

4.6.1. Sample size determination

The s ample size was calculated based on the formula of single population proportion;

n=z2*p(1-p)/d2; n=sample size, z= 1.96 (level of error in 95% confidence interval), d=margin err or=0.05, p= prevalence; based on study done at Felege Hiwot referral hospital, the prevalence of *S aureus* in white coats of healthcare workers (15).

p=20.1%, n=1.962*0.201(1-0.201)/0.0025=247, considering 10% non-response rate, the total sample size= 24+247=271. Because of sudden closed of micro biology laboratory, the processed samples were collected from 222 participants (82% of pre planed sample size).

4.6.2. Sampling method

Convenient sampling technique was used to collect swab samples from working cloths and 53 randomly selected hand swabs.

4.6.3. Data collection

After introducing the objective of the study, the coded information sheet with questioner was provided to the volunteer to sign the consent. Instructions on how to fill the questioners and how to take part during sample collection were described to them. The socio-demographic data and the knowledge, attitude, and practice of participants related to the study were collected by self-administered questioners and direct observation.

4.6.4. Laboratory Methods

4.6 4.1. Specimen collection

The swabs were quickly inserted into a sterilized screw-capped tube and immediately transported to the microbiology laboratory for further processing.

4.6.4.2. Inoculation and Identification

The collected swab samples were inoculated on nutrient broth and incubated at35- 37ºc for overnight. Then subculture on mannitol salt agar and incubate the culture at 35-37ºc for 24 hours. After 24 hours of incubation, examine the culture for colony growth, gram stain, and run the conventional biochemical test for identification. *S aureus* produces golden yellow colony on mannitol salt agar, gram-positive cocci, catalase-positive, coagulase production by converting fibrinogen to fibrin in plasma and *DNase* positive (33,34).

4.6.4.3. Antimicrobial susceptibility (Kirby-Bauer disk diffusion technique)

Antimicrobial susceptibility tests were done for all *Coagulase-positive staphylococcus (S aureus)* isolates and some *Coagulase-negative staphylococcus (CoNS).*

Using a sterile loop, take 3-5 colonies and emulsify in 3-4 ml sterile nutrient broth in the sterile test tube, mix and standardize the turbidity of suspended cells equivalent to the 0.5 McFarland standard. By using a sterile cotton tip swab, streak on the entire surface of Mueller- Hinton (MHA) agar. By using the Kirby-Baur method, place antibiotic discs (Penicillin 10, Cefoxitin 30, Clindamycin 2, Erythromycin 5, Gentamycin 10, and Trimethoprim /sulfamethoxazole 25) evenly distributed (about 30 mm apart) and incubated at 33oc-35oc for 18-24 hours. BY using a ruler measure the inhibited zones and report susceptible, intermediate, and resistance according to the interpreting chart CLSI M100 (35).

Susceptible(S): A pathogen reported as susceptible suggests that the infection it has caused is likely to respond to treatment when the drug to which it is susceptible is used in a normal recommended dose and administered by an appropriate route.

Intermediate (I): A pathogen reported as intermediately susceptible suggests that the infection it has caused is likely to respond to treatment when the drug is issued in larger doses than normal

Resistant (R): A pathogen that the infection it has caused will not respond to treatment with the drug to which it is resistant irrespective of dose. (33)

Detecting *MRSA (Methicillin Resistant Staphylococcus aureus)*

In MHA disk diffusion technique for *S aureus* isolate, if the inhibition zone of cefoxitin is >22mm, the result interpreted as ‘’sensitive’’.

When the inhibition zone of the disk is < 21mm, the result interpretation is ‘’Resistant’’.

The cefoxitine resistant *S aureus* is called as *MRSA* positive or “*MRSA* detected” (35).

4.7. Quality Control

All culture media were prepared according to the instructions of the manufacturers. The sterility test of the culture media was done by randomly picking 2% of the prepared culture media, incubating at 35-370c for 24 hours and observing for any growth. The prepared nutrient broth also tested by incubating at 35-370c for 24 hours and observe for any turbidity. The normal saline was tested for sterility by inoculated on mannitol salt agar media and observes for any growth after incubating for 24 hours at 35-37ºc. Strict bacteriological sample collection procedure was followed at the time of swabbing. Aseptic techniques were observed in all the steps of specimen collection and inoculation to minimize contamination. Immediately after collection, the swab samples were transported to microbiology laboratory for processing. All materials, equipment and procedures were adequately having controlled. The control organism for *S aureus* and *S epidermidis* were obtained from Ethiopia public Health Institute (EPHI) micro biology department. Pre-analytical, analytical and post-analytical stages of quality assurance were incorporated in standard operating procedures (SOPs) of the microbiology laboratory. The complete filling of socio- demographic data and questioner was checked at the site during

collection from the individual participant. The results were recorded carefully and checked before data analysis.

4.8. Data analysis and interpretation.

The micro soft excel was used for data entry and data analysis was done by using SPSS Version21. Age range classification was based on EDHS (Ethiopia Demographic and Health survey) 2016.

4.9. Operational Definition

Good Knowledge: - Correctly answer ≥50% of questioners related to knowledge about bacterial contamination and spread through working clothes.

Good attitude: -Correctly respond ≥50% of attitude questioners related to bacterial contamination through working clothes and hand hygiene.

Good practice: - Correctly answer ≥50% of questioners related to practice on handling and use of working clothes

Healthcare workers: - Who had direct or indirect contact with patients (health professionals and hospital waste handlers).

Workings clothe: - gowns and uniforms worn by staff at federal police hospital

4.10. Ethical considerations.

Ethical clearance was obtained from the department of Research and Ethical Review Committee (DRERC) of the Department of Med]ical Laboratory Science, College of Health Sciences, Addis Ababa University. Permission obtained from Federal Police Commission Health Service Deputy Sector.

The purpose of the study was clearly explained for study participants and the written signed consent was obtained prior to sampling. Confidentiality was strictly maintained during the data collection and processing.

4.11. Dissemination of the results

The finding of this study will be disseminated to Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences and Federal Police Commission Health Service Deputy Sector. Finally, the manuscript will be submitted to peer review journals for possible publication**.**

5. Results

5.1 Socio-demographic data of study participant HCWs at FPH

In this study, a total of 222 health care workers, 131(59.0%) females and 91(41.0%) males from 18 different departments were included. Most of the participants 110(49.5%) were nurses and 56(25.2%) participants were from the outpatient department (OPD). The participants' average age was 34.3 (minimum 21 and maximum 67) years with a standard deviation of ±8.6 and most participants 64(28.8 %) were in the age of 25-29 years. 75(33.8 %) participants had work experience of 5-9 years and the education level of 129 (58.1 %) participants was 1st degree. (Table 1)

Table 1: Socio Demographic characteristics of study participant healthcare workers at FPH from November 2020 – February 2021

Variables		**Frequency N (%)**
SEX	Male	91(41.0)
	Female	131(59.0)
	Total	222(100)
Age		
	18-24	12(5.4)
	25-29	64(28.8)
	30-34	56(25.2)
	35-39	38(17.1)
	≥40	52(23.4)
	Total	222(100)
Experience in year		
	≤4	74(33.3)
	9-May	75(33.8)
	14-Oct	35(15.8)
	≥15	38(17.1)
	Total	222(100)
Sample swab from	Cloths	222(100)
	Hands	53(100)
Education Level		
	Doctor specialist	3(1.4)
	General practitioner	22(9.9)
	Masters	2(0.9)
	1st Degree	129(58.1)
	Diploma	41(18.5)

	Certificate	5(2.3)
	Formal education	20(9.0)
	Total	222(100)
Professions		
	Doctor	25(11.3)
	Health officer	14(6.3)
	Nurse	110(49.5)
	Lab professional	23(10.4)
	Pharmacist	15(6.8)
	Cleaner	20(9.0)
	Physiotherapist	5(2.3)
	Radiologist	4(1.8)
	Anesthetist	3(1.4)
	Ophthalmologist	2(0.9)
	Data encoder (IT)	1(0.5)
	Total	222(100)
Departments		
	Outpatient department	56(25.1)
	Ward	34(15.3)
	Lab & Pathology	26(11.7)
	Gyn &mat ward	15(6.8)
	Pharmacy	15(6.7)
	NICU	9(4.1)
	Operation room	11(5.0)
	Radiology	8(3.6)
	POPD	7(3.2)
	Intensive care unit	6(2.7)
	ANC &Gyn OPD	6(2.7)
	Recovery	5(2.3)
	Pediatric ward	6(2.7)
	Physiotherapy	5(2.3)
	Eaer, Nose,Troat	4(1.8)
	Ophthalmology	4(1.8)
	Dentistry	3(1.4)
	Injection& wound	2(0.9)
	Total	**222(100)**

