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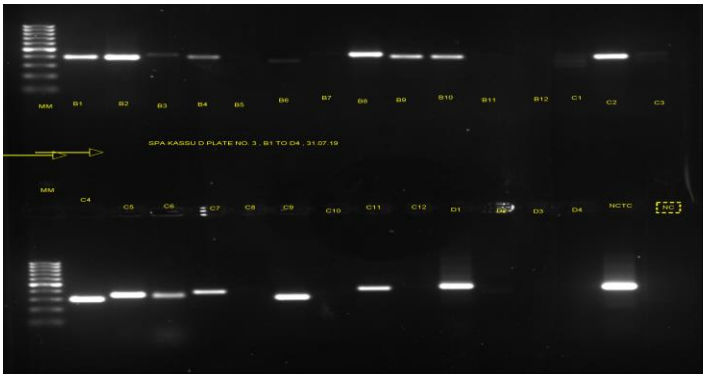
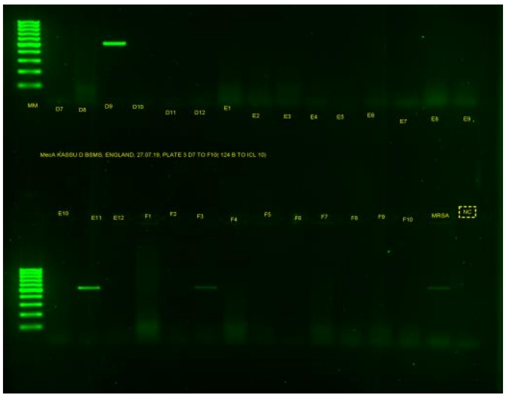
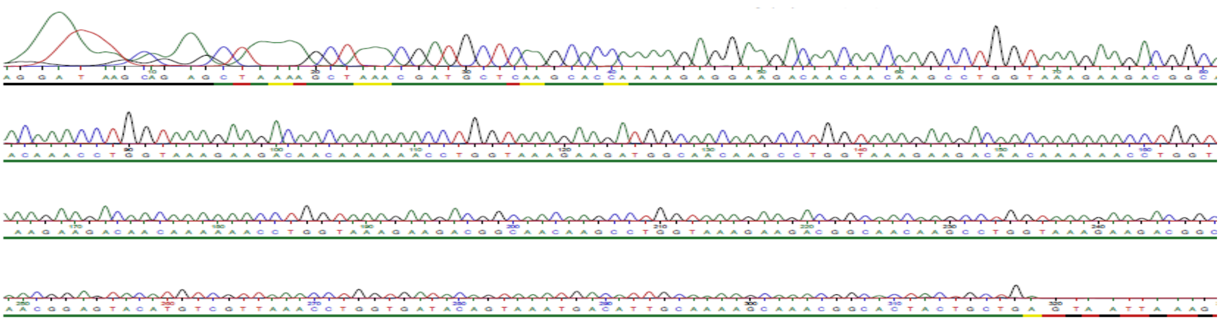
**Burden of Methicillin *Resistant Staphylococcus aureus (*MRSA) and associated factors at TASH: Evidence from colonization of Health Care Workers, Administrative staff, Patients and selected inanimate objects.**

Kassu Desta Tullu (BSc. M.Sc.)







Department of Microbiology, Immunology, and Parasitology, School of Medicine, Addis Ababa University

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**ADDIS ABABA UNIVERSITY**

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**DEPARTMENT OF MEDICAL MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY**

# Burden of Methicillin *Resistant Staphylococcus aureus (*MRSA) and associated factors at TASH: Evidence from colonization of Health Care Workers, Administrative staff, Patients and selected inanimate objects.

**BY: Kassu Desta Tullu (BSc, MSc)**

A research thesis submitted to the Department of Medical Microbiology, Immunology and Parasitology, School of Medicine, College of Health Sciences, Addis Ababa University, in partial fulfilment of the requirements for Doctor of Philosophy in Medical Microbiology.

**December, 2023 ; Addis Ababa, Ethiopia.**

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## Ph.D. Research Thesis

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**Major: Medical Microbiology**

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**Title of thesis**: **Burden of Methicillin *Resistant Staphylococcus aureus (*MRSA) and associated factors at TASH: Evidence from colonization of Health Care Workers, administrative staff, Patients and selected inanimate objects.**

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**II. ACKNOWLEDGMENTS**

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## III. ABBREVIATIONS

ACME: Arginine catabolic mobile element

AST : Antimicrobial Susceptibility Testing

ATCC: American Type Culture Collection

BLAST- Basic Local Alignment Search Tool

CA-MRSA : Community-associated MRSA

CCs: Clonal Complex

CDC-Centres for disease control and prevention

CFU: Coloney forming Units

CLSI : Clinical and Laboratory Standard Institute

*CONS :Coagulase-negative Staphylococcus*

CSA : Central Statistical Agency of Ethiopia

DMIP : Department of Microbiology, Immunology and Parasitology

DNA: Deoxyribonucleic acid

EMRSA: Epidemic Methicillin Resistant *Staphylococus aureus*

ESBL : Extended-spectrum beta-lactamase producing bacteria

FMOH : Federal Ministry of Health

HAI : Health care-associated infections

HA-MRSA : Healthcare-associated MRSA

HCW : Healthcare worker

ICU : Intensive Care Unit

KAP: Knowledge, Attitudes, and Practice

MALDI-TOF-MS- Matrix-Assisted Laser Desorption Ionization Mass Spectrometry

MGE : Mobile genetic element

MHA : Muller Hinton Agar

MLST: Multi Locus Sequence Typing

MRSA : Methicillin resistance *Staphylococcus aureus*

MSSA : Methicillin Sensitive *Staphylococcus aureus*

PBP2a: Penicillin-binding protein 2a

PCR : Polymerase chain reaction

PFGE: Pulse Field Gel Electrophoresis

PPE : Personal protective equipment (PPEs) in a sustained manner.

PVL: Panton Valentine Leukocidin

SCCmec: Staphylococcal chromosome cassette mec

SLVs : Single-locus variants

SOP : Standard Operating Procedure

SpA: Staphylococcal protein A

SPSS : Statistical Package for Social Science

SSTIs: Skin and Soft Tissue Infections

ST : Sequence type

VRE: Vancomycin-Resistant *Enterococcus*

WGS : Whole genomes sequence typing

WHO : World Health Organization

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## VI. ABSTRACT

**Background**: Methicillin-resistant *Staphylococcus aureus* (MRSA) are multidrug-resistant bacteria that are carried harmlessly by humans and also cause severe life-threatening infections in community and healthcare settings worldwide. MRSA colonization of hospitalized patients and healthcare workers (HCWs) is a risk factor for transmission of infection. Furthermore, inanimate objects, such as Mobile phones, hospital gowns, and other fomites can serve as a reservoir for MRSA. Good knowledge, attitudes, and practices (KAP) of HCWs towards MRSA sources, colonization, and transmission is a key strategy for the control of MRSA. In Ethiopia, MRSA is a public health concern and observed rates of MRSA infections are reported from different parts of the country but molecular based data are very scarce or absent.

**Objectives**: This study aimed to determine the burden and associated factors for MRSA colonization among HCWs, administrative staff, patients and selected inanimate objects at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia.

**Methods**: A prospective and retrospective cross-sectional study was conducted between June 2018 to September, 2019 that included 588 HCWs and 468 administrative staff. Nasal swabs and swabs from their mobile phones were collected. Single swab from gown of each HCW was sampled. Samples from inanimate objects from the hospital were also analysed for MRSA. Perceived knowledge, attitudes, and practice (KAP) of HCWs were assessed using a pretested structured questionnaire. In addition, 170 *S. aureus* stored isolates were included mainly for molecular testing. All swabs samples were cultured on Mannitol salt agar and / or blood agar for growth and identification of *S. aureus* and MRSA were made by standard biochemical tests and cefoxitin disc methods respectively. Antimicrobial testing was performed according to CLSI breakpoints and isolates were further tested for MecA , PVL detection, Spa and MLST typing and WGS. Data were analysed using SPSS version 20.0 and statistical testing (Chi-square or Fisher's exact test for categorical variables) was used to assess the difference between MRSA colonization rate among different groups, socio-demographic factors, and KAP level. P-value less than 0.05 was taken Statistically significant

**Results**: A total of 588 HCWs were included in this study and 58.4 % of them were female. The mean age and standard deviation of HCWs were 29.13 ± 6.66 years, The majority of HCWs were nurses by profession accounting for 49.1 % (289/588) followed by Medical doctors (28.4 % ) [167/588]. Among 468 administrative staff , 64.1 % of them were females. The rate of *S.aureus* isolates were 16.32 % (96/588) and 8.97 % (42/468) for nasal swabs and 6.46 % (38/588) and 3,85 % (18/468) for mobile phone contamination for HCWs and administrative staff respectively . For HCWs group the rate of *S. aureus* isolation from gowns was 7.48 % (44/588). Overall, 31 isolates (13.02 %) were sensitive to all antibiotics tested. All isolates were sensitive to rifampicin. About 10 % of isolates including all MRSA isolates were vancomycin sensitive using Van A and Van B genes detection methods. Furthermore ,237 isolates were resistant to at least for one drug. Overall there was a significant difference between drug resistance among MRSA and MSSA isolates (P-value <0.05). The burden of MRSA nasal colonization among HCWs and administrative staff was found to be 4.8 % (28/580) and 0.2 % (1/468) respectively. Nurses were most colonized by MRSA (22/28 total positive) P-value < 0.05). In this study, 2.72 % (16/588) and 1.3 % (6/468) mobile phones of HCWs and administrative staff were contaminated by MRSA respectively which are more frequent for nurses and doctors. Importantly ,2.89 % (17/588) of HCWs gowns were contaminated with MRSA and slightly higher among female HCWs (p-value >0.05). The proportion of MRSA from stored isolate of mother-child pair and diabetic patients was 0.24 % (2/846). While 21.73 % (20/92) stored isolates from clinical samples were MRSA. Overall 53 % (178/336) of *S.aureus* isolates obtained from nasal, clinical, mobile, and HCW's gowns were PVL positive. A significant difference was also seen between pvl status and source of *S.aureus* isolates ( P-value <0.05).Genotyping based on spa typing has resulted in 131 spa types for 189 *S. aureus* isolates from various sources of samples, Forty-two spa types (32.06 %) occurred in 2 or more frequencies, while 89 of them (67.94 %) observed in singleton. The most frequent spa types in this study were t355 which were observed 23 times, followed by t223, t085, t131, and t003. Spa type *t701, t1828, t080, t2235, t2302 and t14805,t314, t380, t3841, t11375, t5338, t14350,*t062, t318 ,t693 and t937 were some of the spa types described in this study. Moreover, spa CC 15 and CC 22 are the most dominant clones observed. MLST Sequence data were available

for 52 representative *S.aureus* isolates and 31 sequence types (ST) were generated. ST 152 is the most dominant ST accounting 20.3 % (11/ 54) followed by ST 4666, ST 5, and ST 744 each accounted 5.55 % (3/54 for each STs) . ST 80, ST88. ST97 and other STs were also found. Twenty-four STs are clustered in 5 MLST CCs and CC5, CC8, and CC15 were the very common clones both for MRSA and MSSA groups. Many spa types are reported for the first time in Ethiopia. Moreover, 18 *S.aureus* were characterized by whole genome sequencing (WGS),and based on the 16 SrRNA sequences, phylogenetic tree were constructed along with known sequences from the NCBI database. It seems our isolates were diverse and mostly poorly separated. Based on the average nucleotide identity and many of our *S.aureus* isolates from the nasal, gown, mobile phone, and clinical sources showed high similarity with *S.aureus* strains identified from various clinical and non-clinical isolates. In this study, the overall knowledge of HCWs about MRSA prevention and control was 85.5 % .The majority of HCWs in TASH had a positive attitude towards the importance of system-wide approach to prevent MRSA in the hospital (95.2 %, 542/ 582). While 54.68 % of HCWs had a positive attitude and 68.4 % of them had good practice about MRSA prevention and control measures at TASH.

**Conclusion:** MRSA nasal colonization and mobile phone contamination of HCWs in TASH was higher than administrative staff. The presence of MRSA from gowns of HCWs is a cause for concern. MRSA was also seen from stored isolates of nasal source of patients and clinical isolates based on Mec A detection. The high rate of pvl among all *S. aureus* isolates is worrisome. There is diverse spa and MLST types among *S.aureus* isolates, t355 and ST 152 were common ST and spa type respectively. Although, HCWs in TASH had high level of knowledge about MRSA control and prevention, their attitude and practice score is inadequate. Hence, it is important to implement a system for continuous surveillance of MRSA in TASH using phenotypic and genotypic methods to take preventive measures and track changes of intervention. Moreover, concerted efforts are needed among HCWs, management body and policymakers to avail and regulate personal protective equipments (PPEs) in a sustained manner.

**Key Words: *S.aureus* , MRSA, TASH, MLST, WGS, Spa typing, HCWs**

# CHAPTER ONE: INTRODUCTION

## 1.1. Background

*Staphylococcus aureus (S.aureus)* is a gram-positive, non-motile, pus-producing coccus with a microscopic appearance of 0.5- to 1.5-μm balls that are clumped together, similar to grapes but could be found in single-cell, pair, short-chain forms. There are more than 35 species of Staphylococcus and 17 subspecies. *S. aureus* has also more than 200 strains. It possesses several virulence factors combined with its increasing antibiotic resistance (Green et al., 2012; Bannerman et al., 2003).

*S.aureus* is , non-spore-forming, usually catalase-positive, typically unencapsulated or with limited capsule formation. It is a facultative anaerobe and grows best at 37°C. The genome size of *Staphylococcus* is in the range of 2000-3000 kb (Bannerman et al., 2003).

*S. aureus* is a major human pathogen that causes a wide range of clinical infections. It is a leading cause of bacteraemia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections. For the past 20 years, an increment in health care-associated infection due to *S. aureus* and community-associated epidemics were seen worldwide. The bacteria are both a commensal and a human pathogen. Approximately 30% of the human population is colonized with *S. aureus*. Concomitantly, it is a leading cause of bacteraemia and a range of systemic and superficial infections both at the hospital and community level (Tong et al., 2015).

Methicillin-resistant *S. aureus* (MRSA) is *S. aureus* that is resistant to many antibiotics including penicillin and cephalosporin. MRSA is significantly associated with health care-associated infection more than community-acquired one (CDC, 2014). The proportion of *S. aureus* infections due to MRSA is increasing and *S. aureus* infections are also increasingly being acquired in the community. In addition, there is growing evidence that patients with MRSA bacteraemia have a worse outcome than patients with infections due to methicillin-susceptible *S. aureus* (MSSA) (Garau et al., 2009).

MRSA can colonize or infect adults and children and being colonized by MRSA is a known risk factor for the development of systemic and superficial MRSA infection (Calfee et al., 2016).

Several immunomodulatory proteins like staphylococcal protein A (SpA) could play a role during *S. aureus* colonization. The persistence potential of *S. aureus* colonization may be due to the ability of these strains to express greater amounts of immunomodulatory proteins which were observed in human and animal models (Mulcahy et al., 2016; Lacey et al., 2016).

Beta- lactam antibiotics have been a mainstay of clinical therapeutics for methicillin-susceptible *S. aureus* (MSSA) infections. Since most *S. aureus* bacteraemia is caused by MSSA, the anti-staphylococcal β-lactams remain a key element of therapeutic strategies for such infections. From clinical trial studies, β-lactams are found to be superior over vancomycin for the treatment of MSSA bacteraemia infections, including endocarditis. The American Heart Association has consistently recommended it as the treatment of choice for MSSA endocarditis. However, large, randomized controlled clinical trials are required to introduce it for day-to-day patient management purposes (Garau et al., 2009; Bayer et al., 2017, Zaghloul et al., 2016).

Panton Valentine Leukocidin (PVL) is a virulence factor of *S. aureus that* was first associated with Skin and Soft Tissue Infections (SSTIs) in 1932 by Panton and Valentine (Melles et al., 2006). Experimental and clinical evidence shows that PVL-producing strains are associated with severe, necrotizing skin infections and pneumonia. Another pore-forming leukocyte toxin, α-toxin does not lyse neutrophils but instead lyses other immune cells such as macrophages and lymphocytes. Chromosomal genetic elements are linked with the acquisition of the mecA gene, located within the large chromosomal element known as the SCCmec. Likewise, Arginine catabolic mobile element (ACME ) is a large mobile genetic element (MGE) that may play an important role in the growth, transmission, and pathogenesis of CA-MRSA. *S. aureus* are capable of producing superantigens that cause serious illnesses, including toxic shock syndrome and necrotizing pneumonia. The ability of MRSA to form biofilms is an important virulence mechanism that complicates infections, especially those involving foreign materials like catheters and prosthetic joints (Laceyet al., 2016, Chessa et al., 2016, Watkins et al., 2012, Kong et al., 2016 10-12).

MRSA is primarily mediated by the over-production of penicillin-binding protein 2a (PBP2a) with a low affinity for β-lactam antibiotics. The mecA gene is part of a 21 kb to 60 kb staphylococcal chromosome cassette mec (SCCmec), a mobile genetic element that may also contain genetic structures as Tn554, pUB110, and pT181 which encode resistance to non-β-lactam antibiotics. The mecA gene which encodes PBP2a is considered a useful molecular marker of putative methicillin resistance in *S. aureus*. *S. aureus* strains tend to accumulate additional resistance determinants, resulting in the formation of multiple-antibiotic resistant MRSA strains, which are creating therapeutic problems and limiting the choice of therapeutic options (Zaghloul et al., 2016).

Accurate and rapid identification and detection of MRSA is vital for effective antimicrobial chemotherapy and infection control practice. Conventional culture methods are the most available methods despite it's long turnaround time. Methods with a short turnaround time for the identification of MRSA have been available in different settings and costs. Moreover, the sensitivity, specificity, positive and negative predictive value of a test is critical to choose and adopt for MRSA testing. Polymerase chain reaction, (PCR) based tests, penicillin-binding protein 2a (PBP2a) antibody agglutination tests, Chromagar base detection, GeneXpert assays, and other methods which are under evaluation could be used for detection of MRSA (Wassenberg et al., 2010; Ghanwate et al., 2016, French et al., 2009).

Hospitalized patients can be colonized not only with MRSA but can also harbour other drug-resistant bacteria such as extended-spectrum beta-lactamase producing bacteria (ESBL) and Vancomycin-Resistant *Enterocoous (VRE)* (Young et al., 2014; Davis et al., 2004).

The burden of multidrug-resistant bacteria including *MRSA* is well described in many European and American long-term care facilities and nursing homes. Molecular characterization showed that the isolates were clonally related and that previous use of antimicrobials and hospital admission were also risk factors for colonization (ECDC,2012, Ludden, et al., 2015; Baldwin et al., 2009, Brugnaro et al., 2009; Denis et al., 2009).

The incidence and epidemiology of MRSA colonization or infection are changing in many hospital settings. In Hospitals of Canada from 1995 to 2007, a total of 37,169 hospitalized patients were newly identified as either infected or colonized with MRSA, and the overall incidence of MRSA colonization and infection increased from 0.65 to 11.04 cases per 10,000 patient days. In Canada, the proportion of community-associated MRSA strains increased from 6 % to 23 % and CMRSA-2 (USA 100/800 sequence type) was the dominant genotype resulting in a 17-fold increment from baseline (Simor et al., 2010).

Similarly, the annual number of MRSA notifications to the National Infectious Disease Register (NIDR) of Finland rose over ten-fold, from 120 in 1997 to 1458 in 2004, and the proportion of MRSA among *S. aureus* blood isolates tripled, from < 1 % during 1997–2003 to 2.8 % in 2004. During the same period, 253 different strains among 4091 MRSA isolates were identified by Pulse Field Gel Electrophoresis (PFGE), PFGE: 215 were sporadic and 38 outbreak/epidemic strains, including 24 new strains. Half of the ten most common strains carried SCC*mec*IV or V (Ibrahem et al., 2007).

Health care workers (HCW) who are colonized with MRSA can transmit the bacteria to patients and the community at large during caring for patients. The burden of MRSA among different HCWs has been described in many countries around the globe (Drago et al., 2015; Maheshwari et al., 2015; Shibabaw et al., 2013; Knahal et al., 2015;Verwer et al., 2011; Amorim et al., 2009; Mathanraj et al., 2009; Askarian et al., 2009; Isibor et al., 2014, Sah et al., 2013). MRSA pharyngeal prevalence was 11.8 % in patients and 7.6 % of HCWs in Serbian University Hospital, where, CC5-MRSA-SCCmec I was the dominant clone among patients and HCWs in the emergency and medical department, while high genetic diversity of CA-MRSA was shown in surgical department especially among HCWs (Cirkovic et al., 2015).

HCWs Hands, instruments, mobile phones, or other inanimate hospital objects used by HCWs may serve as reservoirs for microorganism and MDR bacteria that could be transmitted to patients as health care-associated infection (Brady et al., 2006; Brady et al., 2010; Sing et al., 2010).

Mobile phones are almost handled by everyone and the frequent contact of HCWs with cell phones predisposes them to colonization with MRSA and other MDR microbes. MRSA has been isolated from the cell phone of HCWs and patients and reports are available from different researchers (Rana et al., 2013; Singh et al., 2012; Elkholy et al., 2010,).

The knowledge, attitude, and experience of HCWs towards MRSA and other multi drug-resistant (MDR) bacteria sources and infection control is one of the limiting factors in efforts to reduce the burden of health care-associated infections (HAI) linked with these microorganisms. This could also vary among different HCWs in different health care facilities (Tambe et al., 2012; Moura et al.,2007 ).

Studies have shown that compliance with precautions among nurses to avoid exposure to microorganisms is low due to lack of knowledge, time constraint, the negative influence of the equipment on nursing practice, the conflict between the need to provide care and self-protection (Aftab et al.,2015). On the other hand, wearing gloves and gowns for all patient's contacts in ICU reduced the acquisition of life-threatening diseases caused by MRSA or vancomycin-resistant *Enterococcus* (VRE) compared with traditional care (Efstathiou et al.,2011).

**1.2. Statement of problem**

MRSA Infection could arise endogenously from the normal flora of the individuals since patients and HCWs could carry the microbes in the nasal cavity, nasopharyngeal tract, skin, perineum, and rectum. This is an exposure risk for both patients and HCWs.

Many studies showed that prior colonization of patients and HCWs with MRSA are risk factors for different types of clinical infection in Hospital settings (Davis et al., 2004, Harris et al., 2013). Notably, HCWs are mediators for reserving MRSA in the hospital, which could serve as MRSA sources for hospitalized patients both at the inpatient and outpatient departments. Moreover, colonized HCWs could transmit MRSA to their close families, colleagues, and the community at large when they go out of their working places.

Screening and decolonization of high-risk group patients such as hospitalized patients, patients undergoing surgery or haemodialysis procedures, cancer patients, neonates especially those who are underweight and patients in ICU, is a common practice in many developed countries to minimize the consequence of MRSA associated infection (Antonia et al., 2013;Rao et al., 2008).

Screening of HCWs on regular basis is also practiced in Europe and the USA to follow the status of MRSA in a particular hospital and to take action that prevents MRSA transmission from HCWs to admitted patients. Patients may have the right not to be cared for by colonized and infected HCWs (Verwer et al., 2011; Dulon et al., 2013).

HCWs' hands could also carry MRSA and other microbes which can be transmitted to admitted patients following poor hand hygiene practice. Moreover, various inanimate objects such as cell phone of HCWs, their gowns, shoes, stetescopes, blood pressure apparatus, and many others could harbour MRSA and possibly other microbes which serve as a source of colonization and or infection of HCWs themselves or be transmitted to patients (Coello et al., 1994, Boyce et al., 1997; Bearmanet al., 2014).

The knowledge of HCWs, their attitudes, and practice related to MRSA colonization, source of MRSA, and intervention measures to minimize transmission and carriage is crucial. It is well stated that instituting intervention measures like "MRSA bundles" significantly reduce MRSA burden in different hospitals across the world (Anitha et al., 2015; Nantasit et al.,2015). However, there are no systematic intervention strategies in Tikur Anbesa Specialized Hospital (TASH) and most health care facilities in Ethiopia.

### 1.3. Rationale of the study and Justification

MRSA infection is commonly found in Ethiopian patients with varying degrees among inpatients, outpatients, and also a few at the community level (Tigabu et al., 2018 , Tsige et al., 2020, Mulu et al., 2006, Mullu et al., 2012, Gebremedhin et al., 2016, Efa et al., 2019, Balta et al., 2003 ). To our knowledge, in Ethiopia ,informtions are very scarce on MRSA colonization studies from the HCWs groups , patient, inanimate objects such as mobile phones and gowns of HCWs from the molecular methods perspectives .

Few descriptive studies address MRSA colonization among HCWs in the Ethiopian context. The rate of nasal carriage of MRSA from HCWs in Dessie Referral Hospital was 12.7 % (Shibabaw et al., 2013). However, a study from Dessie Referral Hospital and many other studies on *S. aureus* included simple coagulase and catalase tests to identify *S.aureus* and have used oxacillin /cefoxitin/methicillin as surrogate markers for MRSA. The use of few biochemical tests for identifying organisms and the use of a single antibiotic for identifying MRSA may have an implication that may result in underestimating or overestimating the real burden of MRSA.

Similarly, few studies addressed the isolation of MRSA from inanimate objects like gowns, stetescope, and other objects. Recently, MDR colonization of operating room air and inanimate objects from the operating rooms of public hospitals in Addis Ababa, Ethiopia showed that *S. aureus* is the second most common isolates from inanimate objects and air samples of operating rooms next to *Coagulase-negative Staphylococcus( CONS)*, accounting for more than 16 % of overall bacterial isolates. Out of 33 *S. aureus* isolates, 6 (18.2 %) were MRSA (Kebede et al., 2014). One study in Felege Hiwot Referral Hospital, northwest Ethiopia Bahirdar also depicted that health care workers fomites harbours different microbes ( Ayalew et al., 2019).

In Ethiopia, there is limited information about colonization of patients, HCWs, personal used items like cell phones, gowns, and other inanimate objects with MRSA including the knowledge, attitudes, and practice of HCW towards MRSA colonization and preventive measures in particular. Hence with the growing challenge of antimicrobial-resistant microorganisms infecting hospitalized patients and the need to come up with recommendations for prevention and control activities, it is very important to have a comprehensive research undertaking that uses universally acceptable microbiology methods to confirm the existence of these microbes in the study site and also address the different potential source of MRSA in the Hospital environment.

Therefore, we formulated the following research questions:

- What is the real burden of MRSA colonization in patients at TASH?
- What is the real burden of MRSA colonization in HCWs in TASH?
- How many of the cell phones of HCWS , gown, and other inanimate bodies harbour MRSA at TASH?
- Is there a genetic linkage of MRSA isolates from nasal swabs of patients, HCWs, cell phones, gowns, and other inanimate objects in TASH?
- Is MRSA colonization associated with different risk factors including knowledge, perception, and practice level of HCWs?
- Are HCWs colonized more with MRSA than non HCWs / control groups in TASH?

The emergence and dynamics of microbial resistance genes in *S. aureus* circulating between HCWs, patients, and inanimate objects including cell phones and gowns are not known in the Ethiopian context and this may likely confer survival advantages and further disseminate genes through plasmids and transposons in the hospital and the community.

Addressing the above questions will help hospitals, HCWs, and policymakers to design and implement relevant policy and intervention strategies to minimize the burden of MRSA in hospitalized patients and the community at large.

### 1.4. Literature Review

#### 1.4.1.Over view of *S. aureus*

Among the gram-positive cocci, the genus *Staphylococcus* consists of gram-positive, spherical cocci of about 1 μm in diameter and morphologically it appeared as in pairs, short-chain, tetrad, sarcina, or a bunch of grapes that occurred due to having a different plane of division. This genus is non-motile, non-spore-forming cocci, catalase-positive, and facultative anaerobes.More than 50 species and subspecies of Staphylococcus are currently present and *S.aureus* is one of the major human and animal pathogens that can cause both superficial and systemic infection (Green et al., 2012; Plata *et al.*, 2009; Hennekinne*et al.*, 2012).

Being a commensal, *S.aureus* is a leading cause of a range of infections including endocarditis, bacteraemia, osteomyelitis skin, and soft tissue infections. More importantly upon exposure to antibiotics the organisms become the major etiologies for hospital-acquired or healthcare-associated infection ( Turne et al., 2019). *S. aureus* is one of the successful microbes in causing mild and severe infection as it produces

diverse types of virulence factors including toxins, immune-modulatory factors, and exoenzymes,hemolysin, leukocidins, proteases, enterotoxins, exfoliative toxins are the few among the big lists (Oogai et al., 2011).

It has been said that MRSA colonization could increase infection and the strains that cause infection and the one isolated from the colonization site are matched well indicating the ability of the bacteria to cause endogenous infection. Moreover,*S.aureus* found in our skin and any materials in contact with skin could be contaminated including our clothes, laboratory coats, pens, ties, mobile phones which could serve as a source of infection or contamination. ( Turne et al., 2019).

*S.aureus mecA* gene is responsible for resistance to the drug penicillin including methicillin which is located on a mobile staphylococcal cassette chromosome mec (SCC*mec*) elements and encodes an altered Penicillin Binding Protein (PBP2a), which have a low affinity for all *β*-lactam antibiotics even in the same SCC*mec* type (Turne et al., 2019).

In Ethiopia *S.aureus* and MRSA have been major public health threats that can cause different types of infection and both community-acquired and hospital-acquired infections have been reported. In addition, there is some evidence of MRSA colonization among HCWs, school children, and janitors. More importantly, recent meta-analysis data also indicated that MRSA strains have been reported from different parts of Ethiopia underscoring these bacteria become a public health treats (Shibabaw et al., 2013,Atsebaha et al., 2018, Gebreyesus et al., 2013;Azene and Beyene, 2011; Mulu *et al.*, 2006, Dessalegn *et al.*, 2014; Melaku *et al.*, 2012; Mama et al., 2014; Getahun *et al.*, 2008; Haftu *et al.*, 2012,Negussie et al., 2015 ;Dilnessa et al., 2016; Kahsay et al., 2018; Zenebe et al., 2018; Weldeselassie et al., 2021).

Most of the data regarding *S.aureus* so far are mainly done with conventional microbiologic methods except very few of them (Zenebe et al., 2018) that use mecA and pvl genes; others uses mec A, mec C and or SCCmec typing either from human and or diary sources of *S.aureus* isolates (Abrha 2019;Woldeselassie et al., 2021) which may result in under or overestimation of MRSA both in terms of colonization and in the actual clinical infections which underscore the need for more research.

### 1.4.2. MRSA Colonization of patients and HCWs

A point prevalence of MRSA from 177 adult patients hospitalized in an intensive care unit (ICU) (94 in medical ICUs and 83 in surgical ICUs) of a tertiary care hospital in Taiwan resulted in an overall prevalence of 42 % and 32 % for *S. aureus* and MRSA nasal carriage respectively. MRSA carriage rate of the patients hospitalized in medical ICUs was significantly higher than surgical ICUs patients (47 % vs 16 %, $P < 0.001$). Pneumonia, chronic obstructive pulmonary disease, current MRSA infection, and medical ICU admission were independent predictors for nasal carriage of MRSA. Of the 38 MRSA isolates available for molecular analysis, a total of six pulsed-field gel electrophoresis (PFGE) patterns with two major patterns F,42 %; A, 37 % were identified. Both healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (C-MRSA) were identified from study subjects (Chen et al., 2010).

Prevalence and molecular epidemiology MRSA from a 900-bed tertiary governmental healthcare facility in Bangkok, Thailand showed that 57 of 619 in-patients (9.2%) were positive for MRSA. Being male, long admission, low modified McCabe score, history of MRSA infection, and use of broad-spectrum cephalosporin were the risk factors associated with MRSA carriage. Molecular typing results indicated close relatedness among MRSA isolates and successful epidemic subtypes recovered from many different wards. While those subtypes with different multi-locus sequence types (MLST) were single-locus variants (SLVs) of ST239. The sole ST239 and its SLVs identified in this hospital are striking and a better policy for infection control and prevention was proposed (Jariyasethpong et al., 2010).

MRSA carriage rates and genetic relationships of *S. aureus* strains in children attending daycare centers (DCC) in 14 cities from three geographic regions in Mexico were conducted and 237 children (10.1%) were colonized with *S. aureus* and the burden of MRSA carriage was 9.3 % ( 22/ 237) . Children attending DCCs from cities located in the north and south of Mexico showed a higher prevalence than children from DCCs in the central region. PFGE demonstrated six different restriction profiles of MRSA with a predominant pattern of clone A and B which are closely related representing 45% of the total MRSA isolates. SCCmec type II and type IV strains were common in the regions (Velazquez-Guadarrama et al., 2009).

The Canadian nosocomial infection Surveillance Program comprised of 48 hospitals from 1995 to 2007, a total of 37,169 hospitalized patients were newly identified as either infected or colonized with MRSA, and the overall incidence of both MRSA colonization and MRSA infection increased from 0.65 to 11.04 cases per 10,000 patient-days and 11,828 (32 %) had an MRSA infection which increased from 0.36 to 3.43 cases per 10,000 patient-days. The proportion of community-associated MRSA strains increased from 6 % to 23 %. The most common genotype (47 % of isolates) was CMRSA-2 (USA100/800) followed by CMRSA-10 (USA300) accounting 27 % of isolates which was associated with SCCmec type IV. This finding underscores continuous surveillance to monitor the ongoing evolution of MRSA colonization or infection in Canada and globally (Simor et al., 2010).

In Finland from1997 to 2004, about 253 different strains among 4091 MRSA isolates were identified by PFGE: 215 were sporadic and 38 outbreak/epidemic strains, including 24 new strains. Two epidemic strains resembling internationally recognized MRSA clones accounted for most of the increment: FIN-16 (ST125: IA) from < 1 %

in 1997 to 25 % in 2004, and FIN-21 (ST228: I) from 6 % in 2002 to 28 % in 2004. Half of the ten most common strains were SCC*mec*IV or V type (Salha et al., 2007).

Nasal and Hand Carriage of Multi-Drug Resistant Organisms in a Teaching Hospital in Rural Haryana, India showed that out of 70 HCWs , 13 (18.6%) carried *S. aureus*, 8.6 % ( 6/ 70) were *MRSA*, and 15 (21.4%) colonized by *ESBL* producing Gram-negative bacteria. Considering HCWs categories, Nurses and OT technicians were more colonized by *MRSA* and *ESBL* compared to doctors and laboratory technicians, indicating variation in *MDR* colonization and professional categories even in a health care facility (Maheshwari et al., 2014).

MRSA carriage in 52 HCWs (11 orthopedics doctors and 41 nurses) of spinal surgeries in an Italian Orthopaedic Institute showed a prevalence of *S. aureus* and *CONS* , 42.3% and 98 %, respectively with MRSA rate of 13.5 % (Drago et al.,2015).

MRSA carriage and drug-resistant pattern among 204 HCW at Universal College of Medical Sciences and Teaching Hospital, Nepal, revealed, 32 (15.7 %) HCWs were colonized by *S. aureus* and among them 7 (21.9 %) were MRSA carriers. The overall

nasal carriage rate of MRSA was 3.4 % (7/204). The highest MRSA nasal carriage rate of 7.8 % (4/51) was found among nurses. HCWs of both surgical wards and operating rooms accounted for 28.6 % (2/7) of MRSA carriers each. Among MRSA isolates inducible clindamycin resistance was observed in 66.7 % (2/3) of erythromycin-resistant isolates (Kanahal R et al., 2015).

In a period prevalence study of 1,542 HCWs in Western Australian acute care hospitals, 3.4 % (n = 52) were colonized by MRSA. Colonization was more common inpatient care assistants (6.8%) and nurses (5.2%) than in allied health professionals (1.7 %) and doctors (0.7 %). Working in "high-risk" wards that cared for MRSA colonized / infected patients was the strongest risk factor. Interestingly, community clones, ST1-IV and ST78-IV were frequently colonizing HCWs, capitalizing the need for continuous surveillance of MRSA from HCWs (Verwer et al., 2011).

MRSA colonization study from patients and HCWs in a Portuguese hospital Geral de Santo Antonio (HGSA), revealed that, the prevalence of MRSA carriage among 276 patients screened was 5.1% while it was 4.8 % out of 126 HCWs, with an incidence rate of 61/ 1000 person years. Admission to HGSA or attendance to the Diabetic Foot Outpatient Unit (DFOU) of HGSA within the past 12 months, and previous MRSA isolation were significant risk factors for MRSA carriage for patients. Surprisingly, a clonal identity was found for isolates from patients and HCWs with 88.6 % of isolates belonging to the EMRSA-15 (ST22-MRSA-IV) clone (Castro et al., 2009).

Screening of 200 patients and HCWs from a tertiary hospital in South India showed an overall MRSA carriage rate of 8.5%, with the highest rate in patients (15.6 %) while the lowest was seen in HCWs (1.8%). The commonest site of colonization was the anterior nares (Mathanraj et al., 2009).

Ten EMRSA-15 outbreaks were compared with seven Aus 2/3EMRSA outbreaks in Royal Perth Hospital of Australia and the number of patients colonized was similar between the two groups. While the number of HCWs colonized was significantly higher in EMRSA-15 outbreaks compared to Aus2/3-EMRSA outbreaks. The percentage of HCWs colonized was also higher in EMRSA-15 outbreaks versus Aus2/3-EMRSA outbreaks (median 3.4 % and 0.81%) respectively (Hart et al., 2014 ).

The prevalence of MRSA from nasal and pharyngeal samples of HCWs and patients from a major referral center in Serbian University hospital showed that 7.6 % of the 105 HCWs and 11.8 % of the 195 patients were colonized by MRSA. Being admitted and or working in the emergency department independently associated with an increased risk of MRSA colonization. Surprisingly, CC5-MRSA-SCCmec I was the dominant clone among patients and HCWs in the emergency and medical department while CA-MRSA was shown in the surgical department especially among HCWs (Cirkovic et al.,2015).

The nasal carriage rates of multidrug-resistant (MDR) *S. aureus* in food vendors and hospital workers in Ekpoma, Nigeria showed that 32 % (32/ 100) of hospital personnel had *S. aureus* with 18 (36 %) and 14 (28 %) of the isolates recovered from hospital workers and food vendors, respectively. About 59.3 % and 50.0 % of *S. aureus* were resistant to Ofloxacin and Oxacillin respectively. Out of 17 (53 %) MDR *S. aureus* , only 4 (23.5%) remained MDR after curing with 0.1 mg/ml acridine orange solution (Isibor et al., 2014).

The prevalence of nasal carriage of MRSA among 600 randomly selected HCWs at Namazi Hospital, Shiraz, Iran , showed that 5.3 % of them were MRSA carriers dominating HCWs from surgical wards and the emergency department. The highest resistance rate for both gentamicin and clindamycin (69 %) was noted among the MRSA strains. None of the MRSA strains were resistant to mupirocin, linezolid, fusidic acid, or vancomycin. The existence of the mecA gene in all 32 methicillin-resistant isolates was observed by PCR (Askarian et al., 2009).

A nasal carriage study from 54 HCWs of National Medical College & Teaching Hospital of Nepal showed *S.aureus* carriage rate of 20.37%. All nasal *S. aureus* isolates were sensitive to Amikacin and Vancomycin. Methicillin resistance rate was 45.5 % which is equivalent to MRSA (Sah et al.,2013).

MRSA data from Ethiopia is very scarce and the most available literature from an online source is from Dessie Referral Hospital, Ethiopia. The rate of nasal carriage of MRSA from HCWs was 12.7 % (Shibabaw et al., 2013). However, the sample size is too small, only 118 HCWs, and comprises few groups of HCWs. Moreover, they used a single drug ( oxacillin disc) for characterizing MRSA (Shibabw et al., 2013).

Another cross-sectional study was carried out to determine nasal and hand carriage of MRSA among 177 HCWs of Mekele Hospital, Ethiopia. Among the study participants, 20.3 % (36/177) them had MRSA on their hands and anterior nares. The majority of MRSA was detected in females,25(14.1%). MRSA carriage was significantly higher in nasal swabs than hand swabs (25, 14.1 % vs 11, 6.2 %; $p < 0.05$ respectively). Nurses and medical doctors had MRSA colonization rates of 13. 6 % (26/ 177) and 2.3 % (4/177) respectively. MRSA isolates resistant to ampicillin 88.9 % and tetracycline, 86.1%. In this study 2 (5.6 %) isolates from nasal swabs were vancomycin-resistant (Gebreysus et al., 2013). Similar to a study conducted in Dessie Referral Hospital, which applied oxacillin disc as a surrogate marker for MRSA and the sample size is small. Moreover, they have reported Vancomycin resistance in 2 isolates where, the test was done using a disc diffusion test which may not reflect the actual vancomycin status (Gebreysus et al., 2013).