Note:, Gyn&Mat ward = Gynecology and maternity ward, NIcu=Neonate Intensive care unit, Ped OPD=Pediatric outpatient department, ANC &Gyn OPD=Antenatal care and gynecology outpatient department

5.2 Bacterial Contamination level of study participants' Working Clothes and hand carriage.

Two hundred and twenty-two working cloths swabs and 53 hand swab samples were collected from a total of 222 study participants. A total of 39(17.6%) contamination rate was seen in clothe and hand sample. 41 staphylococcal organisms were isolated that 37(16.7%) from working clothes and 4(7.5) from hands. More contamination (18.4%) was seen in female participants than in male1 (16.5). Participants in the age range of 25-29 were more (23.4%) contaminated. A high contamination rate (50%) was seen in both radiologist and ophthalmologist professionals and a 32.0% contamination rate was seen at laboratory departments. No significant difference (P. value>0.05) in contamination level was seen in any group of the study variables (Table 2).

Table 2. Bacterial Contamination level of participants Working Clothes and hands

Variables		Contamination status			P. value
		Contaminated N(%)	Not contaminated N(%)	Total N (%)	
Sex	Male	15(16.5)	76(83.5)	91(1OO)	0.858
	Female	24(18.4)	107 (81.7)	131(100)	
Age in years					
	≤24	1(8.3)	11(91.7)	12(1OO)	0.192
	25-29	15(23.4)	49(76.6)	64(1OO)	
	30-34	5(8.9)	51(91.9)	56(1OO)	
	35-39	9(23.7)	29(76.3)	38(1OO)	
	≥40	9(17.3)	43(82.7)	52(1OO)	
Experience in year					
	≤4	13(17.6)	61(82.4)	74(1OO)	0.790
	5-9	11(14.7)	64(85.3)	75(1OO)	
	10-14	6(17.1)	29(82.9)	35(1OO)	
	≥15	9(13.9)	29(76.3)	38(1OO)	
Swab sample From	Cloths	35(16.7)	185(83.3)	222(100)	
	Hands	4(7.5)	49(92.5)	53(100)	
Educational Level					
	Gp.Dr	4(18.2)	18(81.8)	22(1OO)	0.693
	1st degree	26(20.2)	103(79.8)	129(1OO)	
	Diploma	7(17.1)	34(82.9)	41(1OO)	
	Certificate	1(20.0)	4(80.0)	5(1OO)	
	Formal education	1(5.0)	19(95.0)	20(1OO)	

Professions					
	Doctor	4(16.0)	21(84.0)	25(100)	0.093
	Health officer	2(14.3)	12(85.7)	14(100)	
	Nurse	20(18.2)	90(81.8)	110(100)	
	LT	8(34.8)	15(65.2)	23(100)	
	Pharmacist	1(6.7)	14(93.3)	15(100)	
	Radiologist	2(50.0)	2(50.0)	4(100)	
	Ophthalmologist	1(50.0)	1(50.0)	2(100)	
	Cleaner	1(5.0)	19(95.0)	20(100)	
Departments					
	OPD	12(21.4)	44(78.6)	56(1OO)	0.287
	Lab& Pathology	8(30.8)	17(69.2)	26(1OO)	
	Ward	7(20.6)	27(79.4)	34(1OO)	
	Gyn&mat. Ward	2(13.3)	13(86.7)	15(1OO)	
	POPD	2(28.6)	5(71.4)	7(1OO)	
	Radiology	2(25.0)	6(75.0)	8(1OO)	
	ANC& Gyn OPD	1(16.7)	5(83.3)	6(1OO)	
	Recovery	1(20.0)	4(80.0)	5(1OO)	
	Pharmacy	1(6.7)	14(93.3)	15(1OO)	
	ENT	1(25.0)	3(75.0)	4(1OO)	
	Ophthalmology	1(25.0)	3(75.0)	4(1OO)	

Note: Gp Dr=General practitioner Doctor, OPD = Outpatient department, Gyn&Mat ward = Gynecology and maternity ward, Ped OPD=Pediatric outpatient department, ANC &Gyn OPD=Antenatal care and gynecology outpatient department, ENT=Ear, nose and throat.

Out of 222 working clothe swabs and 53 participants' hand swabs a total 41 bacterial contaminants (*CoNS* N=23(56.4%) and *S aureus* N=18(43.6%)) were isolated. The *S aureus* was isolated from13 working clothes, 1 participants hand, and from2 participants hand and clothing. Among 18 isolates of *S aureus*, 6 were *methicillin-resistant (MRSA*). The isolation level of *CoNS, methicillin-sensitive S aureus (MSSA*), and *Methicillin-resistant S aureus(MRSA)* were 56.1%, 29.2%, and 14.6% respectively (Table 3).

Table 3. Contamination level and distribution of Isolates in HCWs clothes and hand Carriage.

Sample sources	Isolated organisms						Total isolate N(%)
	CoNS		*MSSA*		*MRSA*		
Cloths N=222	22		10		5		37(16.6)
Hands N=53	1		2		1		4(7.5)
HCW	Clothe	Hand	Clothe	Hand	Clothe	Hand	
Doctors N=25	2	0	1	0	1	0	4(16.0)
HO N=14	1	0	0	0	1	0	2(14.3)
Nurse N=110	9	1	6	1	2	1	20(18.2)
LT N=23	7	0	1	1	0	0	9(39.1)
Pharmacist N=15	1	0	0	0	0	0	1(6.6)
Radiologist N=4	1	0	1	0	0	0	2(50.0)
Ophthalmologist N=2	1		0		0		1(50.0)
Cleaner N=20	0	0	0	1	1	0	2(11.1)
Total	23(56.1)		12(29.2)		6(14.6)		41(100)

Note: - HCW=health care worker,HO=health officer, LT=lab professional

One *methicillin-resistant S aureus (MRSA*) and three *methicillin-sensitive S aureus* (*MSSA)* were isolated from the hand and working clothing of two health care workers (Table 4).

Table 4. Hand and clothe contamination rate

HCW	Type of isolate		Total isolate
	From cloth	From hand	
Lab professional	*MSSA*	*MSSA*	2
Cleaner	*MRSA*	*MSSA*	2

5.3 Anti-microbial Susceptibility Test Pattern of Bacterial isolates

An antimicrobial susceptibility test was done on 23 isolates (4*CoNS* from working clothe,1 *CoNS* from hand, 15 *S aureus* from clothe, and 3from hands) versus 6 antibiotic discs. All 4(100%) *CoNS* which were isolated from clothing were resistant to penicillin and cefoxitin. The hand

isolated *CoNS* was resistant to penicillin, erythromycin, SXT and sensitive to cefoxitin clindamycin and gentamicin. 15(88.2%) and 12(70.6%) of *S aureus* were resistant to penicillin and erythromycin respectively. 6(35.3%) of *S aureus* was resistant to cefoxitin (*MRSA*). All hand swab isolated *S aureus* were 100% resistant to penicillin, erythromycin, SXT however they are100% sensitive to clindamycin and gentamycin (Table 5).