### 1.4.3. MRSA burden in Mobile phones, gowns, and other inanimate objects

Microbial contamination of mobile phones from patients and HCWs at Alexandria University Students ‘Hospital showed that all 40 mobile phones were contaminated with single or multiple pathogens. MRSA and CONS were dominantly found as 53 % and 50 % respectively. The mean bacterial count was 357 CFU/ ml, while the median was 13 CFU/ ml using the pour plate method. Using surface spread methods, the mean and median count were 2,192 and 1,720 organisms/phone respectively (Selim et al., 2015). Many studies showed inanimate objects such as mobile phones of HCWs, their gowns, shoes, stethoscopes; blood pressure apparatus harbour MRSA and other MDR bacteria (Coello etal., 1994,; Boyce et al., 1997; Bearman et al., 2014).

### 1.4.4. Molecular typing of *S.aureus*

Different molecular typing methods of *S. aureus* are available and among these, phage typing. PFGE , spa typing, MLST typing and WGS are among the most commonly used methods. Molecular typing of *S.aureus* isolates gives us additional evidence in the transmission and distribution of specific clones of *S.aureus* population that will be useful for planning intervention (McDougal et al., 2003; Asadollahi et al,, 2018). Spa typing and MLST typing are becoming very common in many parts of the world. The global distribution of spa types of *S. aureus* is illustrated in figure 1.1 below and the diversity of *S.aureus* across countries (Asadollahi et al,, 2018).

As depicted in the figure, there is scarcity of data on the spa types among Ethiopian isolates, although the figure is not very recent. Abrha ( Abrha 2019) analysed the spa types of *S aureus* isolates from clinical, milk, and nasal isolates very recently. Moreover, one spa related publication is also available from animal studies ( Mekonnen et al 2018 ).

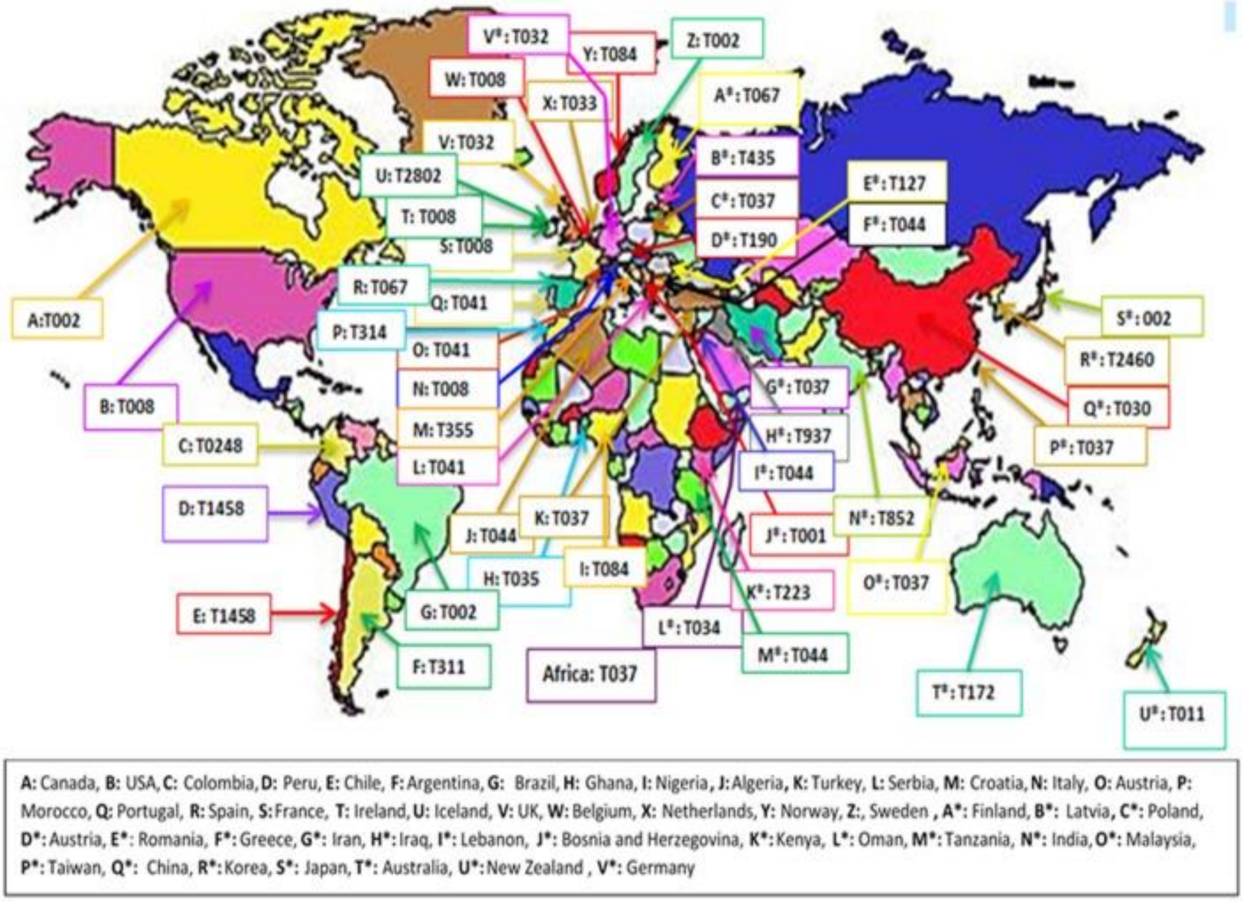


**Figure 1.1.The most prevalent *spa* types across the world. taken from** (Asadollahi et al,, 2018)

The *Spa* typing method involves sequencing of the polymorphic X region or the short sequence repeat region of the protein A gene of *S. aureus* .Likewise, MLST based genotyping is another alternative molecular typing that works based on seven housekeeping genes (Asadollahi et al,, 2018). There are little data from African countries both for MSSA and MRSA isolates of clinical samples. The figure below also shows that many African countries do not have MLST ST for MSSA including Ethiopia.

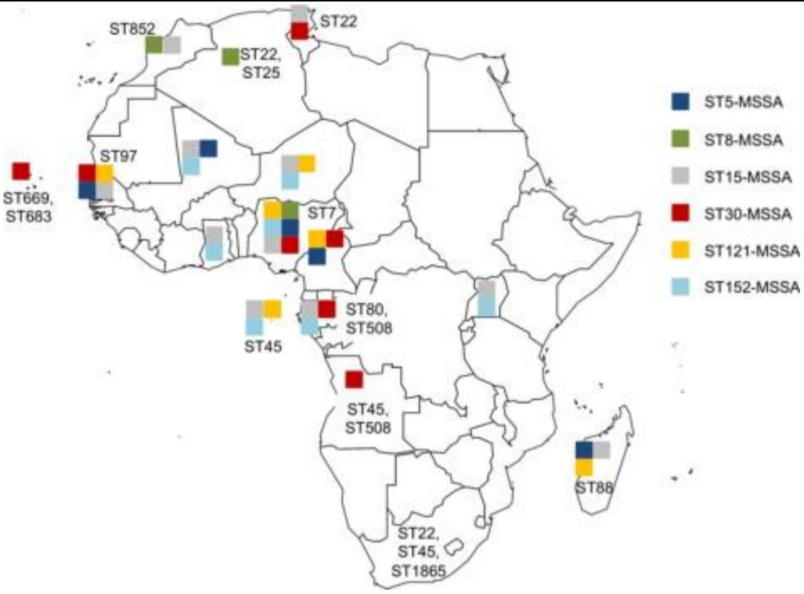


FIG. 1.2. . Distribution of methicillin sensitive *S. aureus* (MSSA) clones in Africa. The three major MSSA multilocus sequence types (STs) of each study were identified. Of these, the six most widely distributed clones in Africa are shown. Sporadic clones are indicated by letters. STs from Uganda and Tunisia were derived from spa types ( taken from Schaumburg F[b] et al., 2014).

As shown in Figure 1.3, the same is true for MLST STs of MRSA data which is scarce in many African nations ( Figure 1.3).

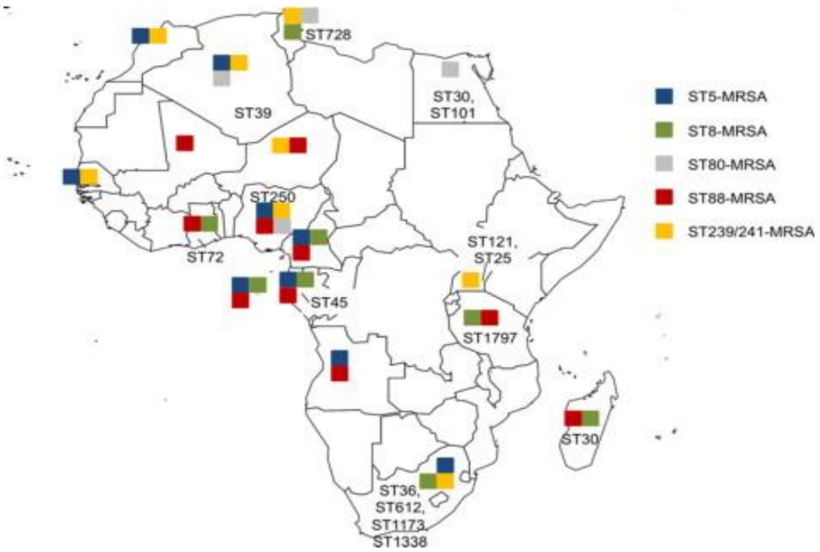


Fig . 1.3. Distribution of methicillin resistant *S.aureus* (MRSA) clones in Africa. The three major MRSA multilocus sequence types (STs) of each study were identified. Of these, the five most widely distributed clones in Africa are shown. Sporadic clones are indicated by letters. STs from Uganda were derived from spa types ( taken from Schaumburg F[b] et al., 2014).

### 1.4.5. KAP of HCWs towards Multidrug resistance bacteria including MRSA

The hand hygiene status of 50 HCWs was assessed by Anitha and collegues using microbiological culture of swabs from HCW's hands before and after alcohol hand rub. At base line 56 % of the HCWs carried *Micrococci*, 12 % carried MRSA, 8 % ESBL producing gram-negative bacteria, 8 % *S. epidermidis* and 2% *Enterococcus faecalis,* which become zero growth after applying alcohol-based hand rub indicating HCWs hand could be colonized with deadly pathogens and proper hand hygiene techniques could minimize transmission of the pathogens to patients and the community at large (Anitha et al., 2015).

Among 42 nursing staff from the medical clinical unit, the nursing staff had unsatisfactory knowledge of the causes of Multidrug-resistant bacteria, chain of transmission of infection, susceptibility to the colonization of multi-resistant bacteria. Lack of knowledge of nursing staff compromised adherence to preventive measures (Tambe et al., 2012).

Knowledge, attitude, and practices of 343 HCWs regarding the transmission of pathogens via fomites at a tertiary care Hospital in Karachi, Pakistan indicated that laboratory coats, stethoscopes, and bedside curtains were most frequently identified as fomites by the participants. Medical students had significantly lower mean scores in the knowledge and attitude sections than consultant physicians, resident physicians, and nurses. Nurses were by far better than consultant physicians, resident physicians, and medical students regarding practice-related matters. Almost 95 % of the participants scored above 50 % on the knowledge component of the questionnaire, but only 32.3% scored above 50 % in the practices section indicating a significant gap in the practice of HCWs (Aftab et al., 2015 ).

A recent systematic review and network meta-analysis showed that the WHO campaign is effective at increasing compliance with hand hygiene in HCWs with additional intervention measures like goal setting, reward incentive, and accountability with adequate resources (Luangasanatip et al., 2015).

A cross-sectional evaluation of HCWs KAP about hand-hygiene and Tuberculosis infection control was conducted on 261 HCWs in two university hospitals of Addis Ababa Ethiopia. Based on their findings, hand hygiene knowledge was fair among

HCWs. Self-reported practice for hand hygiene was suboptimal. Different barriers for performing hand hygiene were lack of hand hygiene agents, (77 %), sinks (30%), proper training (50 %), and irritation and dryness caused by sanitizers (67 %). However, the study does not address the issues of MRSA colonization and prevention measures (Tenna et al., 2013).

The effectiveness of the WHO multimodal hand hygiene program was evaluated in a hospital in Addis Ababa Ethiopia and a significant increment in hand hygiene adherence was noted after implementing the campaign. However, the study is not directly related to MRSA carriage of HCWs, hand hygiene practice, and related intervention measures (Schmitx et al., 2014).

Most studies reviewed above are more fragmented and the actual relationship between MRSA from HCWs, patients, and inanimate objects is not correlated well. The relation between MRSA burden using phenotypic and molecular approaches along with different factors is depicted in the conceptual frame work mentioned in figure 1.4 below and this study will try to fill this important interaction that will contribute to MRSA control program both at the health care facilities and in the community.

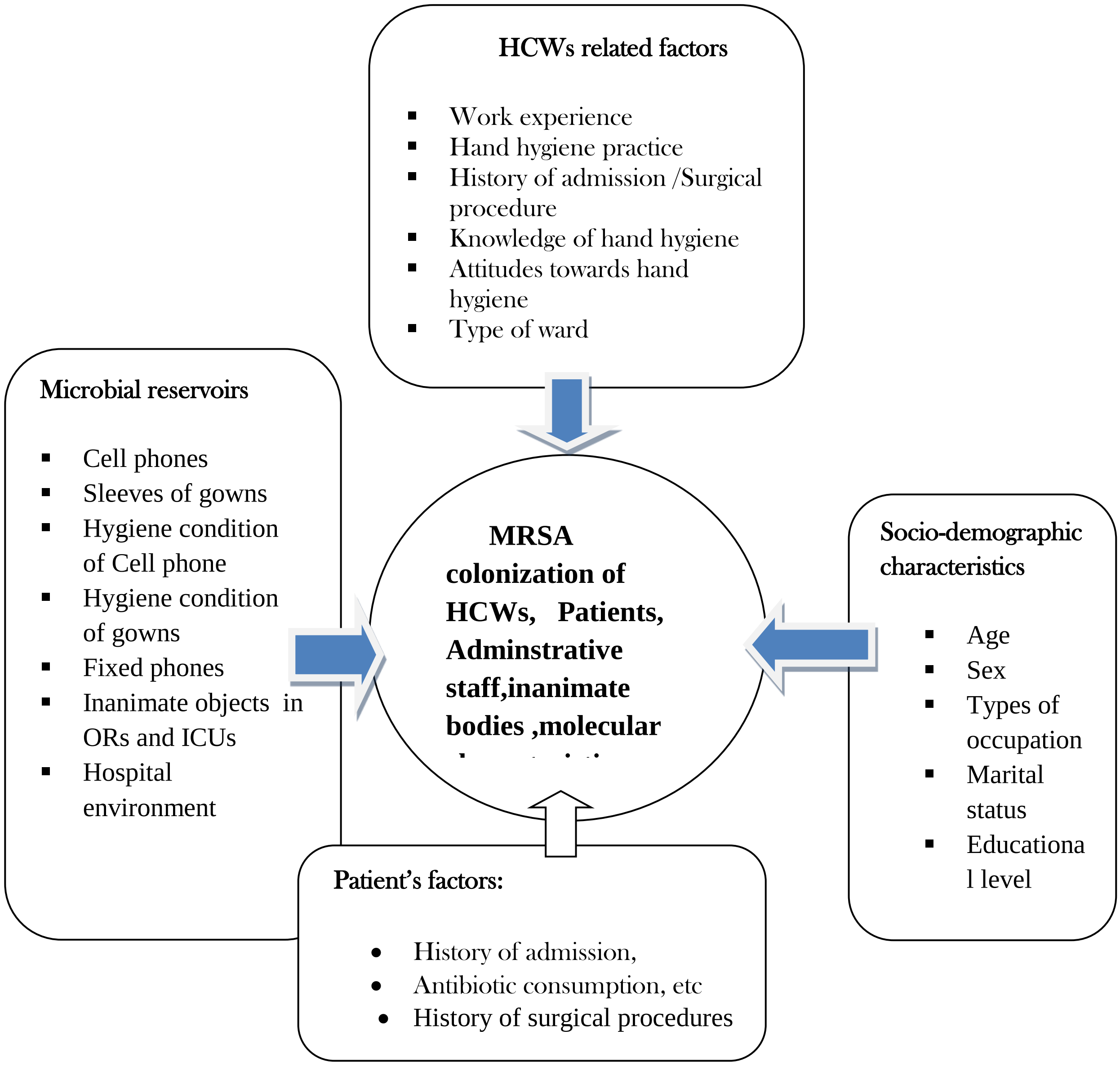


**Fig.1.4** Conceptual frame work for MRSA colonization of patients, HCWs and knowledge and attitudes of personnel at TASH, Addis Ababa, Ethiopia

### 1.5. Significances of the study

In this study, we have generated comprehensive data on the burden of MRSA colonization of patients, HCW, inanimate objects, and associated factors that are potential sources of infections with multidrug resistance microorganisms of hospitalized patients. The data generated from this study could form the ground for recommendations to Hospital-acquired infection control and prevention measures at the study site.

Thestudy also addresses the burden of MRSA from patients, HCWs, and inanimate objects like cell phones, gowns, and other inanimate items in ICU/ Wards. The true burden of MRSA is known along with the molecular characteristics of the agents, as HCWs could be colonized with HA- MRSA and or C-MRSA that will enable us to design appropriate strategies both at the health care facilities and in the community.

Moreover, various factors including gaps in the KAP of HCW about MRSA control and prevention measures were identified that is a foundation for future planning and designing of intervention measures in TASH and possibly to other hospitals in the country with similar set ups.

Above all, HCWs will be equipped with current information about MRSA, sources, colonization status, and appropriate intervention procedures which will help them to take precautions during their routine patient care and minimize transmission of MRSA and other MDR bacteria to patients, themselves, and to the community at large as HCWs could act as reservoirs and sources of infection between hospitalized patients and the community.

This research work can be used as baseline data for future researchers who will work on MRSA colonization study, for surveillance purposes as in case of outbreaks situation and to know the source of MRSA and other multidrug-resistant bacteria in a hospital setting.

### 1.6. Hypothesis of the study

- ✓ There is no difference in the colonization rate of MRSA of HCWs and administrative staff in the study site.
- ✓ The genetic linage of MRSA from health care workers, administrative staff, patients, isolates from cell phones and gown is the same.
- ✓ There is no difference in the AST pattern of *S.aureus* between HCWs and administrative staff.
- ✓ The burden of MRSA is similar for HCWs and *S.aureus* isolates from stored isolates of nasal swabs of patients.
- ✓ The knowledge, attitude, and practice towards MRSA control and prevention is the same among different HCWs in TASH.

### 1.7.0. Objectives

### 1.7.1. General Objective

To assess the burden of MRSA colonization of health care workers, patients, and selected inanimate objects and the knowledge, attitude, and Practice of HCW on MRSA control and prevention measures at TASH.

### 1.7.2. Specific objectives

- ✓ To assess the antimicrobial susceptibility of *S. aureus* isolates from HCWs and administrative staffs of TASH, CHS, AAU
- ✓ To determine the burden of MRSA colonization of HCWs at TASH
- ✓ To determine the burden of MRSA colonization of administrative staffs of TAHS
- ✓ To determine the rate of MRSA from inanimate objects including mobile phones of HCWs , administrative staff and gowns of HCWs, and other inanimate objects from operation rooms and intensive care units of TASH.
- ✓ To determine the rate of MRSA based on MecA gene from stored isolates at TASH
- ✓ To characterize the MRSA and MSSA from nasal isolates of HCWs, patients, administrative staff, HCWs gowns, mobile phones of HCW, and administrative staff using, pvl genes, spa typing, MLST typing, and WGS methods
- ✓ To assess the knowledge, attitude, and perceived practice of HCWs towards MRSA control and prevention measures.

## 2.0. CHAPTER II: MATERIALS AND METHODS

### 2.1. Study Area

The study was conducted in Tikur Anbesa Specialized Hospital (TASH), Addis Ababa, Ethiopia. TASH is located in Addis Ababa, the capital city of Ethiopia. Addis Ababa has a population of 3,384,569 according to the 2007 population census conducted by the Central Statistical Agency of Ethiopia (CSA, 2007) with an annual growth rate of 3.8 % which was estimated at 4478127 by the year 2015. Addis Ababa lies at an altitude of 2324 m (7625 ft.) above sea level and is located at 8°58'N, 38°47'E and has a mean annual temperature and rainfall of 15.9$^{o}$c and 1089 mm, respectively. TASH is a specialized teaching hospital that is found under the College of Health Sciences, Addis Ababa University. The hospital is the largest of its kind in Ethiopia and serves as a referral hospital for the surrounding catchment areas. During the time of data collection, there were about 200 medical doctors, 600 nurses, 70 laboratory personnel, 60 pharmacy personnel, and 115 other HCWs under TASH. Moreover, under CHS, about 1200 administrative staff were working at different departments and units of the CHS at TASH and Sefere-selam campus.

### 2.2. Study design and period

A prospective and retrospective cross-sectional study design was used to determine the burden of MRSA colonization of HCWs, inanimate objects including mobile phones, gowns, and determine associated risk factors such as KAP of HCWs towards MRSA control and preventive measures. In addition to this, stored isolates from *S. aureus* isolates from nasal colonization of Diabetic patients and mother-child pair from TASH and clinical isolates from the bacteriology laboratory of TASH and diabetic foot ulcer project were analyzed for the existence of MRSA and selected molecular typing. The study was conducted between June to September 2018 for phenotypic analysis, while the molecular tests were performed from May to August 2019.

### 2.3. Source of population

All HCWs practicing in TASH, patients, and administrative staff of CHS were our source population for the prospective study.

### 2.4. Study Population

HCWs and administrative staff who were willing to provide nasal swabs, swabs from mobile phones, gowns, and other inanimate objects of the hospital environments and volunteer to participate in this project during the study period were our study population.

**2.5. Selection of study participants**

**2.5.1. Inclusion criteria (for the prospective study):**

- HCWs who are currently working in TASH for at least 6 months.
- For comparator groups: Administrative staff of CHS, AAU, and do not have contact with patient management and support.
- Consenting to be part of the study
- For clinical and patient's colonization study we used stored *S. aureus* isolates from TASH and stored isolates from three previous MSc projects (two MRSA colonization studies and isolates from diabetic foot ulcer infection study ).

**2.5.2. Exclusion criteria: for the prospective study**

- HCWs who were taking antimicrobial drugs for the last 15 days before data collection time
- HCWs on leave of absence during the study period.
- For comparator group: Administrative staff who have been admitted in hospital for the last 1 month before data collection time and taking antibiotics for the last 15 days

**2.6. Study Variables**

**2.6.1. Dependent variables**

- HCWs MRSA colonization status
- MRSA colonization status of mother-child pair and diabetic patients
- MRSA colonization status of administrative staff
- MRSA /contamination level of mobile phone, and gowns,
- MRSA rate of *S.aureus* from clinical sources
- Antimicrobial susceptibility pattern of *S, aureus* from HCWs and administrative staff
- Mec A, Pvl, spa types, MLST types, and WGS of *S.aureus* isolates, Knowledge, attitudes, and practice of HCWs towards MRSA control and prevention

**2.6.2. Independent variables**

- Age
- Sex
- Work experience
- Opportunity of in service training
- Work experience

- Knowledge of HCWs
- Working department
- HCWs source of information for MRSA
- Availability of hand hygiene materials and guidelines for MRSA
- Hygiene status of HCWs
- Cleaning status of cell phone and gown
- History of hospital admission
- History of surgical intervention

**2.7. Sample size and Sampling Technique**

Sample size calculation ws made based on a single population proportion method to calculate the sample size for MRSA colonization of HCWs, administrative staff, MRSA burden from the cell phone, gowns, and for assessing knowledge. Attitude and practice (KAP ) of HCWs. Since there is no such similar study in Ethiopia and other countries with similar settings. We took 50 % proportion for administrative staff, inanimate objects study. The value of p was taken as 50 % (0.5) with 95 % confidence interval, 5 % margin of error with 10 % contingency level.

$$N_1 = \frac{Z\alpha/2^2 \, P(1-P)}{d^2}$$

Where N = Sample sizes z $(\alpha^2)2$ = At 95% confidence interval Z value ($\alpha = 0.05$) = 1.96 Where p = Proportion of occurrence of the events to be studied 50% (0.50) , = Margin of error at (5%) (0.05)

$$N_1 = \frac{(1.96)(1.96)\,0.5(0.5)}{(0.05)(0.05)} = 384.16$$

With 10 % contingency, which resulted in a final sample size of 422.

For colonization of HCWs ($N_2$), we took the prevalence of nasal carriage rate of MRSA among Dessie Referral Hospital HCWs; Dessie, Northeast Ethiopia that was done by Shibabaw and colleagues in 2013 ( Shibabaw et al., 2013).The value of p was taken as 12.7 % (0.127) with 95 % confidence interval, 3 % margin of error with 10 % contingency level.

$$N2 = \frac{(1.96)(1.96)\,0.127(0.873)}{(0.03)(0.03)} = 473$$

With 10 % contingency, 520 HCWs , swabs from cell phones, and gown each was the sample size for the HCWs group . However, to increase precision and power we have included more study participants up to 600, especially for HCWs groups.

Since $N_2$ is greater than, $N_1$, we have considered the largest sample size of 520 both for HCW and administrative staff to compare the burden of MRSA between HCW and administrative staff groups.

For mother-child pair and diabetic patients' MRSA colonization study, we used 70 *S. aureus* stored isolates from MRSA colonization study. Moreover, 100 *S.aureus* clinical isolates were collected from the Clinical Microbiology laboratory and stored isolates from diabetic foot ulcer infected patients for molecular characterization.

We used proportional allocation methods to represent fairly the 200 medical doctors, 600 nurses, 70 laboratory personnel, 60 pharmacy personnel, and 115 other HCWs under TASH. Based on proportional allocation methods, 167 medical doctors, 289 nurses, 36 laboratory personnel, 29 pharmacy personnel, and 67 other types of HCWs were included, though we approached a total of 600 HCWs. using convenient sampling methods for each HCWs group.

For the selection of administrative staff, we have included about 472 staff using convenient sampling methods from 1200 total administrative staff. All administrative staff including secretaries, messengers, drivers, guards, technical personnel working in the various units and departments of the College of health sciences were included.

### 2.8. Data collection procdeures

#### 2.8.1. Socio-demographic and other data for HCWs and administrative staff

Socio-demographic data were collected using pretested self-administered questionnaires which were prepared for this purpose. The socio-demographic data comprises Age, sex, work experience, level of education, current working places, number of gowns/uniforms that an HCW have, how frequently they change their gowns and or uniforms, types of cell phone, availability of guidelines and leaflets about MRSA, their feeling about availability of adequate hand hygiene materials ( alcohol hand rubs, soap, and water), history of hospital admission and surgical intervention to see any impact on MRSA colonization were collected ( Appendix IX).

### 2.9. Sample collection and transport and storage

#### 2.9.1. Sample collection from HCWs and administrative staff

A single nasal swab was collected using sterile moistened swabs (Amie's Oxoid, England) from both anterior nares of informed and consented HCWs and administrative staff and placed in Amie's transport media. After explaining to the participants the procedure for sample collection, nasal swabs were collected by the PI and data collectors. The collected swabs were immediately transported to the microbiology laboratory.

**2.9.2. Sample collection from inanimate objects**

**Samples from mobile phones of HCWs and administrative staff**: We used a sterile moistened swab and both sides of the mobile phones were rubbed and the swabs kept in a sterile tube with a lid and then transported to the microbiology laboratory.

**Samples collection from gowns of HCWs**: Like the mobie phone we have used a moistened cotton swab and we collected samples from the sleeves and pockets of the gown and the swab was kept in a sterile tube which is labelled and transported to the microbiology laboratory.

**Samples collection from other inanimate objects at TASH**: We have collected 210 swabs from various units of TASH including ICUs, operation rooms, and wards were included for 10 weeks (21 swabs per week). Items like beds, coaches, tables, neonatal incubators, chairs, sink; suction machine tips and oxygen cylinder were collected using sterile moistened swabs. Moreover, air sample was tested using open-air settlement methods using blood and mannitol salt agar in the operation rooms and ICUs and transported to the microbiology laboratory for processing.

Each sample was pre-labelled with the identification code of the study participant and sample site. All samples were transported immediately to the microbiology laboratory of the Department of Microbiology, Immunology, and Parasitology (DMIP) for analysis. When there was a delay, samples were kept in the refrigerator for not more than 12 hours.

**2.9.3. Sample processing and Identification of *S. aureus* and MRSA**

All nasal swabs from HCWs and administrative staff, swabs from cell phones and gowns, and swabs from selected inanimate objects of operating rooms and ICUs were

plated on mannitol salt agar and / or MRSA Chrom agar and incubated overnight at 35-36 °C. Typical colonies resembling *Staphylococcus* were gram-stained and those showing gram-positive cocci were further tested for catalase, coagulase enzymes, mannitol fermentation, and or DNAse test. Bacterial colonies confirmed as *S.aureus* were stored in skimmed milk glycerol media at -30 to -70°C until further tests were done (Lozano et al., 2009; CLSI ,2018).

### 2.9.4 Sample processing of the stored isolates

Stored isolates from three previous M.Sc. projects and clinical isolates collected from the routine clinical bacteriology laboratory at TASH were included in this study. One of the M.Sc projects was on MRSA prevalence and antimicrobial drug resistance among diabetic patients at TASH, while the other was a study on the colonization of MRSA in a mother-child pair. In both cases, they have collected nasal swabs and isolates were identified and their antimicrobial susceptibility determined. The third group of isolates was taken from a project which focused on the microbial burden and antimicrobial drug resistance patterns from clinical samples taken from diabetic foot ulcer in selected hospitals of Addis Ababa, Ethiopia. All the stored *Staphylococcus* isolates were subcultured and further identification was performed and those confirmed as *S. aureus* were included for further molecular analysis.

### 2.9.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out for all isolates of *S. aureus*, from nasal swabs of HCWs and administrative staff, mobile phones of HCWs, and administrative staff and gowns of HCWs using the disc diffusion method on Muller Hinton agar (UK) based on CLSI 2018 guideline. Briefly, 3–5 colonies of the test organism were emulsified in 5 ml of sterile nutrient broth and mixed gently. The suspension was incubated at 37 °C until the turbidity of the suspension becomes adjusted to 0.5 McFarland standards. The suspension was then uniformly swabbed onto Muller Hinton agar (MHA). The following antimicrobial disks were used: Rifampin (5 μg), clindamycin (2 μg), Trimethoprim-Sulfamethoxazole (1.25/23.75 μg), erythromycin (15 μg), Tetracycline (30 μg), penicillin (10 Units). Methicillin resistance was detected using cefoxitin (oxacillin) (30 μg) disc diffusion test. The MHA plates were incubated at 36 °C for 18-24 hours and the zone of inhibition around the disc was measured to the nearest millimeter using a graduated caliper and the isolates were classified as sensitive, intermediate, and resistant according to CLSI

methods (CLSI, 2018). Van A and VanB genes were also tested following standard PCR protocol.

## 2.10. Molecular testing

This part of the project was done at the Department of Global Health and Infection, Brighton and Sussex Medical School, University of Sussex, UK. Samples were transported through a material transfer agreement (Annex XII) for further molecular tests. Molecular methods were used to look for the presence of Mec A gene for the existence of MRSA molecular marker, the presence of virulence markers PVL genes, detection of Spa protein genes using conventional PCR protocol and sequencing and PCR for the seven housekeeping genes of *S. aureus* for Multilocus sequence typing (MLST). Whole-genome sequencing (WGS) was done for eighteen *S. aureus* isolates

from various sources including nasal sources from patients, mobile phones of HCWs and administrative staff and gowns of HCWs, and clinical isolates.

### 2.10.1. DNA extraction

The DNA preparation for all PCR protocols ( MecA , van A and Van B genes, PVL gene , Spa gene, seven housekeeping genes of *S. aureus* for MLST analysis) is done based on Instagene matrix solution ( BIO-RAD , Munchen, Germany) following manufacturer instruction. Briefly, 1-2 loopful of overnight growth colonies of *S. aureus* was suspended in a sterile Eppendorf tube and washed with sterile $DDH_2O$ and on the pellets, 200 μl of 6 % Instagene matrix solution was dispensed, and the mixture was heated in a heat block at 56 $^oC$ for 20 min, then vortexed and heated for 8 min at 100 $^0C$ & centrifuged at 8000 x g for 2-3 minutes. The DNA quality was assessed by Nao drop machine for a representative sample. The supernatant solution was kept at refrigerator/ deep freeze temperature until the PCRs were resumed ( Figure 2.1). The same DNA extract was used for all PCR-based tests.

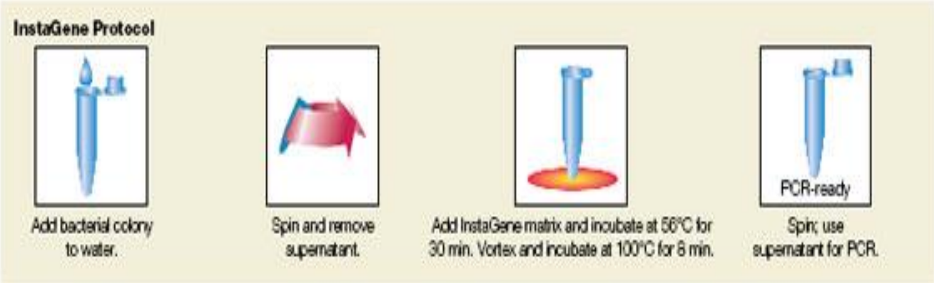



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Figure 2.1: DNA extraction of *S. aureus* isolates using Instagene matrix

**2.10.2.PCR for MecA gene detection**

The presence of *mecA*genes was determined by polymerase chain reaction (PCR) using *mecA F* and *mecAR*, primers (AAAATCGATGGTAAAGGTTGGC;AGTTCTGGAGTACCGGATTTGC) following previously described methods (74). Briefly, a master mix of 22.5 µl was mixed with 0.5 µl of each forward and reverse Mec A primers and 1.5 µl of DNA product . PCR reaction was resumed. The PCR was performed in thermocycler (MRB Research, UK) using the following cycling parameters: Initial 35 cycle of amplification (denaturation at 94°C for 60 s, primer annealing at 60°C for 90 s and primer extension at 70°C for 60 s), and final extension at 72°C for 5 min. 5 µl of PCR products and 1 µl of loading dye was mixed and the band was resolved in 1.2 % agarose gels prepared in 1× TBE buffer containing 0.5 µg/ml of SYBER Green solution. Hundred (100) bp DNA ladder was used as a molecular marker and the amplification products were electrophoresed for 1 h at 100 V. The gel was then visualized under a gel image instrument and the image was saved in the computer.

### 2.10.3.Van A and Van B gene detection

Van A and Van B genes were analyzed using PCR protocols described by ( Sadat et al.,2014) with slight modification. Briefly, we used the GTGACAAACCGGAGGTAATA forward and TCA CCC CTT TAA CGC TAA TA reverse primers for Van A gene and for Van B gene forward primer of CAG TGCATGTGCCATGGATA and reverse primers of CCG CCA TCC TCC TGC AAA AAA .Briefly, a master mix of 22.5 µl was mixed with 0.5 µl of each forward and reverse primers of van A and Van B genes and 1.5 µl of DNA product . PCR reaction was resumed. The PCR was performed in a thermocycler (MRB Research, UK) using the following cycling parameters: Initial 35 cycle of amplification (denaturation at 94°C for 60 s, primer annealing at 60°C for 90 s and primer extension at 70°C for 60 s), and final extension at 72°C for 5 min. 5 µl of PCR products and 1 µl of loading dye was mixed and the band was resolved in 1.2 % agarose gels prepared in 1× TBE buffer containing 0.5 µg/ml of SYBER Green solution. Hundred (100) bp DNA ladder was used as a molecular marker and the amplification products electrophoresed for 1 h at 100 V. The gel was then visualized under a gel image instrument and the image was saved in the computer.

### 2.10.4. Spa Typing

*Spa* typing was done according to the procedure described by Shopsin B and colleagues and James RP and colleagues (Shopsin et al.,2019; James *et al* . 2014 ) with some modification. The principle is based on the amplification of spa genes on the X region using polymerase chain reaction (PCR). We have used the forward and reverse primers; spa-1113f (5'- TAA AGA CGA TCC TTC GGT GAG C -3') and spa-1514r (5'-CAG CAG TAG TGC CGT TTG CTT -3') respectively (Invitrogen, Thermo fisher scientific, Great Britain ). Briefly, a master mix of 22.5 µl was mixed with 0.5 µl of each forward and reverse Spa primers and 1.5 µl of DNA product . PCR reaction was resumed. The PCR was performed in thermocycler (MRB Research, UK) using the following cycling parameters: Initial 35 cycle of amplification (denaturation at 94°C for 60 s, primer annealing at 55°C for 90 s and primer extension at 70°C for 60 s), and final extension at 72°C for 5 min. 5 µl of PCR products and 1 µl of loading dye was mixed and the band was resolved in 1.2 % agarose gels prepared in 1× TBE buffer containing 0.5 µg/ml of SYBER Green solution. Hundred (100) bp DNA ladder was used as a molecular marker and the amplification products were electrophoresed for 1 h at 100 V. The gel was then visualized under gel image instrument and the image was saved in the computer.

### 2.10.5. Spa sequencing

The PCR products of Spa gene were purified using ExoSAP –IT DNA purification kits ( Fisher Scientific, Leicestershire, Great Britain). Briefly, five µl of each PCR Spa product and ExsoSAP-IT reagent was mixed in sterile new PCR tubes and heated at 37 ° C for five minutes and further heated at 80 ° C for 15 minutes using a thermocycler. Then the forward and or reverse Spa primers are mixed with purified PCR products in 1: 2 proportion in a new sterile 1. 5 ml Eppendorf tube which has a unique barcode for sequencing (Figure 2.2). The ready-made pure PCR products were sent to Eurofin genomics, England for sequencing. Using purified PCR products sequencing was done using Agencourt CleanSEQ beads (Beckman Coulter, London, UK). The *spa* types were assessed using RidomStaphType (Ridom GmbH, Germany) software.

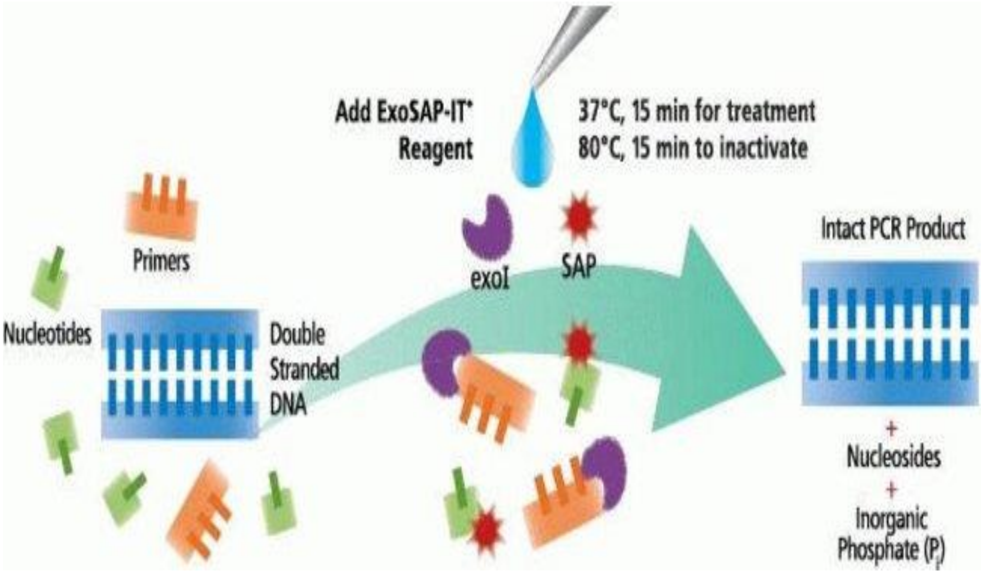


**Figure 2.2..** Spa gene purification methods for sequencing figure taken from manufacturer manual.

### 2.10.6. PVL Detection CHECK REF

Primers used for detection of *PVL* genes were Luk PV-1, ATCATTAGGTAAAATGTCTGCACATGATCCA and Luk PV-2, GCATCAACTGTATTGGATGCCAAAGC which amplify a 433 base pair fragment specific for *lukS/F –PV* genes, encoding the *PVL* S/F bicomponent proteins

(Invitrogen, Thermo fisher scientific, Great Britain ). Briefly, a master mix of 22.5 µl was mixed with 0.5 µl of each forward and reverse PVL primers and 1.5 µl of DNA product .The DNA thermocycler was programmed for initial denaturation at 94 °C for 4 min; 35 cycles of amplification (denaturation at 94 °C for 45 s, annealing at 57 °C for 45 s, and extension at 72 °C for 30 s); and a final extension at 72 °C for 2 min,as decribed by Bastidas and collegues with slight modification (Bastidas et al., 2019). To visualize the PCR product, 5 µl of the PCR amplicon was loaded with loading dye in 1.2 % agarose gel containing SYBERSAFE green followed by electrophoresis at 100 V for 1 h and visualized by using a gel imager instrument with software. Fragments of DNA corresponded with 433 bp corresponded amplification of a fragment to the *PVL* genes was considred as amplification of PVL gene.