Table 5. AST pattern of bacteria isolated from working cloths and hands of HCW at FPH

AST done for bacteria N=23(100%)	Antimicrobial resistance N (%)					
	Penicillin	Cefoxitine	Clindamycin	Erythromycin	Gentamycin	SXT
CoNS from WC N=4(100%)	4(100)	4(100)	1(25.0)	3(75.0)	0	3(75.0)
CoNS from Hand N=1	1(100)	0	0	1(100)	0	1(100)
S aureus from WC N=15(100%)	13(92.8)	5(35.7)	1(7.1)	9(64.2)	1(7.1)	2(14.3)
S aureus from hands N=3(100%0	3(100)	1(33.3)	0	3(100)	0	3(100)
Total	21(91.3)	10(43.5)	2(8.7)	16(69.6)	1(4.3)	9(39.1)

Note; SXT=trimethoprimsulfamethoxazole

Among a total of 18 *S aureus* isolates 12(67%) of them (10 from working cloths and 2 from hands) were sensitive to cefoxitine (*MSSA*). The other 6(33%) *S aureus* isolates (5 from working cloths and 1 from hand) were resistant to cefoxitine (*MRSA*). (Fig.1)

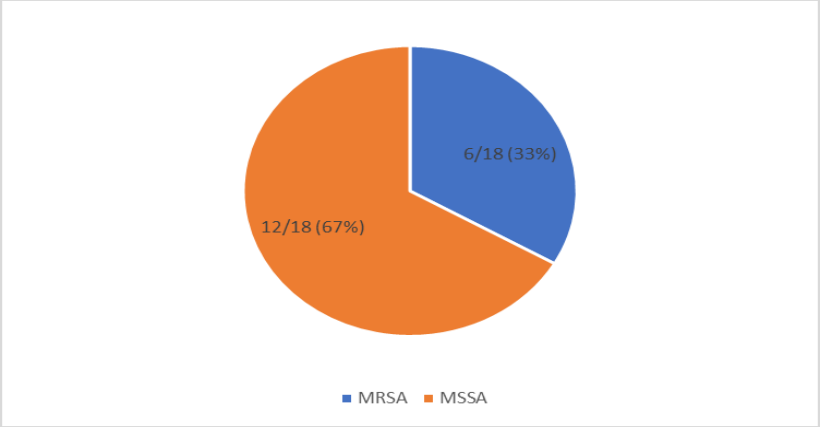

Fig.1 Methicillin sensitive *S aureus* (*MSSA)* and methicillin resistance *S aureus (MRSA)*.

5.4 Knowledge, attitude, and practice

5.4.1 Knowledge of participants on the use of working cloths related to bacterial contamination

There is no significant association (P> 0.05) between the knowledge of study participants and bacterial contamination level. About 86% of study participants have correctly answered the questions related to bacterial contamination through working clothes. It indicates the good knowledge on proper using and handling of working clothes. 32.4% of the participants were not taking any infection prevention training. 7.2% HCWs responded that they don't know about working clothes can protect themselves and others from bacterial contamination and 6.8% of participants were responding that working clothes don't prevent bacterial contamination. (Table 6).

Table6. Knowledge of participants about bacterial contamination and use of working clothes

Characteristics	Contaminated N(%)	Non-contaminated N(%)	TotalN(%of222)	P-value
Ever taken any training on infection prevention?				
Yes	29(13.1)	121(54.5)	150(67.6)	0.318
No	10(4.5)	62(32.4)	72(32.4)	
Infectious organisms may transmit through working clothe?				
TRUE	36(16.2)	171(77.0)	207(93.2)	0.731
FALSE	3(1.4)	12(5.4)	15(6.8)	
Working clothe may protect you and others from bacterial contamination?				
Yes	35(15.8)	156(70.3)	191(86.0)	0.764
No	2(0.9)	13(5.9)	15(6.8)	
I don't know	2(0.9)	14(6.3)	16(7.2)	
Clean working clothe even used for few days are free from micro-organisms?				
TRUE	6(2.7)	35(15.8)	41(18.5)	0.585
FALSE	33(14.9)	148(66.7)	181(81.5)	

5.4.2 Attitude of Study participants on the use of working cloths related to bacterial contamination

The attitude of 94% of study participants towards bacterial contamination through improper use of working clothe was good (responded positively). 3% of them strongly disagreed with the statement "bacterial contamination can occur when drying hands-on working clothes". (Fig 2).

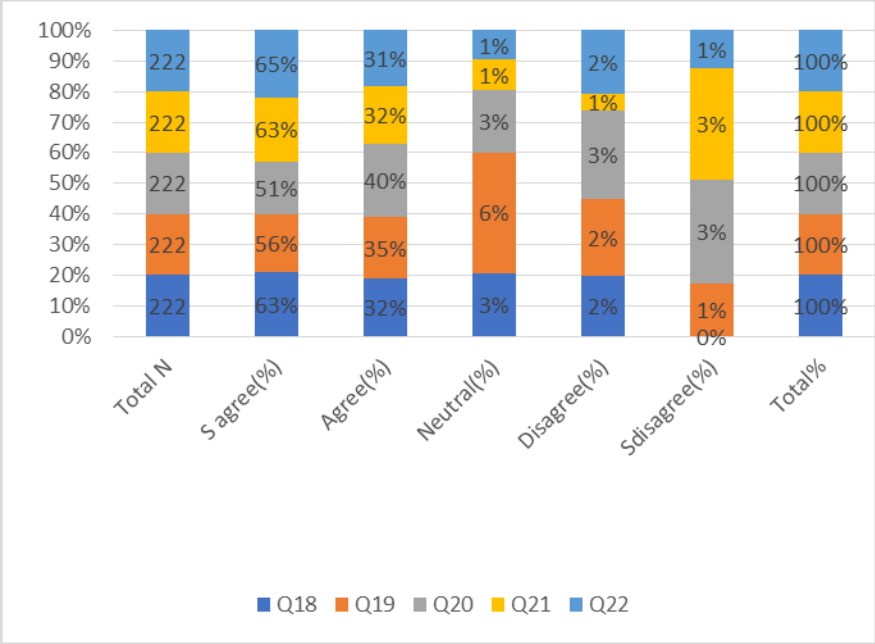

Fig. 2 Attitude of study participants on use of working clothe related to bacterial contamination.

Note: S agree=strongly agree, S disagree=strongly disagree
Q18. Do you agree that pathogens can be transmitted through working clothe?
Q19. Do you agree working clothe can cause nosocomial infection?
Q20. Do you agree drying hands on working clothe can cause bacterial contamination?
Q21. Do you agree wearing working clothe at a tea café/lounge has a contamination risk?
Q22. Do you agree on the washing of hands after each healthcare activity is the best way of infection prevention?

5.4.3 Practice of study participants related to prevention of bacterial contamination.

The questioners to evaluate the healthcare workers on infection prevention practice, those who respond to questions correctly were considered as in good practice. From overall participants, 70% of them responded as they are practicing well on infection prevention related to working clothes. 4.1% HCWs were responded that always they are drying their hands on their working clothes (Table 7).

Table7. Practice of study participants related to prevention of bacterial contamination, 2021

Characteristics	Responses (%)					Total N(%)
	Always	Sometimes	Rarely	Very rarely	Never	
Regular hand washing after each health care activity?	119(53.6)	80(36.0)	21(9.5)	2(0.9)	(0.0%)	222(100)
Wearing of working clothe only at work site?	180(81.1)	38(17.1)	3(1.4)	1(0.5)	(0%)	222(100)
After washing hands, drying on working clothe?	9(4.1)	29(13.1)	17(7.7)	18(8.1)	149(67.1)	222(100)
Regularly wearing of working clothe at work?	173(77.9)	41(18.5)	5(2.3)	2(0.9)	1(0.5)	222(100)

On other practice-related presented questions the responses of participants were, 35.1% of them responded that they go to tea café/lounge/, shop, by wearing the same working clothe. 13.1% kept their working clothes together with other clothe and 12.2% were used to take to their home. 25.7% were clean /wash/ their workings clothe at their home. 89.2% of respondents were kept different personal items in the pocket of their working clothe and 0.9% of them keep foodstuffs in the pocket. The other question about the hinder on regular use of working clothe, 64.4% of participants were responded they do not wear working clothe regularly because of shortage of working clothe, 9.5% responded that it is not comfortable and 4.7% responded that they did not wear regularly because of lack place to keep clothe.

6. Discussion

Identification and drug resistance profiling of common contaminant microorganisms is important to control and intervening in the spread of multi-drug-resistant bacteria.

This study was focused on the *S aureus* contamination level of healthcare workers' working clothe and their hands, the way handling, using, and hand hygiene practice towards preventing self and patient against bacterial contamination.

More than36 reference materials have been used during the study time. During routine clinical care, healthcare providers' clothe often became contaminated (18).In this study, 222swab samples were collected from tips of both sleeves and mouth tips of both front down pockets of working clothe in the pool. 53 hand swabs also have been collected from 53 participants both hands backside with entire palm side. The total contamination rate of health care workers working clothe was 17.6% (N=39/222). It is lower than the studies across different nations (10,11,12,13,20,22,29,30,31) where the contamination rate was (45%, 66%,48%, 77.7%, 91%,61.5%,73.33%,65%,72.8%) respectively and even lower than that of previously done in Ethiopia (57.7%) (15). At the time of sample collection, there was an emerging covid-19 pandemic. This significant dissimilarity might be associated with health care workers frequently using alcohol-based hand rubs to protect themselves and others against covid-19 infection and it might be lower the hand contamination rate as 4/53(7.5%). The availability of hand washing sinks at most healthcare provision sites in FPH enhances the practicing of regular hand hygiene and contamination level could be lower.

Overall study participants were 131(59%) female and 91(41%) males. The contamination level was shown in 24(18.4%) female and 15(16.5%) male participants. There is no statistically significant difference (P-value >0.05) of contamination level in gender. This might be due to equal use of hand cleaning opportunities in male and female HCW. Because of often close contact between patients and healthcare providers in healthcare settings, the sharing of infection-causing agents could have occurred (18).

High contamination rate (197/200) in healthcare workers clothe was seen in a study done by Bechtold M *et al*. Innsbruck, western Austria on 200 swab samples which collected from white coats of 100 doctors (20). Even though a high contamination rate of HCWs' clothes was seen, *S*

aureus was isolated from 4/200 samples which are significantly lower than this study (15/222) finding. The difference might be due to the mentioned samples were collected from single professionals (doctors) coats. The finding of *S aureus* was high (64.7%) in the study done by Robati R *et a*l. in Iran, Shiraz, and *MRSA* was isolated from 4/50 white coats (11), which is soundly higher than this study found. The probability of difference might be by different sample collection methods that samples collected from 4 different parts of white coats were showed a high isolation rate of *S. aureus*.

The bacteria hand carriage level in this study,53 hand swab samples have proceeded and hands of 4 participants were contaminated. *S aureus* was isolated from 3 hands swabs (3/53=5.6%) and 1 isolate (1/53=1.9%) was *MRSA. CoNS* was also isolated from one(1.9%) participant and 4/53(7.5%) total hand carriage seen. This hand carriage level is significantly low when compared with those studies done by Kanayama AK *et al*. in Tokyo, Japan (25), and Gogoi M *et al*.in North East India (26), the finding of *S aureus* and *MRSA* were 55/221(24.9%), 13/221(5.9%), and 18/100(18%), 5/100(5%) respectively. It is also different from another study done in India by Mundhada SG *et al.* (27) that *MRSA* contamination level was 5/122(4.46%). The reason for this significant difference could be using a low sample size. A great difference was seen when comparing with the study done by Chaka ET *et al*. at Addis Ababa Black Lion Hospital that total hand carriage was 78% (16). In the study of Chaka ET *et al*. sample size was 100 and the findings were 56.4% *S aureus*,62.4% *CoNS*, and 40% *MRSA*. This difference could be due to the small sample size of this study and Chaka's study was limited to a single department (pediatric ward). The hand isolate profile in this study was 1/3(33.3%) *methicillin-resistant (MRSA*) and 2/3(66.7%) *methicillin-sensitive (MSSA).* This finding is slightly close to the finding of a study done by Sharman BK *et al.* in Pokhara, Nepal that was 25% *MRSA* and 75% *MSSA* (28). It is also closer to the study of Chaka ET *et al.* of Black Lion hospital that *MRSA* was isolated 40% (16).

In this study, there is no significant difference (p-value >0.05) in bacterial contamination levels in different professions. Although the contamination rate percentage in radiologists (N= 2/4 (50%)) and ophthalmologists (N=1/2(50%) followed by laboratory professionals (N=8/34 (34.1%)) were higher than in other professionals. The high percentage of contamination rate in radiologists and ophthalmologists could be due to practicing poor hand hygiene and the low

number of these professional participants. Since the difficulty of hand washing after processing each patient sample, the bacterial contamination level is highly seen in laboratory professionals because of their work nature. Even though a high contamination rate was seen in these three professionals, the *MRSA* was isolated from none of them. From 4 samples of radiologist participants, *CoNS* was isolated from 2 samples and *S aureus* from the other 2 samples. Out of 2 samples that had been collected from ophthalmologists, *CoNS* was isolated from a single sample. The next high contamination rate (34.1%) was seen in laboratory professionals. Here also among 23 swab samples been collected from 23 lab professionals, *CoNS* isolated from 7 samples and *S aureus* from 2 of them.

In this study, low contamination rate was seen in cleaner and pharmacy professionals. Due to the risk full nature of their work, cleaners strictly practicing good hand hygiene and properly handle and use their working clothes. Because of the work nature of their profession, pharmacists had lesser close contact with patients, and this condition could lower the bacterial contamination rate in pharmacists. No contamination was seen in anesthetists and physiotherapist professionals. The work behavior of anesthetists restricted their movement in a limited environment by wearing working clothe. In FPH hospital, they were strictly practicing good hand hygiene moreover good handling and use of working clothing. Even though physiotherapists had close contact with the patient when providing professional service, the contamination level was down to zero. This could be by applying good hand hygiene practice and proper use of working clothing. This study was limited to the detection of *S aureus*. This limitation could be a factor for appearing absent or low bacterial contamination rate in some professions and work departments. Due to lack of detail previous study data, comparing the finding was difficult.

In this study the contamination rate in doctors and nurses were slightly similar that was 16.7% and 17.5% respectively. This finding was low when compared to a study done by DU ZY *et a*l. in Suzhou, China (22) contamination rate in doctors and nurses was 85% and 79.3% respectively. In both studies, the contamination rate of doctors and nurses were alike in contamination proportion, and it indicated the similar exposure and hand hygiene practicing of doctors and nurses in both studies. In this study, the *S aureus* contamination rate in doctors, nurses, and laboratory professionals was more similar 2/25(8%),9/110(8.2%), and 2/23(8.7%) respectively.

This study samples had been collected from HCWs who worked at 18 different departments. A high contamination level (30.8%) was seen in the laboratory department followed by pediatric OPD (28.6%). The processing of different biological samples in the laboratory could be the predisposing factor for the high contamination rate in the laboratory department.

Among 41 isolates in this study, an anti-microbial sensitivity test was done on 23 isolates (5 *CoNS* & 18 *S aureus)*. The test was done on 4 *CoNS* and 15 *S aureus* which were isolated from clothing swab samples and 3 *S aureus* and 1 *CoNS* isolated from hand swab. The antimicrobial sensitivity test was done against penicillin, cefoxitin, clindamycin, gentamycin, erythromycin, and trimethoprim -sulfamethoxazole (SXT) disc on Muller Hinton agar by using the Kirby Bauer disk diffusion method. The result was interpreted according to the CLSI M100 guideline (35). *CoNS* which from working clothes were 100% resistant to penicillin, cefoxitin, and 100% sensitive to gentamicin. All isolated *CoNS* were 100% resistant to penicillin and 100% sensitive to gentamicin. *S aureus* which was isolated from working clothes were resistant to penicillin 92.8%, cefoxitin 35.7%, erythromycin 64.2%, SXT 14.3%, clindamycin, and gentamycin 7.1%. The *CoNS* and *S aureus* isolated from hand swabs were showed 100% resistance to penicillin, erythromycin, SXT, and 100%sensitive to clindamycin and gentamicin.

In this study, the *CoNS* isolated from working clothe were 100% resistant to penicillin and it is similar to BK Sharma *et al*. study that *CoNS* was resistant to penicillin 100% (28). This finding is higher when compared to a previous study done by Ayalew W. et al at FHRH northwest Ethiopia that *CoNS* resistant to penicillin 77.55% (15). In this study all isolated *S aureus* showed resistance to penicillin 88 .9%, erythromycin 66.7% cefoxitin 33.3%, SXT 27.8%, clindamycin, and gentamycin 5.6%. High resistance to penicillin and erythromycin was seen. This finding is closer to the finding of Ayalew W *et al*. that *S aureus* is highly resistant to penicillin (82.7%), erythromycin (60.5%), and less resistant to clindamycin (2.5%).