**2.10.7. Multilocus Sequence Typing (MLST)**

**2.10.7.1. PCR for 7 housekeeping genes**

The principle was based on the amplification of *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL* (Invitrogen, Thermo fisher scientific, Great Britain )( www.mlst.net). Briefly, a master mix of 22.5 µl was mixed with 0.5 µl of each forward and reverse primers and 1.5 µl of DNA product. PCR reaction was resumed. The PCR was performed in thermocycler (MRB Research, UK) using the following cycling parameters: Initial 35 cycle of amplification (denaturation at 94°C for 60 s, primer annealing at 55°C for 90 s and primer extension at 70°C for 60 s), and final extension at 72°C for 5 min. 5 µl of PCR products and 1 µl of loading dye was mixed and the band was resolved in 1.2 % agarose gels prepared in 1× TBE buffer containing 0.5 µg/ml of SYBERSAFE Green solution. Hundred (100) bp DNA ladder was used as a molecular marker and the amplification products were electrophoresed for 1 h at 100 V. The gel was then visualized under a gel image instrument and the image was saved in the computer.

**2.10.7.2. MLST sequencing and Typing**

MLST analysis was carried out by sequencing fragments of 7 housekeeping genes each (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*), as described in the mlst website (available from www.mlst.net) (Table 2.1). All PCR products were purified by using ExoSAP –IT DNA purification kits ( Fisher Scientific, Leicestershire, Great Britain) . Briefly, five µl of each PCR MLST gene product and ExsoSAP-IT reagent was mixed in sterile new PCR tubes and heated at 37 ° C for five minutes and further heated at 80 ° C for 15 minutes using thermocyler machine. Then the forward and or reverse

MLST primers each were mixed with purified PCR products in 1 : 2 proportion in a new sterile 1. 5 ml epindorf tube which has a unique barcode for sequencing. The ready-made pure PCR products were sent to Eurofin genomics, England for sequencing using an ABI Prism sequence (Applera) with Big Dye reaction mixes, using the primers chosen for the initial amplification, and analyzed on the BioEdit biological sequence editor 5.0.6. Each allele of the 7 housekeeping genes was assigned to a number, and each isolate was characterized by a sequence type (ST), defined by the allelic profile of the housekeeping genes. These profiles were compared to those present in the *S. aureus* MLST database (available from www.mlst.net (Lozano et al., 2009).

**Table 2.1. MLST gene primers used for MLST sequence typing**

| Genes | Primers | Sequences |
|---|---|---|
| Carbamate kinase (*arcC)* | arc C Up<br>arc C Dn | TTGATTCACCAGCGCGTATTGTC<br>AGGTATCTGCTTCAATCAGCG |
| Shikimate dehydrogenase (*aroE*) | aro E UP<br>aro E Dn | ATCGGAAATCCTATTTCACATTC<br>GGTGTTGTATTAATAACGATATC |
| Glycerol kinase (*glpF*) | glp F Up<br>glp F Dn | CTAGGAACTGCAATCTTAATCC<br>TGGTAAAATCGCATGTCCAATTC |
| Guanylate kinase (*gmk*) | gmkUp<br>gmkDn | ATCGTTTTATCGGGACCATCTC<br>ATTAACTACAACGTAATCGTA |
| Phosphate acetyltransferase (*pta*) | pta Up<br>ptaDn | GTTAAAATCGTATTACCTGAAGG<br>GACCCTTTTGTTGAAAAGCTTAA |
| Triosephosphate isomerase (*tpi*) | tpi Up<br>tpiDn | TCGTTCATTCTGAACGTCGTGAA<br>TTTGCACCTTCTAACAATTGTAC |
| Acetyl coenzyme A acetyltransferase (*yqiL)* | *yqiL Up*<br>*yqiLDn* | CAGCATACAGGACACCTATTGG C<br>CGTTGAGGAATCGATACTGGAA C |

### 2.10.8. Whole-Genome Sequencing of representative *S. aureus* isolates

Eighteen selected *S. aureus* isolates from nasal colonization of HCWs , administrative staff, and patients and clinical isolates were sent to the University of

Cologne, Germany for whole-genome sequencing. DNA extraction, library preparation, and sequencing were done according to their protocols and sequence data were sent back for analysis in Fastaq format. On the Fastq data quality control was made using FASTQC tool and genome assembly was performed using Spades tool. The 16 SrRNA phylogeny was prepared by annotating sequences using Prokka tool and sequence comparison of 16 SrRNA was made using BLASTn tool. Sequence similarity of more than 98 % was retrieved to construct the phylogeny tree and to compute divergence time using MEGA 7 tool. Similarly, the Whole-genome phylogeny was compared using BLASTn tool and JSpeciesWS tool (James et al., 2014).

Genome comparison was done with 16S rRNA phylogeny. The analysis workflow is shown in Appendix 1. The 16S rRNA sequence-based evolutionary relationship with previously characterized strains was evaluated with MEGA7 (Kumar et al., 2016). The evolutionary history was inferred with the Maximum Likelihood method based on the Tamura-Nei model and represented with a bootstrap consensus tree from 1000 replicates. Initial trees were built with Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. Only the tree with the highest log-likelihood value was generated with 95% site coverage (Kumar et al., 2016).

Whole-genome sequence comparison was also done with an average nucleotide identity calculated with JSpeciesWS (Richter et al., 2016). The analysis workflow was shown in appendix 1. The sequences obtained in this study will be deposited in the NCBI GenBank database.

### 2.11. Data collection for Knowledge, Attitude, and practice (KAP) of HCW

KAP of HCWs towards MRSA control and prevention measures were collected using pretested self-administered questionnaires prepared for this project (annex IXa to IXc). KAP questionnaire is adopted from different guidelines and published literature (Eldridge et al., 2006; Huskins et al., 2011; Patel et al., 2008 ; CDC, 2007[a]; CDC 2007 [b].; CDC 2006 ). The Data were collected by trained data collectors.

For knowledge questions, each question has 1 point score and a total score of 8. The correct answer had one point and the incorrect answer was given 0 points. The score varies from 0-8. Based on mean scores, the individual was grouped good level knowledge, if they scored 60 % and above out of 8 questions and poor knowledge if they scores < /= 59 % .

For attitude questions, 5 point Likert scale answers were used. For positive questions a mark of 5 to 1 is given for Strongly Agree, Agree, Undecided, Disagree, and strongly disagree responses respectively. For negative questions, the reverse scale mark of 1 to 5 was given for strongly disagree, Disagree, Undecided, Agree, and Strongly agree responses respectively. All participants' results were summed up to obtain a mean score and categorized as positive attitude and negative attitude level if they fall above and below the mean attitude score respectively.

For practice questions, 5 points likert scales were used and for positive questions, a mark of 5 to 1 is given for always, sometimes, rarely, very rarely, and never responses and for negative practice questions, the reverse score 1 up to 5 ws given. All practice related response is summed up to obtain mean score and categorized as good attitude if they scored above the mean and poor attitudes if they scored below the mean score.

The overall data collection process is summarized in the flow chart shown below ( Figure 2.3).

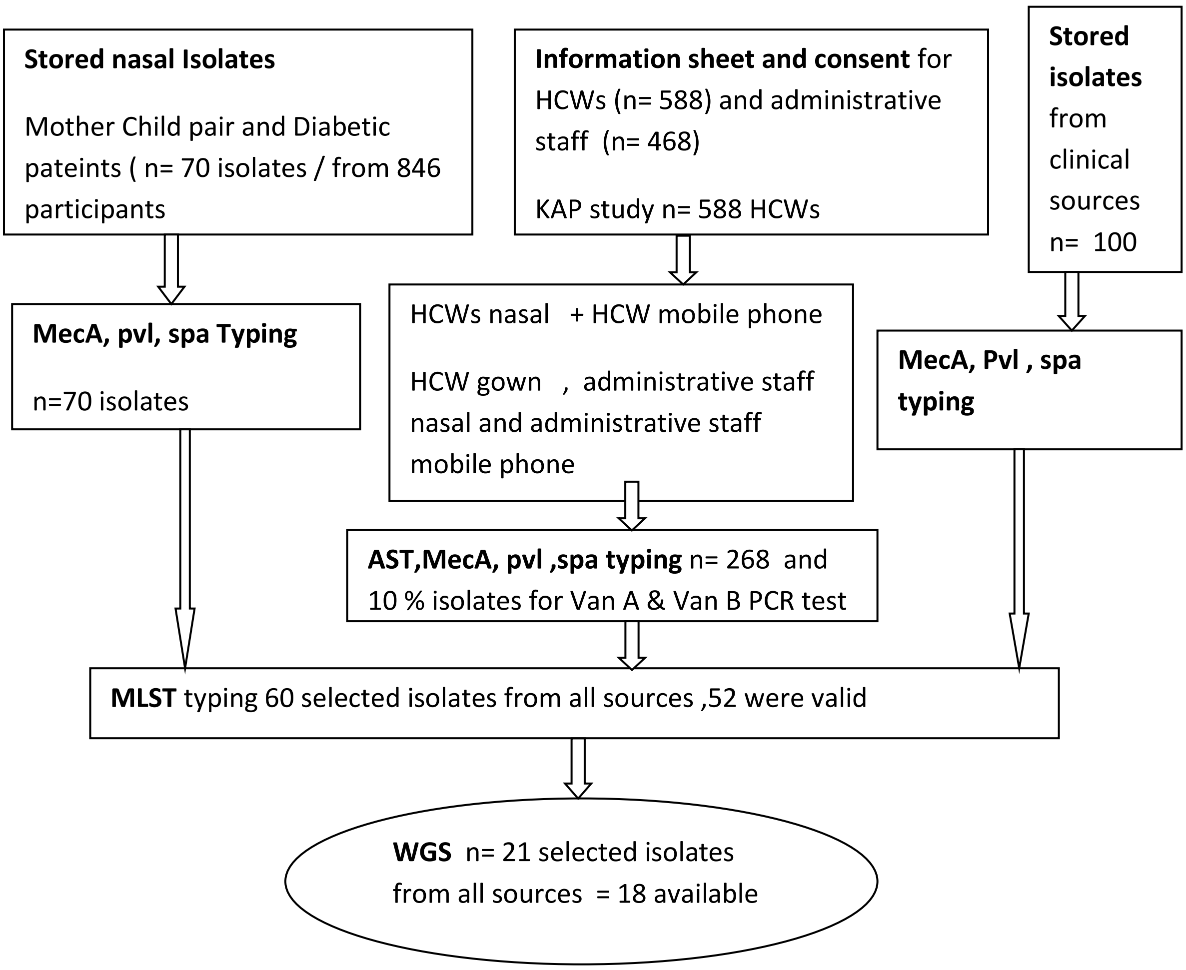

**Figure 2.3.** Flow chart for data collection for assessing “ Burden of Methicillin-*Resistant S.aureus (*MRSA) & associated factors at TASH: Evidence from colonization of HCWs ,patients, administrative staff, selected inanimate objects”, and clinical isolates

### 2.12. Quality assurance measures

Pre-analytical, analytical, and post-analytical quality control measures were taken throughout the research work.

**2.12.1. Pre-analytical measures**: Data collection tools were pretested and standardized. A one-day training was given for data collectors before data and specimen collection. Aseptic procedures have been followed and all culture media were tested for sterility testing ahead of the actual procedures. Before the actual data collection, a pre-test was done on the KAP questionnaires for HCWs other than TASH, and adjustment was made based on the feedback. The temperature of the incubator and refrigerator was monitored. A sterility test was done during the preparation of each culture media.

**2.12.2. Analytical measures**: we have used SOP for isolation, identification, AST, and molecular testing. Quality control strains were used, *S. aureus* (ATCC 25923) and MRSA 252 Newman strain as control strains. About 5 % of AST was duplicated to check for any difference in performances. We have also used known gram-positive and gram-negative organisms for assessing the QC of gram staining reagent, coagulase test, DNAse, and catalase test. Every day, questionnaires were checked to maintain uniformity and consistent data collection. Positive and negative controls were implemented for all PCR reactions and gel electrophoresis reading.

**2.12.3. Post-analytical measures:** All results and activities were registered in a registration book and data were entered properly. as required. The temperature of the refrigerator was monitored regularly to maintain the quality of stored isolates.

### 2.13. Data quality management

Training on data collection procedures was given for data collectors. Pre-testing of the questionnaire also was made to improve the data collection tool. There was supervision during data collection time to check for completeness, accuracy, clarity, consistency of questionnaires on daily basis. Corrective measures were taken accordingly, and then special care was taken during data entry, and data cleaning and analysis. Howeevr, we have encountred some missing vraibles from HCWs groups and we could not fix it as the questionares were anonymous. The data was kept on a secured, password-protected computer including a backup system.

**2.14. Data Analyses**

Data analyses and cleaning was done using SPSS version 20.0 software. Frequency count and Comparison were made for different variables among MRSA colonizers and non-colonizers and statistically tested using Chi-square or Fisher's Exact Test. Mean score of knowledge, attitudes, and perceived practice have been calculated and HCWs scored above average was considered as having good knowledge, attitudes, and practice if not it is labeled as poor knowledge, attitudes and practice. Multivariate analyses were done to predict the potential risk factors for colonization of MRSA. A comparison was made for MRSA colonization rate of HCWs and administrative staff. We have also determined the genetic relationship of *S. aureus* isolates from HCWs, patients, and inanimate objects using different MecA, PVL status, Spa typing. For few isolates, we did also MLST and WGS. A P-value of < 0.05 was considered statistically significant.

**2.15. Ethical Consideration**

Ethical approval was obtained from the Department Review and Ethics Committee (DREC) of the Department of Microbiology, Immunology, and Parasitology (DMIP) and the Institutional Review Board of the College of health sciences (IRB-CHS) (U (Ref. no. AAUMF 03-008) (Ref. no. MoST 310/160/18). We have also secured ethical clearance from the national research and ethics review committee (Ref. no. MoST 310/160/18).). A support letter was written by the clinical director of CHS to all departments of the hospital including the bacteriology laboratory to use clinical isolates. Written informed consent was obtained from each HCW and administrative staff. Once they have read the information sheet, all their questions were explained to them. They were also informed about their right to refuse to participate in the study. The privacy and confidentiality of the information gathered were maintained. No name identifiers were made on the questionnaires. All information was locked in a drawer and secured computer-using password. HCWs were informed of the overall findings of MRSA carriage in the hospital and some awareness has been made about MDR bacteria including MRSA in the hospital settings.

### 2.16. Dissemination of results

After the conclusion of the study, the results of the study with concrete recommendations will be shared by using different means. The information will be shared with the Hospital administration as input for HAI prevention measures in the Hospital; results will also be shared with the Hospital community through presentations at Department morning meetings and workshops. In addition to this, abstracts will be presented at scientific conferences, and manuscripts will be submitted for publications in peer-reviewed journals.

### 2.17. Operational definitions

The following operational terms are used in this proposal. They are adopted from various literature and contextualized to this work (Eldridge et al., 2006; Huskins et al., 2011; Patel et al., 2008; CDC ,2007[a]; CDC,2006, Sax [a] et al., 2007).

**Colonization:** the existence of the microorganism in the nasal area without showing clinical signs and symptoms.

**Inanimate objects**: objects which are non-living but there is potential contact with HCW and patients that can serve as reservoirs of MRSA, in this case, mobile phone, gowns, trolleys, fixed phones, operating beds, Intravenous fluid stands, and other similar items in the operating rooms, intensive care units will be considered.

**Good knowledge**: knowledge level of HCW about MRSA control and prevention who have got above the average score otherwise it will be poor. This means those who scored 60 % and above were labelled as good knowledge.

**Good practice:** perceived level of practice of HCW who have got above the mean practice score.

**Good attitudes:** The attitudes of HCW who have got above the mean attitudes score from the five Likert scale.

**Health care workers**: In this context, health care personnel have close contact with patients for diagnosis, management, and other intervention measures. Medical doctors, nurses, laboratory personnel, pharmacy personnel, and other related .

**Adminstrative staff :** These are staff of TASH and CHS, AAU who do not have direct contact with patient management and or support.

**MRSA:** *S.aureus* which are resistant to penicillin and third-generation cephalosporin drugs. An isolate is MRSA if *S. aureus* isolate was resistant to cefoxitin and positive for Mec A gene.

MSSA : *S.aureus* starins which are sensitive to cefoxitin and mecA gene.

# 3.0. CHAPTER THREE : RESULTS

## 3.1. Socio-demographic characteristics of HCWs and administrative staff

### 3.1.1. Socio-demographic data of Health Care Workers in Tikur Anbessa Specialized Hospital (TASH)

A total of 588 HCWs were included in this study though there are few missing variables in the responces of HCWs that result in different in the total number of HCWs in some responcses . More female HCWs participated in the study than their male counterparts (58.4 %, 339/580) making the male to female ratio 1: 1.4.

The mean age and standard deviation of HCWs were 29.13 ± 6.66 years with minimum and maximum age of 20 and 57 years respectively. Seventy-five percent of HCWs in TASH are within the age group of 20-26 years. About 40.3 % (237/586) HCWs were married; the majority were nurses accounting for 49.1 % (289/588) followed by Medical doctors 28.4 % (167/588) including residents, interns, and senior consultant staff (Table 3.1).

In terms of education level, 63 % (369/586) of HCWs have a Bachelor of Science degree and 9.6 % ( 56/586) of the HCWs have a Medical degree. Regarding work experience, 60.5 % (355/ 587) of the HCWs have 1-4 years of work experience in TASH (Table 3.1).

**Table 3.1. Socio-demographic characteristics and departments of HCWs in TASH, 2019**

| Variables | Frequency | Percent (%) |
|---|---|---|
| **Sex ( n= 580)** | | |
| Male | 241 | 41.6 |
| Female | 339 | 58.4 |
| **Age group ( n= 574)** | | |
| 20-26 Years | 441 | 76.8 |
| 27- 33 Years | 85 | 14.8 |
| 34- 40 Years | 21 | 3.7 |
| >= 41 Years | 27 | 4.7. |
| **Marital status (n = 586)** | | |
| Single | 344 | 58.7 |
| Married | 237 | 40.4 |
| Divorced | 5 | 0.9 |
| **Professional Category (n= 588)** | | |
| Medical doctors | 167 | 28.4 |
| Nurses | 289 | 49.1 |
| Medical Laboratory Personnel | 36 | 6.1 |
| Pharmacy personnel | 29 | 4.9 |
| Others | 67 | 11.4 |
| **Educational level (n= 586)** | | |
| Diploma | 6 | 1.0 |
| Degree | 369 | 63.0 |
| Medical Doctor | 56 | 9.6 |
| MSc | 39 | 6.7 |
| Specialty certificate | 112 | 19.1 |
| Others | 4 | 0.7 |
| **Work experience (n = 587)** | | |
| 1-2 Years | 197 | 33.6 |
| 3-4 Years | 158 | 26.9 |
| 5-7 Years | 111 | 18.9 |
| 8-10 Years | 45 | 7.7 |
| More than 10 Years | 76 | 12.9 |

NB: n is different for variables since there were missing values from the participants responses

In this study , 16.4 % (96/585) of HCWs were working in out-patient and emergency departments. Likewise, 15.38 % (90/585) of HCWs were working in the Gynecology and Obstetrics department of TASH (Figure 3.1).