Among 53 hand swab samples, 3(5.7%) isolates were positive for *S aureus*. These 3 isolates were 100% resistant to penicillin, erythromycin, SXT and 100% sensitive to clindamycin and gentamycin. One (33.3%) of the isolate was resistant to cefoxitin *(MRSA*). The finding is similar to a study done by Sharma BK *et al* (28) that *S aureus* which was isolated from hand were 100% resistant to penicillin while unlikely resistant to clindamycin and gentamycin 66.66%. 33%

MRSA and 67% *MSSA* were identified in this study which slightly alike to that of Sharman *et al.* finding 75% *MSSA* and 25% *MRSA.*

In this study, there is no statistical significance (p-value >0.05) seen in responses of HCW participants regarding the knowledge of proper use and handling of their working clothe and contamination level. 72/222(32.4%) participants were responded that they did not take any training consider to infection prevention. 15/222(6.8%) participants responded "no" to the question "infectious organisms can be transmitted through working clothe?". Similar questions were presented in studies of Sreejith VP *et al* and 11.11% of participants responded "no" (12). Another alike question was presented in these both studies "clean looking working clothes are free from infectious agents? "in this study and "coats are clean if it has no stain?" in Sreejith VP et al. 18.5% were responded “true” in this study and 50% in the study of Sreejith VP study. When comparing the response in both studies, FPH HCWs have more awareness about contamination through working clothe. 82% of this study participants correctly responded about working clothes can protect them and others against bacterial contamination. Generally, 86% of the study participants have good knowledge about their working clothes related to bacterial contamination.

In this study, five questions were presented to assess the attitude of participants towards contamination through their working clothe. For the question "Do you agree that pathogens can be transmitted through working clothe?". 95% of study participants showed a good attitude for this question. 91% of respondents also showed a good attitude for the question "Do you agree working clothe can cause nosocomial infection?" The other presented question "Do you agree that drying hand on working clothe can cause bacterial contamination?", 91% participants responded good attitude to the question. For the question "Do you agree wearing of working clothes at tea café/lounge has contamination risk?" 95% were showed their attitude that the risk full of working clothe when worn out side working site. The other critical question "Do you agree on the washing of hands after each healthcare activity is the best way of infection prevention?", 96% participants were correctly responded to the question. Comparing these results was not possible because of a lack of alike data. These responses indicated that study participant HCWs have a good understanding and positive attitude about contamination through working clothes.

The practice of study participants related to bacterial contamination prevention was assessed by the following questions either they are practicing or not.

"Practicing regular hand washing after each health care activity? Wearing of working clothe only at the worksite? Drying of hand on working cloths? Regularly wearing of working clothe when at work?" 53.6% of participants responded that always practicing regular hand washing after each healthcare activity. In a study conducted by Chaka ET *et al*. at Black Lion Hospital (BLH), 4% male & 37.5% female participants (total 41.5%) were always practicing hand cleaning (14). When comparing the findings, this study participant HCWs more practices regular hand washing than those of BLH HCWs participants. 81% of this study participant was responded that always practicing wearing working clothe only at the worksite. This finding showed better practicing than the findings of Rabati R *et al.* in India(11) that 32/50 (64%) study participants had practicing wearing of working clothe at work site only. Although this finding is less when comparing to the study done by Qaday J.et al.(29) that only 4.3% of participants wore their working clothe outside the working place. These findings indicated that this study participants good practicing than in Rabati R *et a*l. and had less application on proper use of working clothe when compared to Qaday *et al*. study. 4% of this study participants were practicing wrong by drying their hands on the working clothes which potentially exposed them to bacterial contamination. 78% of this study participant always practicing the regular wearing of working clothe when they are at work.

In this study, 13.3% respondents were keeping their working clothe together with other clothes (fomites) and 12.3% take it to their homes. By taking contaminated clothes to home or keeping with other fomites, HCWs could spread contaminants to their family. The other 3.3% of respondents have washed their working clothes together with other clothes. These respondents were poorly understood about the transmission of infectious agents from contaminated clothes to others during washing of all together. 189(89.6%) of this study participants responded that they keep different personal items in the pocket of their working clothes. 4(1.9%) respondents have kept food Stuff (edible materials) in their pocket. The life threatening food poison could happen to those health care workers who keep foodstuffs in the pocket of working clothes. These responses indicated the importance of creating awareness in those respondents about contamina-

tion and the spread of micro-organisms through contaminated working clothes. In this study 136(64.5%) participants responded that they would not wear working clothes regularly because of a shortage of working clothes. Working clothes keep safe against contamination of the other HCWs clothes. Therefore, healthcare workers must have worn their working clothes during each health care activity. 20(9.5%) respondents don't wear work clothes because of not comfortable with them and 10(4.7%) were not wearing because of a lack of keeping places (locker).

Health issue and dissemination of contaminant to other are more expensive than accommodation of comfortable working clothes and lockers to keep working clothe**.**

7. Strength and Limitation

7.1. Strength

The study participants were included from almost all health care departments and different professionals in the Federal police hospital.

7.2. Limitation

There is scarcity and unavailability of study materials (culture media, antibiotic discs, petridishes…,) and local studied articles especially on HCWs' hand carriage and working clothe contamination to review and compute the studies. The misunderstanding and misconception of few laboratory management staff of FPH, Sudden and pre-mature closed of microbiology laboratory was influenced this study to proceed limited sample size (less than pervious planed) and prolonged the study time.

8. Conclusion and Recommendation

8.1. Conclusion

In this study 17.6 % total contamination rate was seen. 85% of all tested organisms were multidrug-resistant. 100% tested *CoNS* and 82.4% isolated *S aureus* were showed *MDR*. From isolated *S aureus*, 33%*MRSA* was identified. Therefore, strictly applying of incorporated knowledge, attitude and practice are mandatory requirements for preventing and reducing the spread of multidrug resistance contaminants at health care providing institutions**.**

8.2. Recommendations

Controlling the spread of multi-drug-resistant micro-organisms is the responsibility of each healthcare worker. Working clothes protect health care workers against infectious contaminants. Contamination of working clothe could be occurred by direct contact with the patient, contaminated environment, and hand. At each health care provision site, appropriate use and handling of working clothes incorporate with thorough hand washing before and after each healthcare activity are the main ways to control the spread of multidrug-resistant micro-organisms. Accommodation of comfortable working clothes, increase awreaness of staff and updated information, and training on infection prevention might contribute to controlling the spread of infectious organisms in hospitals. The bacterial contamination rate in this study seems low since it focused on detecting the limited type of bacteria hence further study will be needed to assess the contamination rate in HCWs with other contaminants.

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10. Annexes

Annex I: - General information sheet for the study participants

Introduction

Hello dear participants!

My name is Zewditu Kaba; I am an MSC student of Addis Ababa University, School of Medical Laboratory Sciences. I am doing research entitled '*Staphylococcus aureus* and *MRSA* Contamination level of Working Clothes, Hand Carriage and Knowledge, Attitude and Practice Among Healthcare Workers at Federal Police Hospital; Addis Ababa, Ethiopia.''.

The objective of the study:- The objective of this study is to assess the *Staphylococcus aureus* and *MRSA* Contamination level of Working Clothes, Hand Carriage and Knowledge, Attitude and Practice Among Healthcare Workers at Federal Police Hospital; Addis Ababa, Ethiopia.

Duration: - The duration of this study depends upon the availability of study subjects. It might take about three months or more.

The risk associated with the specimen collection: -The risk associated with the sample collection is free of any risk and associated factors.

The procedure of the study: If you agree to participate in the study, the sample will be collected from the desired part of your working clothes with a sterile swab moistened in normal saline by the principal investigator.

Confidentiality: All records will be kept strictly confidential. Participants' identifications will not be collected for the study purpose instead code numbers will be used. Personal identifying information will not be shared outside of the study and it will not be used in any of the publications.

The benefit of the study: - There is no payment or direct benefit for participating.

Withdrawal rights: -Your participation in this study is purely voluntary and you may stop the 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 will not involve any penalty or loss of benefits.

If you are not comfortable please feel free to stop it at any level of the study. I appreciate your cooperation to a great extent.

If you have any question regarding to this study

Principal Investigator: Zewditu Kaba

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AAU College of health sciences department of medical laboratory science Tel: +251-112755170

Participant signature _____Date_____

Annex II: General information sheet for the study participants in Amharic

አጠቃላይ መረጃ

አዲስአበባዩኒቨርሲቲየድህረምረቃት/ቤትየላቦራቶሪሳይንስትምህርትክፍል

በጥናቱየሚሳተፉየህክምናባለሙያዎችየፈቃድመጠየቂያእናመቀበያፎርም

መግቢያ

ጤና ይስጥልን

ዘውዲቱ ካባእባላለሁ:: በአ.አ.ዩ. የላበራቶሪሳይንስትምህርትክፍልየማስተርስዲግሪተማሪነኝ። ዘውዲቱካባእባላለሁ::

በአዲስአበባዩኒቨርስቲየላቦራቶሪሳይንስትምህርትክፍልየማስተርስዲግሪተማሪነኝ።

”*Staphylococcus aureus* and *MRSA* Contamination level of Working Clothes, Hand Carriage and Knowledge, Attitude and Practice Among Healthcare Workers at Federal Police Hospital; Addis Ababa, Ethiopia.”**በሚልርእስየመመረቅያጥናትእየሰራሁነው።**

የጥናቱዋናአላማ

የጥናቱአላማበህክምና አገልግሎት ሰጪዎች የእራስን መከላከያ የስራ ልብስእና እጅ ላይ ስታፊሎኮከስ አውረስ የሚባለውን ታህዋስ መለየት እና የጤና አገልግሎት ሰራተኛ የግንዛቤና አተገባበርሁኔታለማወቅ።

የጥናቱጊዜ

የጥናቱጊዜበሚሰበሰበውየናሙናመጠንየሚወሰንሲሆን 3 ወርእናከዛምበላይሊቆይይችላል።

ስጋቶችናየምቾትመጓደል

ለጥናቱበሚወሰደዉናሙናምክንያትበተሳታፊላይየሚከሰትየተለየችግር ወይም የምቾትመጓደልአይኖርም።

የጥናቱሂደት

እርስዎበጥናቱላይለመሳተፍፍቃደኛከሆኑከራስንመከላከያየስራልብስዎላይበንጹህጥናሙናዎችን ጠርገን እንወስዳለን።

የጥናቱምስጢራዊነት

የሚሰጡትመረጃምስጥጢራዊነቱየተጠበቀነው፡
በስምአይጻፋምየዚህኮድመፍቻበፋይልተቆልፎየሚቀመጥሲሆንየተፈቀደለትሰውብቻፋይሉንማየትይ ችላል።
ከዚህጥናትበሚወጡዘገባዎችወይንምየህትመትውጤቶችላይስምወይምሌላየእርስዎንማንነትየሚገል ጽመረጃአይኖርም።ኮምፒውተርላይያሉመርጃዎች
ምስጢራዊነታቸውየተጠበቀሲሆንበወረቀትያሉመረጃዎችምደህንነቱበሚጠበቅቦታየሚቆለፉናየተፈ ቀደለትሰውብቻሊያያቸውእንድችልተድርጎይጠበቃል።

የሚያስገኘውጥቅም

በጥናቱበመሳተፈዎምንምአይነትክፍያአይጠይቁምወይምየሚያገኙትገንዘብአይኖርም።

ከጥናቱስለ መውጣት ወይም ማቋረጥ

በጥናቱየሚሳተፉትፈቃደኛከሆኑብቻነው።ስለዚህመሳተፍወይምአለመሳተፍ፤ከጀመሩበኋላማቋረጥ ወይምመመለስ፤የማይፈልጉትንጥያቄላለመመለስ፡ሙሉ፡መብት፡አለዎት።
በጥናቱመሳተፍወይምአለመሳተፍአገልግሎትላይምንምአይነትጥቅምምሆነጉዳትአይኖረውም።
ጊዜዎትንመስዋዕትአድርገውስለተባበሩኝአመሰግናለሁ።

ስለጥናቱሕጋዊነት ለመጠየቅ ከፈለጉ

ይህንንጥናትአስመልክቶጥያቄካለዎትወይምየጥናቱንየመጨረሻውጤትለማወቅከፈልጉበሚከተለዉ አድራሻሊያገኙንይችላሉ።

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የተሳታፊው/ዋ ፊርማ _____
ቀን_____

Anex III: Questionnaire

The title of this study is *Staphylococcus aureus* and *MRSA* Contamination level of Working Clothes, Hand Carriage and Knowledge, Attitude and Practice Among Healthcare Workers at Federal Police Hospital; Addis Ababa, Ethiopia." We are grateful for your agreement to participate in this study. All of the answers you provide in this study will be kept confidential. The information you give us is very essential for this study. Therefore, we respectfully ask you to give us the right answer.

Demographic data

Date_____/_____/_____ Candidate number/code _____

1. Age _____

2. Sex_____

3. Occupation/profession_____

Education level_____

4. Currently assigned/Department_____

5. Length of practice/experience in the hospital (number of years/months? _____

Using and Handling of Working Clothes

6. Do you go out of work site /to café, shop or lounge / by wearing the same gown or uniform or scrub you worn when working?

A. Yes B. No

7. When or at what time do you change your working clothes/gown/scrub/uniform? Your answer can be more than one.

A. when it looks dirty B. every day C. Every week D. When it Contaminated with blood, body fluid, or other clinical contaminants

E. other (specify) _____

8. When did you wear/change/ the working clothes / gown or uniform/ you are wearing now? Please specify. _____

9. Where do you keep your protective clothes /gown, uniform, scrub / when you are not engaged at healthcare activities?

A. Keeps in the locker together with other fomites. B. keeps in the locker separately.

C. Take to home D. Hang at work site. E. Keeps at special place preserved to keep gowns. F. keeps on working bench G. Other (specify) ____

10. How do you clean your working clothes/gown/uniform/apron/scrub?

A. Wash at working place/at hospital laundry B. wash at home. C. Wash separately D. Washes together with other clothes.

11. Do you keep things in the pocket of your gown/uniform? A. Yes B. No

12. If yes, what materials or things do you keep? Your answer can be more than one. A. Mobile phone B. wallet C. money D. food staffs like biscuit, kolo, chewing gum.

E. others (p/s specify)_____

13. Any hinder on the way of using personal protective clothes properly?

A. Shortage of materials/working cloths/ B. Lack of knowledge C. Not comfortable D. No hinder E. Other (p/s specify) _____

Knowledge

14. Have you ever taken any training on infection prevention? A. Yes

B. No

15. Infectious organisms transmitted through working clothes/gown/. A. True B. False

16. Dose working clothes or gowns may protect you and others from infection-causing agents?

A. Yes

B. No C. I do not know

17. The clean working clothes are free from infectious micro-organisms even they were used for few days. A. True

B. False

Attitude

18. Do you agree that pathogenic organisms can be transmitted from person to person through personal protective working clothes/gown, uniform, apron, scrub/ can spread?

A. Strongly agree B. Agree

C. Neutral D. Disagree E. Strongly disagree

19. Do you agree that the working clothes/gown, uniform, apron/ can be causes for nosocomial infections?

A. Strongly agree on B. Agree C.

Neutral D. Disagree E. Strongly disagree

20. Do you agree that you may get bacterial contamination when drying hands-on working clothes? A. Strongly agree on B. Agree C. Neutral

D. Disagree E. Strongly disagree

21. Do you agree that wearing working clothes at tea cafes or lounges has a risk of bacterial contamination and spread of infection?

A. Strongly agree B. Agree

C. Neutral D. Disagree E. Strongly disagree

22. Do you agree that washing hands after each health care activity is the best way to prevent bacterial/nosocomial infection?

A. Strongly agree B. Agree

C. Neutral D. Disagree E. Strongly disagree

Practice

23. Do you wash your hands regularly after touching each patient, or biological sample, or cleaning the working environment?

A. Always B. Sometimes C. Rarely D. Very rarely E. Never

24. Do you put off your working clothes when leaving the working site?

A. Always B. Sometimes C. Rarely D. Very rarely E. Never

25. After washing hands, do you dry your hands on your gown/working clothes/? A. Always B. Sometimes

C. Rarely D. Very rarely E. Never

26. Do you wear your protective working clothes regularly when you are at work?

A. Always B. Sometimes C. Rarely D. Very rarely E. Never

Thank you for your time.