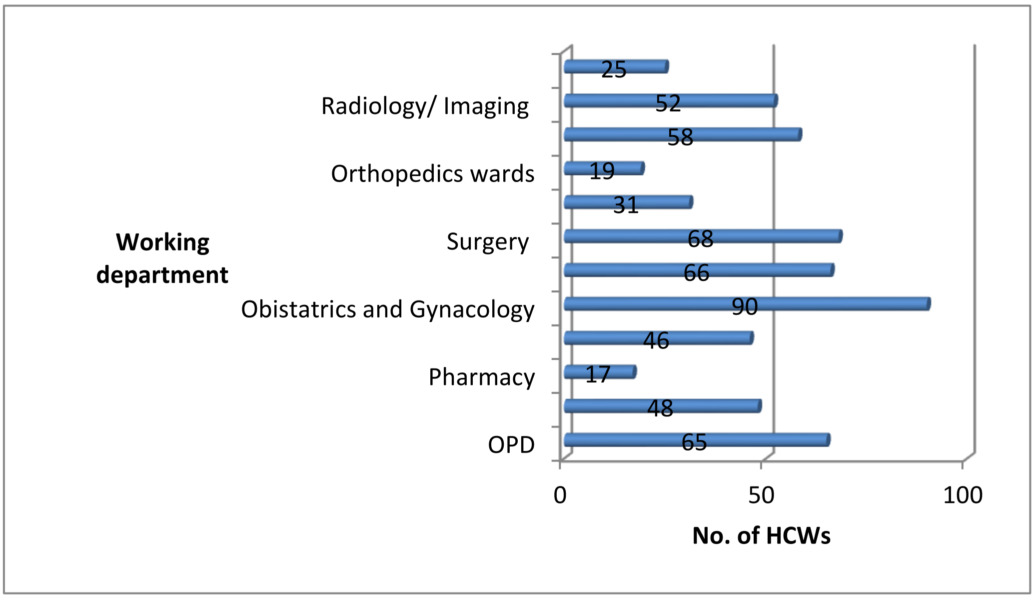


**Figure 3.1.**Distribution of the HCW in the various Departments of TASH , 2019.

Assessment on availability, type of gowns, and frequency of changing gowns among HCWs in TASH, showed that 15 % (88/588) of HCWs had only a single gown and 2 % ( 12 / 588) of HCWs have six or more gowns for their clinical practice in the hospital. The majority of HCWs 57.5 % ( 338/588) had long sleeve gowns and 48.1 % ( 282/586) of the HCWs in TASH change their gowns on weekly basis (Table 3.2).

Regarding mobile phones types, 81.1 % ( 476/ 587) of HCWs had a smartphone , while 52.5 % ( 306/583) of the HCW's mobile phones did not have cell phone cover. Most HCW, 66.4 % (387/ 583) in TASH use their mobile phones during their clinical activities like during operations, other procedures, and or examining patients and other similar activities in the hospital (Table 3.2).

**Table 3.2.Availability of gown, mobile phone use, and MRSA-related training among HCWs in TASH, 2019.**

| Variables | Frequency | Percent |
|---|---|---|
| **Possession and no. of Gowns by HCWs ( n= 588)** | | |
| Only one gown | 88 | 15.0 |
| Two gowns | 231 | 39.3 |
| Three gowns | 176 | 29.9 |
| Four gowns | 58 | 9.9 |
| Five gowns | 23 | 3.9 |
| Six or more gowns | 12 | 2.0 |
| **Type of Gowns ( n= 588)** | | |
| Short sleeves | 209 | 35.5 |
| Long sleeves | 338 | 57.5 |
| Both types | 41 | 7.0 |
| **Frequency of changing gown (n= 586)** | | |
| Every day | 11 | 1.9 |
| Every other day | 143 | 24.4 |
| Weekly | 282 | 48.1 |
| Every other week | 99 | 16.9 |
| As required | 51 | 8.7 |
| **mobile phone type used by HCWs (n= 587)** | | |
| Ordinary ( Key pad type) | 97 | 16.5 |
| Smart mobile phone | 476 | 81.1 |
| Both types | 14 | 2.4 |
| **Presence of mobile phone cover (n= 583)** | | |
| Present | 277 | 47.5 |
| No Cell phone cover | 306 | 52.5 |
| **Use of mobile phone during clinical practice ( n= 583)** | | |
| Yes | 387 | 66.4 |
| No | 111 | 19.0 |
| Some times | 85 | 14.6 |
| **Training on MRSA prevention and** | | |

| | | |
|---|---|---|
| **control in general (n=585)** | | |
| Trained | 70 | 12.0 |
| Untrained | 515 | 88.0 |
| **Presences of guidelines and leaflet about MRSA (n = 585)** | | |
| Present | 81 | 13.8 |
| Absent | 433 | 74.0 |
| Do not know | 71 | 12.1 |
| **Sufficient hand hygiene materials in TASH ( n= 586**) | | |
| Present | 190 | 32.4 |
| Absent | 383 | 65.4 |
| Do not know | 13 | 2.2 |

NB: n is different as there are missing values in the responses from participants responses. Clinical Practice: activities related in operation rooms, wards, examining patients, and other similar activities.

Concerning whether HCWs in TASH took training in MRSA-related prevention and control, 88 % (515/585), did not take such training during their practice in TASH. Seventy-four % (433/ 585) and 65.4 % (383/386) of HCWs did not know or have not heard about the presence of guidelines and leaflets about MRSA and they did not feel that there were sufficient hand hygiene materials in TASH respectively (Table 3.2).

HCWs in TASH got MRSA-related information from various sources, such as 21.2 % (124/ 586) and 15.5 % (91/586) from radio / TV and other health personnel respectively. Only 5.1 % of HCWs (30/586) obtain such information through reading books and journals (Figure 3.2).

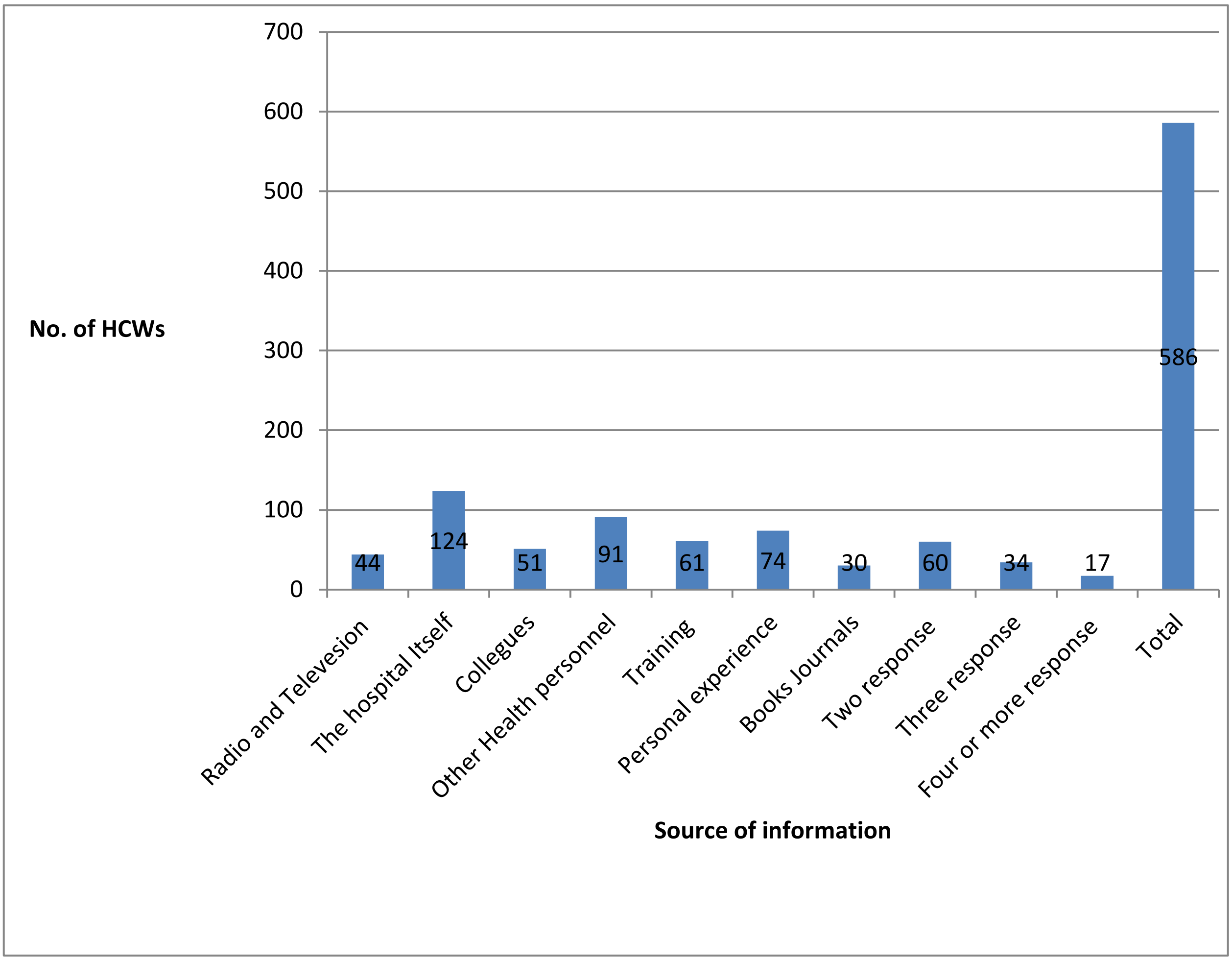


Figure 3.2 Source of MRSA related information perceived from HCWs of TASH, 2019

### 3.1.1.2. History of hospital admission and surgical intervention among HCWs in TASH

Among the HCWs working in TASH, 82.5 % (484/587) did not have a history of hospital admission and only 11.5 % (67/585) of the HCWs had surgical intervention in the hospital for the previous three years of the data collection time (Table 3.3).

**Table 3.3.History of Hospital admission and surgical intervention by HCWs in TASH, 2019**

| Variables | Frequency | Percent |
|---|---|---|
| **Hospital admission ever since (n= 487)** | | |
| Admitted | 99 | 16.9 |
| Not admitted | 484 | 82.5 |
| Do not remember | 4 | 0.7 |
| **Surgical intervention for the last three years (n= 585)** | | |
| Yes | 67 | 11.5 |
| No | 512 | 87.5 |
| Do not remember | 6 | 1.0 |
| **Surgical intervention for the last one year (583)** | | |
| Yes | 42 | 7.2 |
| No | 526 | 90.2 |
| Do not remember | 15 | 2.6 |

### 3.1.2. **Socio demographic characterstics of Administrative staff of CHS, AAU**

A total of 482 administrative staff of the College of Health Sciences, Addis Ababa University were approached in this study, and 468 staff responded with a response rate of 97 %. The majority of the participants were females (64.1 %) and only 4.7 % (22/468) fall within the age group of 56 years and above. More than 65 % of the staff had secondary level education or below. Nearly 25 % of the staff had a history of hospital admission and less than 5 % had a history of surgical intervention (Table 3.4).

**Table 3.4. Sociodemographic characteristics of Administrative staff of CHS, AAU, 2018**

| Variables | Frequency in No. ( %) |
|---|---|
| **Sex** (n = 468) | |
| Male | 168 (35.9 %) |
| Female | 300 (64.1%) |
| **Age group (n=468)** | |
| 17-25 Years | 127 (27.13 %) |
| 26-35 Years | 140 (29.91%) |
| 36-45 Years | 120 (25.64 %) |
| 46-55 years | 59 (12.6 % ) |
| >= 56 years | 22 (4.7 %) |
| **Marital status (n= 468)** | |
| Single | 177 (37.82 %) |
| Married | 288 ( 61.54 %) |
| Divorced | 3 (0.64 %) |
| **Educational category ( n= 468)** | |
| Diploma | 64 (13.67 %) |
| Degree | 52 (11.11 %) |
| MSc | 7 ( 1.49 %) |
| Certificate | 36 (7.69 %) |
| Secondary level education | 142 (30.34 %) |
| Primary level education | 155 ( 33.11%) |
| Read and write | 12 (2.56 %) |
| **The current working area in the CHS (n=468)** | |
| CHS | 271(57.90 %) |
| Sefere-Selam | 65 (13.88 %) |
| TASH offices | 19 (4.05 %) |
| TASH wards | 113 ( 24.15 %) |
| **History of Hospital admission last 3 years during data collection (n=468)** | |
| Yes | 115 (24.57 %) |
| No | 353 (75.43 %) |
| **History of surgical intervention for last 3 year during data collection(n=468)** | |
| Yes | 24 (5.13 %) |
| No | 444 (94.87 %) |

Out of 468 administrative staff, 25 % (117/468) were cleaners and laundry workers, followed by drivers and guards, accounting for 19.01 % (89/468) (Figure 3.3. ).

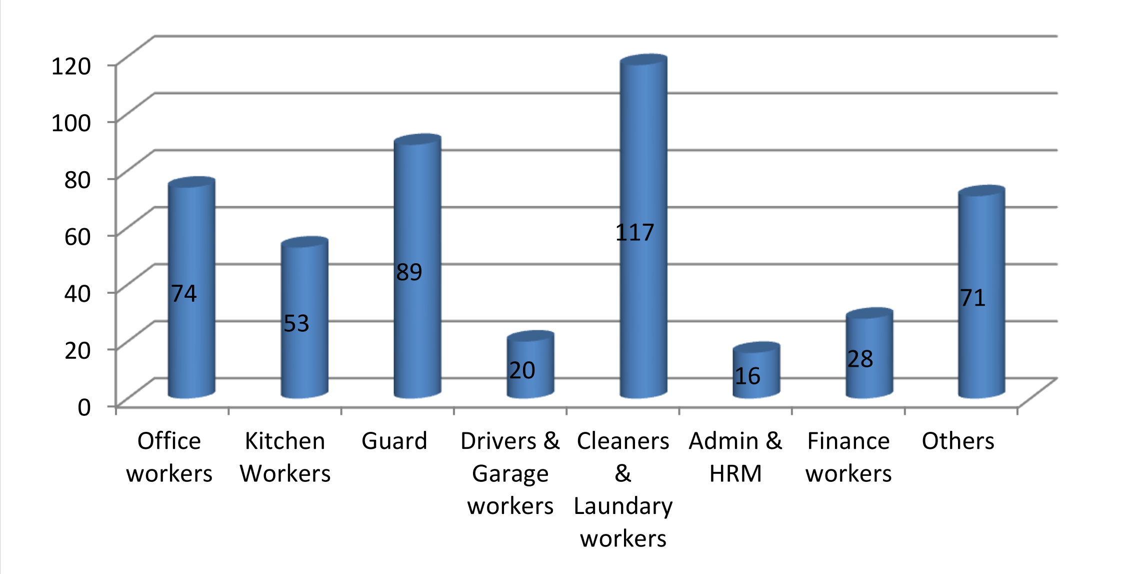


**Figure 3.3.** Distribution of administrative staff of CHS, AAU, by professional category, 2019

Based on work experience in CHS, AAU, more than 37 % of the staff served at least 8 years. Out of the total, 48 .07 % ( 225/468) of administrative staff had the ordinary type of mobile phone (Table 3.5).

**Table 3.5. Distribution of administrative staff by service year and mobile types of CHS, AAU, 2019.**

| Variables | Frequency in No. (%) |
|---|---|
| **Service year in CHS, AAU** | |
| 1-2 years | 179 (38.24%) |
| 3-4 years | 63 (13.46 %) |
| 5-7 years | 49 (10.47 %) |
| 8-10 years | 25 (5.34 %) |
| More than 10 years | 152 (32.47 %) |
| **Mobile phone types** | |
| Ordinary | 225 (48.07 %) |
| Smart phones | 216 (46.15%) |
| No phones during survey date | 27 (5.76 %) |
| **Cell phone cover** | |
| Present | 26 (5.55 %) |
| Absent | 415 (88.67 %) |
| Not applicable | 27 (5.76 %) |

## 3.2. Isolation rate of *S .aureus* from HCWs and administrative staff

More than 280 presumptive *S. aureus* were isolated from nasal swabs of HCWs and administrative staff, mobile phones of HCWs, and administrative staff and gowns of HCWs. Upon further identification, 238 of them were confirmed as *S .aureus* ( Figure 3.4) and the remaining 42 were identified as coagulase-negative *Staphylococcus*( CONS). The rate of *S.aureus* isolates from nasal swabs of HCWs and administrative staff was16.32 % (96/588) and 8.97 % (42/468) respectively. While the rate of *S.aureus* among HCWs and administrative staff mobile phones were 6.46 % (38/588) and 3.84 % (18/468) respectively. On the other hand, the rate of *S.aureus* isolates among gowns of HCWs was 7.48 % (44 /588).

Two hundred and ten (210) swabs collected from different inanimate objects were included in this study, and no *S .aureus* was detected. Rather, 28 coagulase-negative *Staphylococcus* ( CONS) were identified and 4 of them were harbouring mec A gene.

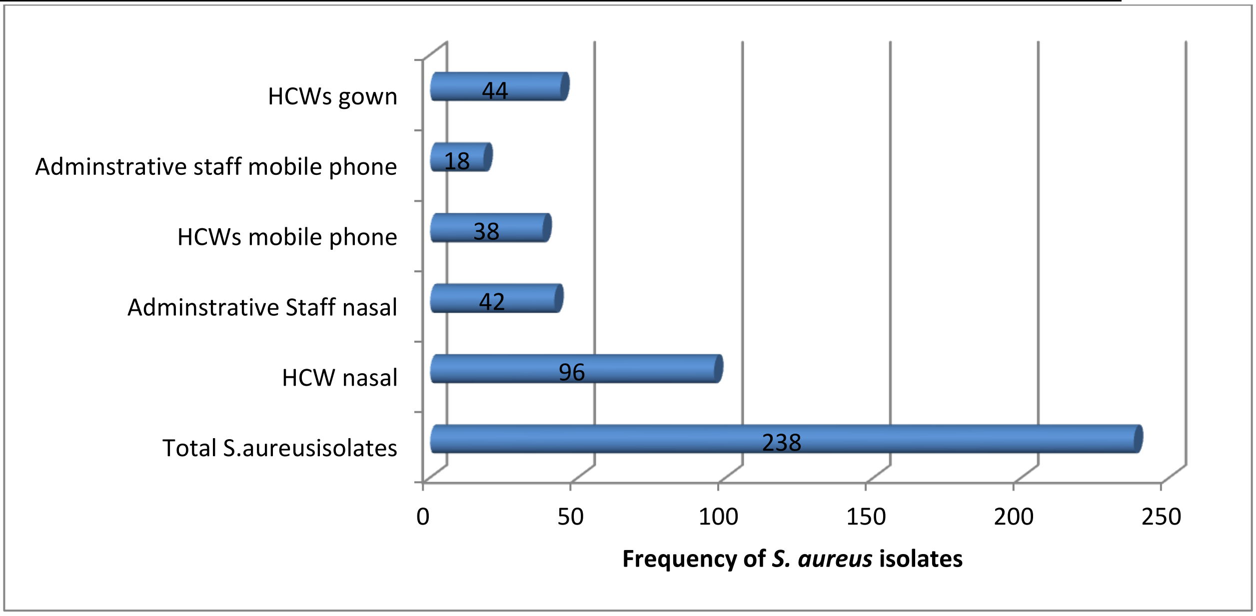


Figure 3.4. Source *S.aureus* isolates from HCWs and administrative staff of CHS, AAU, 2019

### 3.2.1. Antimicrobial susceptibility testing (AST) Pattern of *S. aureus* isolates

Disc diffusion test was done for 238 *S.aureus* isolates from nasal swabs of HCWs and administrative staff, mobile phones of HCWs, and administrative staff and gowns of HCWs. Overall, 31 isolates (13.02 %) were sensitive to all antibiotics tested. All isolates were sensitive to rifampicin. About 10 % of isolates including all MRSA isolates were vancomycin sensitive using Van A and Van B genes by PCR methods.

Penicillin is the least sensitive drug for *S. aureus* isolates and 75.2 % of the isolates were resistant to this drug. In this study, 68 isolates were resistant to cefoxitin (oxacillin) and all of them were mec A positive (Table 3.6).

**Table 3.6. Antimicrobial susceptibility profile of *S. aureus* isolates from nasal swabs, mobile phones, and gowns of HCWs, TASH, CHS, Addis Ababa, Ethiopia,2019**

| Antibiotics tested | Sensitive n (%) | Resistant n (%) | Intermediate n (%) |
|---|---|---|---|
| Penicillin ( 10 ug) | 59 (24.8%) | 179 (75.2%) | - |
| Oxacillin (Cefoxitin ) (30µg) | 167 (70.2) | 68 (28.6%) | 3 (1.2%) |
| Erythromycin (15µg) | 199 (83.6%) | 36 (15.1%) | 3 (1.3%) |
| Clindamycin (2µg) | 217 (91.2 %) | 21 (8.8%) | - |
| Tetracycline (30µg) | 89 (37.4 %) | 149 (62.6%) | - |
| Rifampin (5µg) | 238 (100%) | - | - |
| Trimethoprim-Sulfamethoxazole (1.25/23.75 µg) | 74 (31.1%) | 164 (68.9%) | - |

All MRSA isolates and 10 % of methicillin sensitive isolates were sensitive to vancomycin as we did not amplifay vanA and Van B genes.

*S. aureus* isolated from HCWs had a higher resistance rate of the antibiotics tested compared to isolates from administrative staff and the difference is statistically significant p-value <0.05 (Table 3.7).

**Table 3.7. Antibiotics resistant profile of *S.aureus* isolates from HCWs groups versus administrative staffs of TASH, CHS, AAU, 2019**

| Antibiotics tested | Number and percent ( % ) of resistant *S.aureus* | | | |
|---|---|---|---|---|
| | HCWs groups | Administrative Staff | $X^2$ | P value |
| Penicillin ( 10 ug) | 139 (78.08) | 40 (66.66) | 3.14 | 0.076 |
| Oxacillin (Cefoxitin (30µg) | 61 (34.26 ) | 7 ( 11.66) | 12.8 | 0.002 |
| Erythromycin (15µg) | 178 (14.04) | 11 (18.33) | 1.59 | 0.451 |
| Clindamycin (2µg) | 20 (11.23) | 1 (1.66) | 5.1 | 0.024 |
| Tetracycline (30µg) | 80 (44.94) | 9 (15.0) | 17.1 8 | 0.000 |
| Trimethoprim-Sulfamethoxazole (1.25/23.75 µg) | 58 ( 32.58) | 16 ( 26.66) | 0.773 | 0.392 |

In addition, statistically significant resistant rates to all drugs described in Table 3.7 were generally seen among MRSA isolates than MSSA isolates with a p-value < 0.05, except the drug erythromycin which did not have statistical significance.

### 3.2.2. Multidrug-resistant pattern of *S. aureus* isolates

Over all 237 isolates (88.4%) of *S. aureus* isolates were resistant to at least a single antibiotic. Sixty-one isolates were resistant to two different classes of antibiotics and one isolate was resistant to six antibiotics ( Figure 3.5). The level of MDR *S.aureus* found to be 28.9 % (69/238).

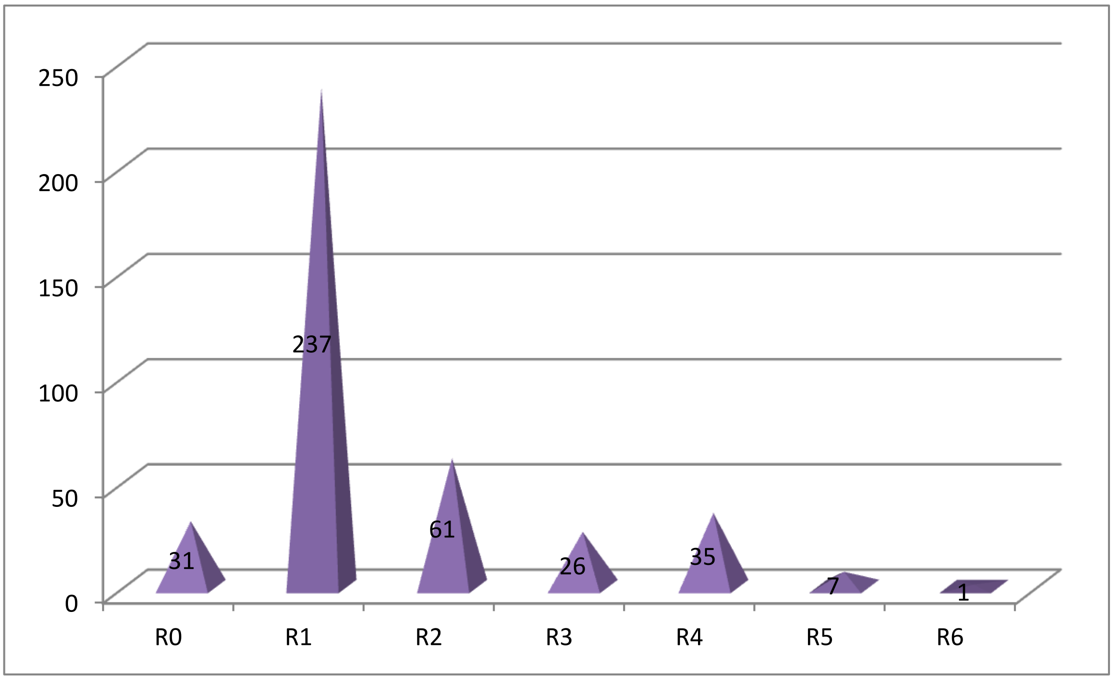


**Fig 3.5.** Multiple antibiotics resistant pattern of *S aureus* isolates at TASH, CHS, AAU, 2019

### 3.3.The burden of MRSA: Mec A gene detection from different source samples

In this section, the burden of MRSA is presented for *S.aureus* isolates from nasal colonization of HCWs and administrative staff, mobile phones of HCWs, and administrative staff and gowns of HCWs. Moreover, we have also determined the burden or proportion of MRSA from stored isolates from mother-child pairs and diabetic patients nasal isolates and clinical isolates ( diabetic foot ulcers infection and other clinical isolates) to estimate the true burden of MRSA based on cefoxitin disc diffusion and mec A gene amplification ( Figure 3.6).

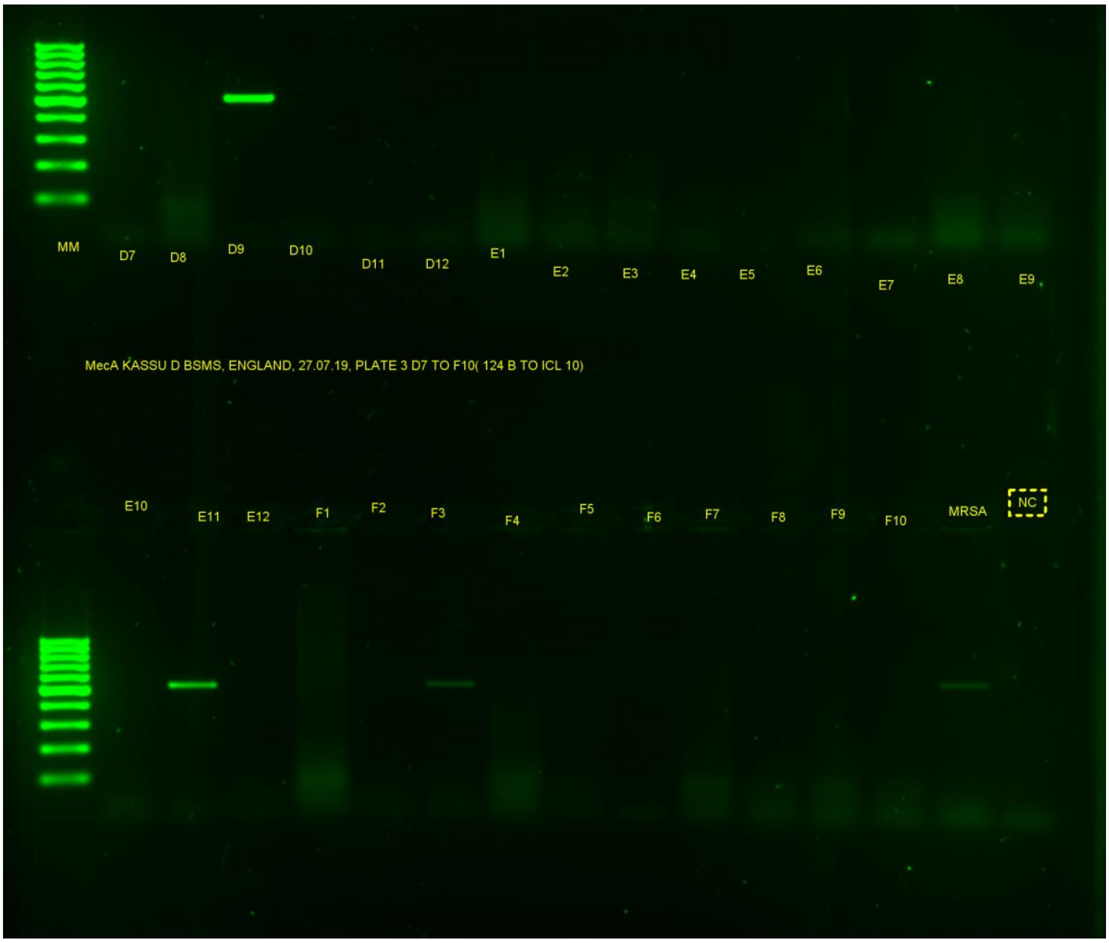


**Figure 3.6**.Gel band result of MecA genes . MM is for molecular markers of 100 bp, the letter from D7 to F10 are PCR products of *S.aureus* isolates, MRSA is a positive control and NC is a negative control.

### 3.3.1.Nasal Colonization of HCWs and administrative staff with MRSA at TASH,CHS, Addis Ababa University

#### 3.3.1.1.Nasal colonization of HCWs at TASH

The burden of MRSA nasal colonization in HCWs is found to be 4.8 % (28/580). The burden of MRSA is more or less the same among males and females with no significant difference. HCWs within the age group of 20-26 years were the dominant group and the burden is also higher among these age groups compared to others (Table 3.8). Similarly, Nurses were the highest HCWs in TASH and the proportion of nasal colonization of MRSA is higher among nurses than other HCWs ( 22/28 total positive). Twenty-three HCWs have first-degree among the 28 MRSA-positive nasal carriers.

**Table 3.8. Nasal Colonization of HCWs with MRSA working at TASH, CHS, AAU, 2019.**

| Variables | MRSA Positive No. (%) | MRSA Negative No. ( %) |
|---|---|---|
| **Sex ( n= 580)** | | |
| Male | 12 (4.97 %) | 229 (95.03 %) |
| Female | 16 (4.72 %) | 323 (95.30 %) |
| **Age Group *** | | |
| 20-26 years | 26 (5.89 %) | 415 (94.11 %) |
| 27-33 years | 0 (0 %) | 85 (100 %) |
| 34-40 years | 1 (4.76 %) | 20 (95. 24 %) |
| >/= 41 years | 1 (3.70 %) | 26 (96.30 %) |
| **Marital status** | | |
| Single | 19 (5.52%) | 325 (94.48%) |
| Married | 9 (3.79 %) | 228 (96.21%) |
| Divorced | 0 (0%) | 5 (100 %) |
| **Professional category **** | | |
| Medical doctor | 4 (2.39 %) | 163 (97.61 %) |
| Nurses | 22 ( 7.61 %) | 267(92.39 %) |
| Medical Laboratory personnel | 0 (0 %) | 36 (100 %) |
| Pharmacy personnel | 1(3.44%) | 28 (96.55%) |
| Others | 1(1.49%) | 66 (98.51%) |
| **Educational level** | | |
| Diploma | 0 (0 %) | 6 (100 %) |
| Degree | 23 (6.23 %) | 346 (93.77 %) |
| Medical doctor | 1 (1.78 %) | 55 (98.22 %) |
| MSc | 1 (2.56 %) | 38 (97.44 %) |
| Speciality certificate | 3 (2.67 %) | 109 (97.33%) |
| Others (PhD, Pos graduate diploma) | 0 (0 %) | 4 (100 %) |

*$x^2$ = 5.4; p value = 0.023; ** $x^2$ =10.7; p value = 0.03

HCWs having 1-2 years of work experience are the largest group and 6.59 % (13/ 197) of them have nasal MRSA colonization. There is no significant difference between the number of gowns possessed by HCWs and nasal colonization by MRSA (Table 3.9). However, a statistically significant difference was seen between HCW's frequency of changing gowns and nasal colonization with MRSA. On the other hand, the proportion of MRSA nasal colonization is not different among HCWs having smartphones or ordinary type cell phones.

**Table 3.9. MRSA burden of HCWs with gowns and mobile phone use at TASH, CHS, AAU, 2019.**

| Variables | MRSA Positive No. (%) | MRSA Negative No. ( %) |
|---|---|---|
| **Work experience year** | | |
| 1-2 | 13 ( 6.59 %) | 184 ( 93.41 %) |
| 3-4 | 6 ( 3.79 %) | 152 (96.21 %) |
| 5-7 | 6 ( 5.40 %) | 105 (94.60 %) |
| 8-10 | 2 ( 4.44 %) | 43 ( 95.56%) |
| More than 10 years | 1 ( 1.31 %) | 75 ( 98. 69 %) |
| **No. of gown you have** | | |
| One | 5 (5.68 %) | 83 (94. 32 %) |
| Two | 5 (2.16 %) | 226 (97.84%) |
| Three | 10 ( 5.68 %) | 166 (94. 32 %) |
| Four | 5 ( 8.62 %) | 53 (91.38 %) |
| Five | 2 ( 8.69 %) | 21 ( 91.31 %) |
| Six or more | 1( 8.33 %) | 11 ( 92.67 %) |
| **Types of gown** | | |
| Short sleeves | 13 ( 6.22 %) | 196 ( 93.78 %) |
| Long sleeves | 13 ( 3.84 %) | 325 ( 96.16 %) |
| Both | 2 ( 4.87 %) | 39 ( 95. 13%) |
| **Frequency of changing gown *** | | |
| Every day | 2 (18.18 %) | 9 (81. 82 %) |
| Every other day | 12 (8.39 %) | 131 (91. 61 %) |
| Weekly | 6 (2.12 %) | 276 (97.88 %) |
| Every other week | 6 (6.10 %) | 93 (93.90%) |
| As required | 2 (3.92 %) | 49 (96.08%) |
| **Type of cell phone** | | |
| Ordinary cell phone | 6 (6.18 %) | 91 (93.82 %) |
| Smart type phone | 21(4.41 %) | 455 (95.59%) |
| Both types | 1 (7.14 %) | 13 (92.86%) |
| **Cell phone cover** | | |
| Present | 12 ( 4.33 %) | 265 (95. 67 %) |
| Absent | 15 ( 4.93 %) | 289 (95.07 %) |
| Not applicable | 0 ( 0 %) | 2 (100 %) |
| **Do you use mobile phone during clinical practice** | | |
| Yes | 18 ( 4.65 %) | 369 ( 95.35 %) |
| No | 6 ( 5.40 %) | 105 ( 94.60 %) |

| | | |
|---|---|---|
| Some times | 3 ( 3.52 %) | 82 ( 96.48 %) |

*Fisher exact test**13.24; p value 0.010**

Nasal colonization of HCWs is not different whether they took MRSA-related training or not, availability of guidelines or leaflets, history of hospital admission, and surgical intervention (Table 3.10 ).

**Table 3.10. Nasal colonization of HCWs with MRSA with related factors at TASH, CHS, AAU, 2019.**

| Variables | Nasal MRSA Positive No. (%) | Nasal MRSA Negative No. ( %) |
|---|---|---|
| **Training on MRSA** | | |
| Yes | 6 (8.57%) | 64 ( 91. 43 %) |
| No | 22 ( 4.27 %) | 493 ( 95.73 %) |
| **Availability of guidelines & leaflets for MRSA** | | |
| Present | 5 ( 6.09 %) | 76 ( 93. 91 %) |
| Absent | 19 ( 4.38 %) | 414 ( 95.62 %) |
| Do not know | 4( 5.63 %) | 67 ( 94. 37 %) |
| **Availability of sufficient hand hygiene materials in TASH** | | |
| Present | 13 (6.84 %) | 177 ( 93.16 %) |
| Absent | 14 (3.39 %) | 369 ( 96.61 %) |
| Do not know | 1 (7.69 %) | 12 ( 92. 31 %) |
| **History of hospital admission ever** | | |
| Yes | 3 (3.03 %) | 96 ( 96. 97 %) |
| No | 25 (5.16 %) | 459 ( 94. 84 %) |
| Do not know | 0 (0 %) | 4 ( 100 %) |
| **History of surgical intervention** | | |
| Yes | 1 (2.38 %) | 41 (97.62 %) |
| No | 27 (5.13 %) | 499 (94. 87 %) |
| Do not know | 0 (0 %) | 15 (100 %) |

Moreover, there is no significant difference between the current working department and the Source of information with nasal colonization of HCWs by MRSA ( P-value > 0.05; data not shown).

#### 3.3.1.2. The burden of Methicillin-Resistant *S. aureus* among Administrative staff of CHS, AAU

Among 468 administrative staff of the College of Health Sciences, Addis Ababa University, only one individual had MRSA carriage in the nasal cavity, making a magnitude of 0.2 % (1/468). The MRSA-positive administrative staff was a female, between the age of 26-35 years and married. She has primary level education, works as a cleaner, has no history of hospital admission and surgical intervention for the last three years before data collection with a work experience of 4 years. She had a smartphone without a phone cover.

### 3.3.2. The burden of MRSA Contamination of HCWs and administrative staffs' mobile Phone practicing at TASH, CHS, AAU

#### 3.3.2.1. Burden of MRSA Contamination of HCWs mobile phones

We have determined the MRSA contamination level of the mobile phone of HCWs and it is found to be 2.89 % (17/ 588). Fifty percent of HCW's mobile phone contamination belonged to females ( P-value > 0.05). The majority of mobile phone contamination is seen among the very young age group, single HCWs, nurses, and medical doctors (Table 3.11).

**Table 3.11. MRSA contamination level mobile phones of HCWs practicing at TASH, CHS, AAU, 2019.**

| Variables | MRSA Positive No. (%) | MRSA Negative No. Percent ( %) |
|---|---|---|
| **Sex (N= 580)** | | |
| Male | 5 (0 %) | 236 (100 %) |
| Female | 10 (2.94 %) | 329 (97.06 %) |
| **Age Group ( N= 574)** | | |
| 20-26 years | 11 (0 %) | 430 (100 %) |
| 27-33 years | 3 (3.52 %) | 82 (96. 48%) |
| 34-40 years | 1 (4.76 %) | 20 (95.24%) |
| More than 40 years | 0 (0 %) | 27 (100 %) |
| **Marital status ( N= 586)** | | |
| Single | 10 (2.90 %) | 334 (97.10 %) |
| Married | 6 (2.53 %) | 231 (97.47 %) |
| Divorced | 0 (0%) | 5 (100 %) |
| **Professional category ( N= 588)** | | |
| Medical doctors | 7 (4.37 %) | 160 (95. 63%) |
| Nurses | 7 (2.42 %) | 282 (97.58%) |
| Medical Laboratory personnel | 1 (2.77 %) | 35 (97.33%) |
| Pharmacy personnel | 0 (0 %) | 29 (100 %) |
| Others | 1(1.49 %) | 66 (98.51%) |
| **Educational level ( N= 586)** | | |
| Diploma | 0 ( 0 %) | 6 (100 %) |
| Degree | 8 ( 2.16 %) | 361 (97. 84 %) |
| Medical doctors | 0 ( 0 %) | 56 (100 %) |
| MSc | 1 (2.56 %) | 38 (97.44%) |
| Speciality Certificate | 7 (6.25 %) | 105 (93.75) |
| Others | 0 (0 %) | 4 (100 %) |

**NB:** n is different as there were missing values from the responses of participants.

In this study, 62.5 % ( 10/16) mobile phone MRSA contamination is observed among HCWs who had work experience of 1-4 years, and interestingly, 12 of the 16 MRSA mobile phone contamination was seen among HCWs who have only two gowns, although the difference is not statistically significant ( P-value > 0.05). Similarly, the contamination level is high among those who change their gown every week and among HCWs who had a smartphone, but it is not statistically significant (Table 3.12).