Annex IV: Questioners in Amharic version

ጥያቄዎች

በመጀመሪያበጥናቱላይለመሳተፍፈቃደኛስለሆኑልንምስጋናችንየላቀነው።

የጥናቱርእስ*Staphylococcus aureus* and *MRSA* Contamination level of Working Clothes, Hand Carriage and Knowledge, Attitude and Practice Among Healthcare Workers at Federal Police Hospital; Addis Ababa, Ethiopia.'''.**ሲሆንይህመጠይቅየተዘጋጀውየህክምናአገልግሎትሰራተኞችግላዊመረጃእናበስራላይየራ ስንከበሽታአምጪተዋህስመከላከያየስራልብስአጠቃቀምእናበንጽህናአያያዝግንዛቤናአተገባበርለማዎቅ የተዘጋጀመጠይቅነው።**

እርስዎየሚሰጡንመረጃምስጢራዊነቱየተጠበቀሲሆንለጥናቱእጅግጠቃሚበመሆኑትክክለኛመረጃይሰ ጡንዘንድበትህትናእንጠይቃለን።

ግላዊ መረጃ

ቀን_____/____/_____

ኮድ_____

1. **ዕድሜ** ————
2. **ጾታ** ————
3. **ስራ/ሙያ**_____
4. **የስራክፍልዎ**_____
5. **የስራልምድ** ————

የስራ ልብስ አጠቃቀም እና አያያዝ

6. **በስራ ላይ ሆነው የለበሱትን ጋዎን ወይም ሸርጥ ወይም የስራ ደንብ ልብስ እንደለበሱ ከስራ ቦታ ውጭ ይሄዳሉ? /ወደ ሻይ ቤት፣ሱቅ፣ማረፊያ ክፍል/ ? ሀ. አዎ ለ. አይ**
7. **የስራ ልብስዎትን መች/ምን ጊዜ ይቀይራሉ? (ከአንድ በላይ መምርጥ ይችላሉ)።ሀ. ሲቆሽሽ ለ. በየቀኑ ሐ. በየሳምንቱ መ. በበካይ ነገሮች/ደም፣ከበሽተኛ ሰውነት የሚወጡ ፈሳሾች እና በመሳሰሉት ሲበከል ሠ.ሌላ(ይግለጹ)_____**

8. አሁንለብሰውትያለውንጋወን/ደንብልብስ/ሽርጥመቼነውየቀየሩት እባክዎትንመልሱንይግለፁ._____

9. ከስራውጭበሚሆኑበትጊዜየስራልብስዎትን/ጋወን፣ሽርጥ፣የስራደንብልብስ/የትያስቀምጣሉ?

ሀ. ከሌሎችልብሶችጋርበመቀላቀልአስቀምጣለሁ.ለ. ወደቤቴይገፍእሄዳለሁ.ሐ. ለብቻለይቼአስቀምጣለሁ

መ. በስራቦታልብስመስቀያላይእሰቅላለሁሠ. ለዚሁበተዘጋጀቦታላይአስቀምጣለሁ

ረ. በስራቦታጠረጴዛላይአስቀምጣለሁሰ. ሌላቦታ(ይግለጹ)_____

10. የጋዎን/የስራደንብልብስ/ሽርጥ/ ንጽህናእንዴትይጠብቃሉ ሀ. በስራቦታ/በሆስፒታልአጠባክፍልበማጠብለ. ቤትወስዶበማጠብሐ. ለብቻውለይቶበማጠብ መ. ከሌሎችልብሶችጋርቀላቅሎበማጠብሠ.ሌላ(ይግለጹ)_____

11. በጋዎን/ስራ ደንብ ልብስ/ሽርጥ/ ኪስ ውስጥ ዕቃ ያስቀምጣሉ ሀ. አዎ ለ. አይ

12. ምልስዎት አዎ ከሆነ ምንድነው የምያስቀምጡት ሀ. ሞባይል ስልክ ለ. የገንዘብ ቦርሳ ሐ.ገንዘብ መ. የሚበሉነገሮች/ቆሎ፣ብስኩት፣ ማስቲካ / ሠ. ሌላ(ይግለጹ)_____

13. በህክምናስራላይከበሽታአምጪተዋህስያንራስንመከላከያየስራልብስበአግባቡለመጠቀምእንቅፋ ትየሚሆኑነገሮችምንድናቸው? ሀ. እጥረትስላለለ. ጥቅማቸውንስለማላውቅሐ. ስለማይመቹኝ መ እንቅፋት የለም ሠ ሌላ(ይግለጹ)

ግንዛቤን በተመለከተ

14. በበሽታ አምጪ ተህዋስያን መበከልን ስለመከላከል ስልጠናወስደው ያውቃሉን? ሀ. አዎ ለ. አይ

15. በስራ ልብስ አማካኝነት በሽታ አምጪ ተዋህስያን ወደ ሰዎች ይዛመታሉ/ሊዛመቱ ይችላሉ/ ። ሀ. እውነት ለ. ሐሰት

16. በስራ ላይ እራስን መከላከያ ልብስ/ጋወን፣ሽርጥ፣የስራ ደንብልብስ/ ከበሽታ አምጪ ተዋህስያን እራስንና ሌሎችንም ይከላክላል ሀ. አዎ ለ. አይ ሐ. አላውቅም

17. ጥቂት ቀናት የተለበሰ ቢሆንም ያልቆሸሸ የስራ ልብስ ከበሽታ አምጭ ተዋህስ የጸዳ ነው። ሀ. እውነት ለ. ሐሰት

አመለካከት

18. በሽታ አምጭ ጀርም በስራ ልብስ አማካኝነት ከአንዱ ሰው ወደሌላ ሰው ይተላለፋል ብለው ይስማማሉ?

ሀ.በጣም እስማማለሁ ለ. እስማማለሁ ሐ. አስተያትየለኝምመ. አልስማማም ሠ. በጣም አልስማማም

19. የስራ ልብስ በሆስፒታል ውስጥ ለሚተላለፉ በሽታዎች መተላለፊያ ምክንያት ሊሆን ይችላል ብለው ይስማማሉ?

ሀ.በጣም እስማማለሁ ለ. እስማማለሁ ሐ. አስተያየትየለኝምመ. አልስማማም ሠ. በጣም አልስማማም

20. እጅን በስራ ልብስ ማድረቅ በበሽታ አምጪ ባክቴሪያ መበከል ምክንያት ሊሆን ይችላል ብለው ይስማማሉ?

ሀ.በጣም እስማማለሁ ለ. እስማማለሁ ሐ. አስተያትየለኝምመ. አልስማማም ሠ. በጣም አልስማማም

21. የስራ ልብስን ከስራ ቦታ ውጭ ሻይ ቤት፣ ወይም ምግብ ቤት መልበስ የባክቴሪያ ብክለትን ያስከትላል ብለው ይስማማሉ?

ሀ.በጣም እስማማለሁ ለ. እስማማለሁ ሐ. አስተያትየለኝምመ. አልስማማም ሠ. በጣም አልስማማም

22. ማንኛውንም ወይም እያንድንዱን የጤና አገልግሎት ስራ እንዳከናወኑ እጅን መታጠብ የበሽታ አምጭ ተዋህስን ለመከላከል ዋነኛ መንገድ ነው ብለው ይስማማሉ?

ሀ.በጣም እስማማለሁ ለ. እስማማለሁ ሐ. አስተያት የለኝም መ. አልስማማም ሠ. በጣም አልስማማም

ተግባር

23. እያንዳንዱን በሽተኛ ወይም ከበሽተኛውጋር ንክኪ ያለው ነገር ከነኩ ወይም የስራ አካባቢን ባጸዱ ቁጥር እጅዎትን ይታጠባሉ? ሀ. ሁልጊዜ ለ. አንድ አንድ ጊዜ ሐ. አልፎ አልፎ መ.በጣም አልፎአልፎ ሠ. በፍጹም

24. የጤና አገልግሎት ከሚሰጡበት ቦታ ከመውጣትዎ በፊት የስራ ልብስዋትን ያወልቃሉ?

ሀ. ሁልጊዜለ. አንድአንድጊዜሐ. አልፎአልፎመ.በጣምአልፎአልፎ ሠ. በፍጹም

25. እጅዎትን ከታጠቡ በሁዋላ በስራ ልብስዎት ያደርቃሉ?

ሁልጊዜለ. አንድአንድጊዜሐ. አልፎአልፎመ.በጣምአልፎአልፎ ሠ. በፍጹም

26. ስራ ላይ በሚሆኑበት ጊዜ ሁሉ የሰራ ልብስዎትን በቋሚነት ይለብሳሉ?

ሀ. ሁልጊዜለ. አንድአንድጊዜሐ. አልፎአልፎመ.በጣምአልፎአልፎ ሠ. በፍጹም

ጊዜሰጥተውስለተባበሩንእናመሰግናለን።

Annex V: Laboratory Standard Operational Procedure (SOP)

1. Sample collection

· **Necessary materials: -** Sterilized normal saline (physiological saline), sterilized cotton tips, sterilized test tube with screw cap, marker**.**

· **Sample collection procedure**

1. Moisten the sterile cotton tip by rinsing in sterilized normal saline

2. Roll the wet cotton tip on tips of right and left sleeves, mouth tips of both front down pockets of sample clothe (for clothe sampling), and rolling backside and palm side of both hands.

3. Immediately put the swab sample in a sterilized screw cap test tube. (Keep clothe sample and hand sample in different test tubes)

4. Label with participant code and sample source (clothe/hand)

5. Transport to microbiology laboratory within 30minutes.

2. Culture media preparation and storage

Necessary materials: - Culture media powder (mannitol salt agar, tryptone soya broth, Mueller Hinton agar, DNAse agar, skimmed milk) 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.

Fluid culture media: - Tryptone soya broth and skimmed milk

Tryptone soya broth: - Used as enrichment media where organisms are likely to be few and to prepare bacterial suspension for antimicrobial susceptibility test**.**

Skimmed milk: - Used to preserve the isolated organisms.

Solid culture media: - Mannitol salt agar, Mueller Hinton agar, DNase agar

Mannitol salt agar (MSA): - is selective and differential media for Staphylococcus aureus.

Mueller Hinton agar: -used for Kirby-Bauer disc diffusion technique antimicrobial susceptibility test**.**

DNase agar: - Used for identification of S aureus. (Bio-chemical test)

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

3. **Quality control (QC): -** Sterility and growth test**.**

Sterility test: - Incubate5% of the batch at 35–37oC overnight. Contamination by microorganisms capable of overnight growth will be 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.

Sterility test of normal saline done by inoculating it on mannitol salt agar culture media and incubate at 35-37oC for 24 hours and check for any growth.

Growth test: - The growing ability of culture was tested by inoculating known microorganisms (S aureus and S epidermdis) which were obtained from Ethiopia Public Health Institute (EPHI), incubate at 35-370c for overnight, the growth tested, and compa**re** with the previous result.

4. Sample processing

Note: Before starting sample processing, the disinfecting of the working area with 10% bleach and performing of all sample processing procedures near to flame of the Bunsen burner are mandatory.

Culturing

Culturing in liquid culture media (Tryptone soya broth): - The collected swab samples direct inoculate in tryptone soy broth to increase the number of bacteria.

Procedure: - On the disinfected working table, near to the flame of the Bunsen burner,

1. Bring the broth to room temperature.
2. Look for any turbidity (turbidity occurs due to contamination)
3. Remove the caps of collected swab and broth,
4. Direct inoculate the collected swab into tryptone soya broth aseptically,
5. Immediately recap the broth sample. (Note that never put the cap of the broth on an unsterile object or surface.)
6. Label the broth sample with the previous sample code (participant code)
7. Incubate at 35-37**0**C overnight.

Culture in mannitol salt agar (MSA): - This culture media is selective and differential media for the growth of Staphylococcus aureus. It contains 5% sodium chloride which inhibits the

growth of micro-organisms other than S aureus and S aureus produces a golden yellow colony by breaking down the mannitol.

Procedure: - On the disinfected working table, near to the flame of the Bunsen burner,

1. Remove the broth sample from the incubator,

2. Bring the MSA to room temperature,

3. Look for crack, any growth, or color change of the culture media.

4. Label the backside of the media with sample code,

5. Remove the cap of the broth sample, by using a sterile loop transfer the loop full growth from the broth to mannitol salt agar media and streak.

6.Incubate at 35-37oc for 18-24 hours(33).

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

Colony characteristics: -S aureus produces a yellow colony with a yellow background on mannitol salt agar(34).

Gram stain: -The Gram staining reaction is used to help identify micro-organisms in cultures by their Gram reaction (Gram-positive or Gram-negative) and morphology. Differences in Gram reaction between bacteria are thought to be due to differences in the permeability of the cell wall of Gram-positive and Gram-negative organisms during the staining process. Since the cell walls of gram-positive organisms are less permeable, the dye is not easily removed from them. The acidic protoplasm of gram-positive organisms binds to basic dye with help of iodine.

S aureus is gram-positive (Stain dark purple with crystal violet and are not decolorized by acetone or ethanol within a specific staining time (10 seconds).

Necessary materials(requirements) for gram stain: -Crystal violet stain, Lugol's iodine, Acetone–alcohol decolorizer, Safranin, objective slide, normal saline**.**

Gram stain procedure (36**)**

1. Label slide with sample code

2. Emulsify a colony in sterile distilled water and make a thin preparation on a slid and let it air dry.

3. check for the expiry date of each staining solution.

4. Fix the dried smear with heat or alcohol.

5. Cover the fixed smear with a crystal violet stain for 10 seconds.

6. Rapidly wash off the stain with clean water.

7. Tip off all the water, and cover the smear with Lugol's iodine for 10 seconds.

8. Wash off the iodine with clean water.

9. Decolorize rapidly (few seconds) with acetone–alcohol.

10. Wash immediately with clean water.

11. Cover the smear with safranin stain for 10 seconds.

12. Wash off the stain with clean water.

13. Wipe the back of the slide clean, and place it in a draining rack to air-dry.

14. Examine the smear microscopically, with the oil immersion objective to report the bacteria.

Interpretation

Staphylococcus = Dark purple color, spherical shape

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. Chek 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 (Results)

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.

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 (Results)

Clumping within 10 secs =S. aureus

No clumping within 10 secs = No bound coagulase

DNAse test

This test is used to help in the identification of S. aureus which produces deoxyribonuclease (DNase) enzymes. The DNase test is particularly useful

when plasma is not available to perform a coagulase test or when the results of a coagulase test are difficult to interpret.

Principle

Deoxyribonuclease hydrolyzes deoxyribonucleic acid (DNA). The test organism is cultured on a

medium that contains DNA. After overnight incubation, the colonies are tested for DNase

production by flooding the plate with a weak

hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. DNase-producing colonies are therefore surrounded by clear areas due to DNA hydrolysis.

Necessary materials (Required):-DNase agar plate,(Up to six organisms may be tested on the same plate.), Hydrochloric acid Reagent 1 mol/1 (1 N)

Procedure (Method)

1. Check for any crack and growth on DNase media

2. Divide a DNase plate into the required number of strips by marking the underside of the plate.

3. Using a sterile loop or swab, spot-inoculate the test and control organisms. Make sure each test area is labeled clearly.

4. Incubate the plate at 35–37OC overnight.

5. Cover the surface of the plate with 1 mol/l hydrochloric acid solution. Tip-off the excess acid.

6. Look for clearing around the colonies within 5 minutes of adding the acid.

Interpretation (Results)

Clearing around the colonies = DNase positive strain (S aureus)

6. **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**):- Mueller Hinton agar, Antimicrobial discs, Turbidity standard equivalent to 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 3–5 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, rotating the plate approximately 60o to ensure even distribution.

5. With the petri dish lid in place, allow 3–5 minutes (*no longer than 15 minutes*) for the surface of the agar to dry.

6. Using sterile forceps, 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 35oC for 16–18 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 zones sizes of each antimicrobial, reporting the organisms 'Resistant', 'Intermediate/Moderately susceptible', 'Susceptible (33)

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: **Zewditu Kaba (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Kassu Desta (MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Asegedech Asmamaw (MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.