**Table 3.12. MRSA contamination level of mobile phones of HCWs practicing at TASH, CHS, AAU, 2019.**

| Variables | MRSA Positive No. (%) | MRSA Negative No. (%) |
|---|---|---|
| **Service year (N= 581)** | | |
| 1-2 years | 5 (2.53 %) | 192 (97.43 %) |
| 3-4 years | 5 (3.28 %) | 147 (96. 72 %) |
| 5-7 years | 4 (3.60 %) | 107 (96.40%) |
| 8-10 years | 0 (0 %) | 45 (100 %) |
| More than 10 years | 2 (2.63 %) | 74 (97. 37 %) |
| **No. of gown you have (N= 588)** | | |
| One | 2 (2.27 %) | 86 (97.73 %) |
| Two | 12 (5.19 %) | 219 (94. 81%) |
| Three | 2 (1.13 %) | 174 (98.87%) |
| Four | 0 (0 %) | 58 (100 %) |
| Five | 0 (0 %) | 23(100%) |
| Six or more | 0 (0%) | 12 (100 %) |
| **Types of gown ( N= 588)** | | |
| Short sleeves | **8 (3.82 %)** | **201 ( 96.18%)** |
| Long sleeves | 6 (1.77%) | 332 ( 98. 23 %) |
| Both | 2 (4.87 %) | 39 ( 95. 13 %) |
| **Frequency of changing gown (N= 586)** | | |
| Every day | 0 (0 %) | 11 (100 %) |
| Every other day | 5 (3.49 %) | 138 (96.51 %) |
| Weekly | 9 (3.19 %) | 273 (96. 81 %) |
| Every other week | 2 (2.02 %) | 97 (97.98 %) |
| As required | 0 (0 %) | 51 (100 %) |
| **Type of mobile phone ( 587)** | | |
| Ordinary cell phone | 3 (3.09 %) | 94 (96. 91%) |
| Smart type phone | 13 (2.73 %) | 463 (97.27 %) |
| Both types | 0 (0 %) | 14 (100 %) |
| **Cell phone cover ( N= 583)** | | |
| Present | 8 (2.88 %) | 269 (97.12 %) |
| Absent | 8 (2.63 %) | 296 (97.37 %) |
| Not applicable | 0 (0 %) | 2 (100 %) |
| **Do you use mobile phone during clinical practice (N= 583)** | | |
| Yes | 14 (3.61 %) | 373 (96.39 %) |
| No | 0 (0%) | 111 (100 %) |
| Some times | 2 (2.35 %) | 83 (97.65 %) |

All mobile phone MRSA contamination is found among HCWs who do not have MRSA-related training. At the same time, 10 out of 16 MRSA mobile phone contamination is found among HCWs who said there are no guidelines and leaflets about MRSA in TASH, and there is no significant difference ( P-value = 0.052). MRSA mobile phone contamination is not different concerning the history of hospital admission and surgical intervention (Table 3.13).

Moreover, there is no significant difference between the current working department and source of information for MRSA /*S.aureus* with the level of mobile phone contamination of HCWs by MRSA ( P-value > 0.05; data not shown).

**Table 3.13. MRSA mobile phone contamination level of HCWs practicing at TASH, CHS, AAU, 2019.**

| Variables | MRSA Positive No. (%) | MRSA Negative No. ( %) |
|---|---|---|
| **Training on MRSA (n= 585)** | | |
| Yes | 0 ( 0 %) | 70 ( 100 %) |
| No | 16 ( 3.10 %) | 499 ( 96. 90 %) |
| Availability of guidelines & leaflets for MRSA (n= 585) | | |
| Present | 1 ( 1.23 %) | 80 (98.77%) |
| Absent | 10 ( 2.3 %) | 423 (97.7%) |
| Do not know | 5 ( 7.01 %) | 66 (92.99 |
| **Availability of sufficient hand hygiene materials in TASH (n=586)** | | |
| Present | 6 ( 3.15 %) | 184 ( 96.85 %) |
| Absent | 9 ( 2.34 %) | 374 ( 97. 66%) |
| Do not know | 1 ( 7.69 %) | 12 ( 92.31 %) |
| History of hospital admission ever ( n= 587) | | |
| Yes | 5 (5.05 %) | 94 (94.95 %) |
| No | 11(2.27 %) | 473 (97.73%) |
| Do not know | 0 (0 %) | 4 (100 %) |
| **History of surgical intervention ( n= 585)** | | |
| Yes | 4 (5.97 %) | 63 (94.13 %) |

| | | |
|---|---|---|
| No | 11 (2.14 %) | 501 (97. 86 %) |
| Do not know | 0 (0 %) | 6 (100 %) |

### 3.3.2.2. MRSA contamination level of Mobile phones of Administrative staffs of CHS, AAU

In this study, six administrative staff mobile phones harboured MRSA (MecA) making MRSA isolation rate of 1.3 % (6/468). Four of them were male participants, four of the staff had a history of hospital admission and all of them did not have a history of surgical intervention at all (Table 3.14).

**Table 3.14. MRSA contamination of mobile phone with socio-demographic factors of administrative staffs of CHS, AAU, 2018**

| Variables | MRSA Positive No. (%) | MRSA Negative No. ( %) |
|---|---|---|
| **Sex** (n = 468) | | |
| Male | 4(2.38 %) | 164 (97.62 %) |
| Female | 2 (0.30 %) | 298 (99.7 %) |
| **Age group (n=468) *** | | |
| 17-25 Years | 1 (0.78 %) | 126 (99.21%) |
| 26-35 Years | 1 (0.71%) | 139 (99.28 %) |
| 36-45 Years | 0 (0%) | 120 (100 %) |
| 46-55 years | 3 (5.10 %) | 56 (94.90 %) |
| >= 56 years | 1 ( 4.45 %) | 21 (95.45 %) |
| **Marital status (n= 468)** | | |
| Single | 2 (1.13 %) | 175(98.87%) |
| Married | **4(1.38%)** | 284 (98.61 %) |
| Divorced | 0 (0 %) | 3 (100 %) |
| **Educational category ( n= 468)** | | |
| Diploma | 3 (4.68 %) | 61 (95.31%) |
| Degree | 0 (0 %) | 52 (100 %) |
| MSc | 1 (14.3%) | 6 (85.71%) |
| Certificate | 0 ( 0 %) | 36 (100 %) |
| Secondary level education | 0 (0 %) | 142 (100 %) |
| Primary level education | 2(1.29 %) | 153 (98.70 %) |
| Read and write | 0 (0 %) | 12 (100 %) |

| | | |
|---|---|---|
| **Current working area in the CHS (n=468)** | | |
| CHS | 3 (1.10 %) | 268(98.89 %) |
| Sefere-Selam | 1 (1.54% ) | 64(98.46 %) |
| TASH offices | 0 (0 %) | 19 (100 %) |
| TASH wards | 2(1.77 %) | 111 (98.23 %) |
| **History of Hospital admission (n=468)** | | |
| Yes | 2 (1.74 %) | 113 (98.26 %) |
| No | 4 (1.13 %) | 349 (98.86 %) |
| **History of surgical intervention (n=468)** | | |
| Yes | 0 (0 %) | 11 (100 %) |
| No | 6 (1.31%) | 451 (98.68 %) |

*FET 10.7, P value 0.029

Two of the six administrative staff whose mobile was contaminated with MRSA were office workers, 5 of them had 10 years or more of work experience in CHS. Among the six administrative staff whose mobile phones were contaminated with MRSA, three of them each had ordinary and smartphone and all MRSA-positive individuals except one had no mobile phone cover (Table 3.15).

**Table 3.15. MRSA contamination level of mobile phone with professional category, service year and mobile types of Administrative staff of CHS, AAU, 2018.**

| Variables | MRSA Positive No. (%) | MRSA Negative No. ( %) |
|---|---|---|
| **Professional category** | | |
| Office workers | 2 (2.70 %) | 72 (97.29 %) |
| Kitchen workers | 1 (1.89 %) | 52 (98.11 %) |
| Guard | 1 (1.12 %) | 88 (98.88 %) |
| Drivers and garage workers | 0 (0 %) | 20 (100 %) |
| Cleaners and laundry workers | 0 (0 %) | 117 (100 %) |
| Administrative and human resource management | 0 (0 %) | 16 (100 %) |
| Finance workers | 1 (3.57%) | 27 (96.43 %) |
| Others | 1 (1.41%) | 70 (98.59 %) |
| **Service year in CHS, AAU *** | | |
| 1-2 years | 1 (0.55%) | 178 (99.44%) |
| 3-4 years | 0 (0 %) | 63 (100 %) |
| 5-7 years | 0 (0 %) | 49 (100 %) |
| 8-10 years | 0 (0 %) | 25 (100 %) |
| More than 10 years | 5 (3.28%) | 147 (96.71%) |
| **Mobile phone types** | | |
| Ordinary | 3 (1.33% ) | 222 (98.67 %) |
| Smart phones | 3 (1.38%) | 213 (98.61%) |
| No phones during survey date | NA 0 ( 0 )%) | NA 27 (100 %) |
| **Mobile phone cover** | | |
| Present | 1 (3.84 %) | 25 (96.15 %) |
| Absent | 5 (1.20%) | 410 (98.79 %) |
| Not applicable | 0 (0 %) | 27 (100 %) |

*FET 18.3; P-value 0.005

### 3.3.3.The burden of MRSA Contamination of HCWs gowns practicing at TASH, CHS,AAU

In the same manner, the level of MRSA contamination level of HCWs gowns was determined and 17 out of 588 (2.89 %) were contaminated. All MRSA contamination is found among the age group of 20- 33 years, slightly higher among female than male HCWs (12 vs 5). No significant difference was seen between sex, age, marital status, educational status, and level of education (P-value > 0.05) (Table 3.16).

**Table 3.16. MRSA contamination level HCWs gowns practicing at TASH, CHS, AAU, 2019.**

| Variables | MRSA Positive No. (%) | MRSA Negative No. ( %) |
|---|---|---|
| **Sex ( n= 580)** | | |
| Male | 5 (2.07 %) | 236 (97.93 %) |
| Female | 12 (3.53 %) | 327 (96.47 %) |
| **Age Group ( n= 574)** | | |
| 20-26 years | 15 (3.40 %) | 426 (96.60 %) |
| 27-33 years | 2 (2.35 %) | 83 (97.65 %) |
| 34- 40 years | 0 (0 %) | 21 (100 %) |
| More than 40 years | 0 (0 %) | 27 (100 %) |
| **Marital status (n= 586)** | | |
| Single | 8 (2.32%) | 336 (97. 68%) |
| Married | 9 (3.79%) | 228 (96.21%) |
| Divorced | 0 (0%) | 5 (100 %) |
| **Professional category )n= (588)** | | |
| Nurse | 4 (2.39 %) | 163 ( 97.61%) |
| Medical doctors | 10 ( 3.46 %) | 279 ( 96.54%) |
| Medical Laboratory personnel | 0 | 36 ( 100 %) |
| Pharmacy personnel | 0 | 29 (100 %) |
| Others | 3 ( 4.47 %) | 64 ( 93.53%) |
| **Educational level (n= 586)** | | |
| Diploma | 0 (0 %) | 6 (100 %) |
| Degree | 12 ( 3.25 %) | 357 ( 96. 75%) |
| Medical doctorate degree | 2 ( 3.57 %) | 54 ( 96.43%) |
| MSc | 0 (0 %) | 39 (100 %) |
| Speciality certificate | 2 ( 1.78 %) | 110 ( 98. 22%) |
| Others | 0 (0 %) | 4 ( 100 %) |

Thirteen out of seventeen (76.47%) gowns contamination found among HCWs who had work experience of 1- 4 years. In the same way, 16 out of 17 MRSA is found among HCWs who had 1-3 gowns, 10 of 17 gowns MRSA contamination was seen among HCWs who change their gowns on weekly basis.

Most of the MRSA contamination was observed in 13 out of 17 (76.47%) mobile phones of HCWs who use their mobile phones during any of their clinical practice. However, none of these differences is statistically significant (P-value > 0.05) (Table 3.17).

**Table 3.17. MRSA contamination level of gowns of HCWs practicing at TASH, CHS, AAU, 2019.**

| Variables | MRSA Positive No. (%) | MRSA Negative No. ( %) |
|---|---|---|
| **Service year ( n= 587)** | | |
| 1-2 years | 6 ( 3.04 %) | 191 ( 96.96 %) |
| 3-4 years | 7 ( 4.43 %) | 151 ( 95.57 %) |
| 5-7 years | 2 ( 1.80 %) | 109 ( 98.20 %) |
| 8-10 years | 0 (0 %) | 45 ( 100 %) |
| More than 10 years | 2 ( 2.63 %) | 74 ( 97.37 %) |
| **No. of gown you have (n=588)** | | |
| One | 3 ( 3.40 %) | 85 ( 96.60%) |
| **Two** | 8 ( 3.46 %) | 223 (96.54%) |
| Three | 5 ( 2.84 %) | 171 (97.16%) |
| Four | 1 ( 1.72 %) | 57 (98.28%) |
| Five | 0 (0 %) | 23 (100 %) |
| Six or more | 0 (0 %) | 12 (100 %) |
| **Types of gown (n= 588)** | | |
| Short sleeves | 8 ( 3.82 %) | 201 (96. 18%) |
| Long sleeves | 9 ( 2.66 %) | 329 (97.34 %) |
| Both | 0 ( 0 %) | 41 (100 %) |
| **Frequency of changing gown ( n= 586)** | | |
| Every day | 0 (0 %) | 11 (100 %) |
| Every other day | 2 ( 1.39 %) | 141 (98. 61%) |
| Weekly | 10 ( 3.54 %) | 272 (96.45%) |
| Every other week | 2 ( 2.02 %) | 97 (97.98%) |
| As required | 1 (1.96 %) | 50 (98.04%) |
| **Type of mobile phone (n= 587)** | | |
| Ordinary mobile phone | 1 ( 1.03 %) | 96 (98.97 %) |
| Smart type mobile phone | 15 ( 3.15 %) | 461 (96.85 %) |
| Both types | 1 ( 7.14 %) | 13 (92.86 %) |
| **Mobile phone cover ( n= 583)** | | |
| Present | 7 ( 2.52 %) | 270 ( 97.48%) |
| Absent | 9 ( 2.94 %) | 297 ( 97.06%) |
| **Do you use mobile phone during clinical practice(n=583)** | | |
| Yes | 13 (3.35 %) | 374 (96.65%) |
| No | 1 (0.90%) | 110 (99.10%) |
| Sometimes | 2 (2.35 %) | 83 (97.65%) |

Mobile phone contamination is higher among HCWs who did not take MRSA training and the availability of sufficient hand hygiene materials. MRSA contamination is higher among those HCWs who had no history of surgical intervention and this is statistically significant (p-value < 0.05) (Table 3.18).

**Table 3.18. MRSA contamination level of gowns of HCWs practicing at TASH, CHS, AAU, 2019.**

| Variables | MRSA +ve No. (%) | MRSA -ve No. ( %) |
|---|---|---|
| **Training on MRSA (n=585)** | | |
| Yes | 2 (2.85 %) | 68 ( 97.15 %) |
| No | 15 (2.91 %) | 500 ( 97.09%) |
| Availability of guidelines & leaflets for MRSA( n=585) | | |
| Present | 2 (2.46 %) | 79 ( 97.54%) |
| Absent | 11( 2.54 %) | 422 ( 97.46%) |
| Do not know | 4 ( 5.63 %) | 67 ( 94.37 %) |
| **Availability of sufficient hand hygiene materials in TASH (n=586)** | | |
| Present | 7 ( 3.68 %) | 183 ( 96. 32%) |
| Absent | 8 ( 2.08%) | 375 ( 97. 92%) |
| Do not know | 1 ( 7.69 %) | 12 ( 92. 31%) |
| **History of hospital admission ever ( n= 587)** | | |
| Yes | 5 ( 5.05%) | 94 ( 94. 95%) |
| No | 12 ( 2.47 %) | 472 ( 97. 43%) |
| Do not know | 0 ( 0 %) | 4 ( 100 %) |
| **History of surgical intervention (Last three years) (n=585)*** | | |
| Yes | 6 ( 8.9%) | 61( 91. 10%) |
| No | 11 ( 2.14 %) | 501 ( 97.86%) |
| Do not know | 0 (0%) | 6 (100 %) |
| **Knowledge level of HCWs ( n= 587 ) **** | | |
| Good | 15 (2.98%) | 488 (97.02 %) |
| Poor | 2 (2.38 %) | 82 (97.62 %) |
| **Attitudes of HCWs towards MRSA control & prevention(n=584)** | | |
| Good | 8 ( 2.51 %) | 310 (97.49%) |
| Poor | 9 ( 3.38 %) | 257 (96.62%) |
| **Practice of HCWs towards MRSA control & prevention(n=582)** | | |
| Good | 8 (2.01 %) | 390 (97.99%) |
| Poor | 9 (4.89 %) | 175 ( 95.11%) |

*$X^2$ 9.9; P value 0.007;**$X^2$ 6.08; P-value 0.048

Moreover, there is no significant difference between the current working department and the source of information for MRSA /*S.aureus* with the level of gowns contamination of HCWs by MRSA (P-value > 0.05).

### 3.4. MRSA burden: Evidence from nasal colonization of Mother-child pair and Diabetic patients (stored samples)

*S.aureus* isolates from the stored samples from the other projects were re-analyzed to know whether the isolates were methicillin-resistant or not, based on MecA detection. Accordingly, 70 stored isolates obtained from nasal swabs of 422 diabetic patients and 212 mother-child pairs (total of 846 swabs ) were retested. Originally in the previous projects, three MRSA isolates were reported from both groups based on the cefoxitin disc test, one from the diabetic patient group and two from the mother-child pairs group. In this Ph.D.work, we have repeated identification and cefoxitin disc tests and there was 100 % concordance in terms of MRSA detection. This results in an overall MRSA colonization rate of 0.35 % (3/ 846). All three isolates were MecA positive. However, one of the *Staphylococcus* isolate was found to be *S.epidermidis* on further identification (MALDI-TOF) methods making the real burden of MRSA 0.24 % (2/846).

Two of the MRSA isolates were from nasal swabs of the mother-child pair group (only mothers were MRSA positive) and the remaining one is from the diabetic patient group (Figure 3.8). The burden of MRSA among diabetic patients and mother-child pairs was 0.236 % (1/422) and 0.235 % (1/424) respectively (Figure 3.7).

The diabetic patient is a 67 years old male patient with type II diabetes with a duration of 19 years. The person has uncontrolled blood sugar and diabetic foot ulcer infection. While the other MRSA nasal colonization is reported from a mother, 36 years with no history of diabetes, renal, or liver diseases, but no MRSA was detected in her child .

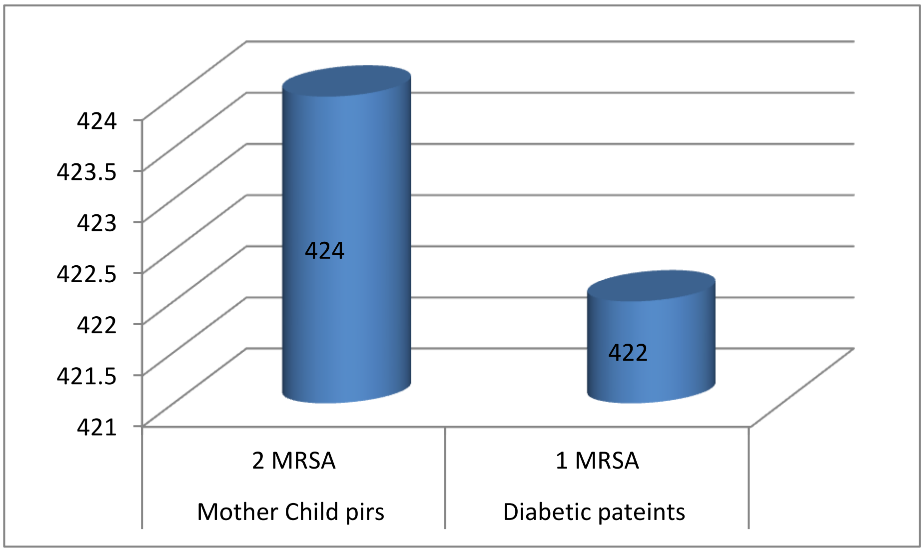


**Figure 3.7. Source of Nasal swabs and MRSA isolation rate among stored isolates of diabetic patients and mother-child pairs in TASH, CHS, AAU, 2019.**

## 3.5. MRSA Burden: Evidence from various clinical isolates in TASH

We have also collected about 100 *S. aureus* clinical isolates at TASH microbiology laboratory and isolates from a diabetic foot ulcer (DFI) infection project (stored isolates) (the Ph.D. candidate supervises the M.Sc. student of this project). The majority of the clinical specimen were wound samples ( 63 isolates), followed by 15 isolates from diabetic foot ulcer, 9 isolates from ear discharge, 3 from urine, and 2 from other body fluids. Accordingly, 92 isolates were found to be *S.aureus* upon further identification, eight were identified as coagulase-negative *Staphylococcus*. Cefoxitin screen also revealed that 23 isolates were found to be MRSA which was later found to be MecA positive using PCR. However, upon further identification with MALDI-TOF, two of these isolates were identified as *S. hemolyticus* and one as *S. epidermidis.* Hence the burden of MRSA from these clinical isolates was found to be 21.73 % (20/92).

The highest proportion of MRSA is observed in diabetic foot ulcer isolates, that is 11 out of 15 isolates were found to be MRSA and only four of them were MSSA, followed by isolates from wound samples (Figure 3.8).

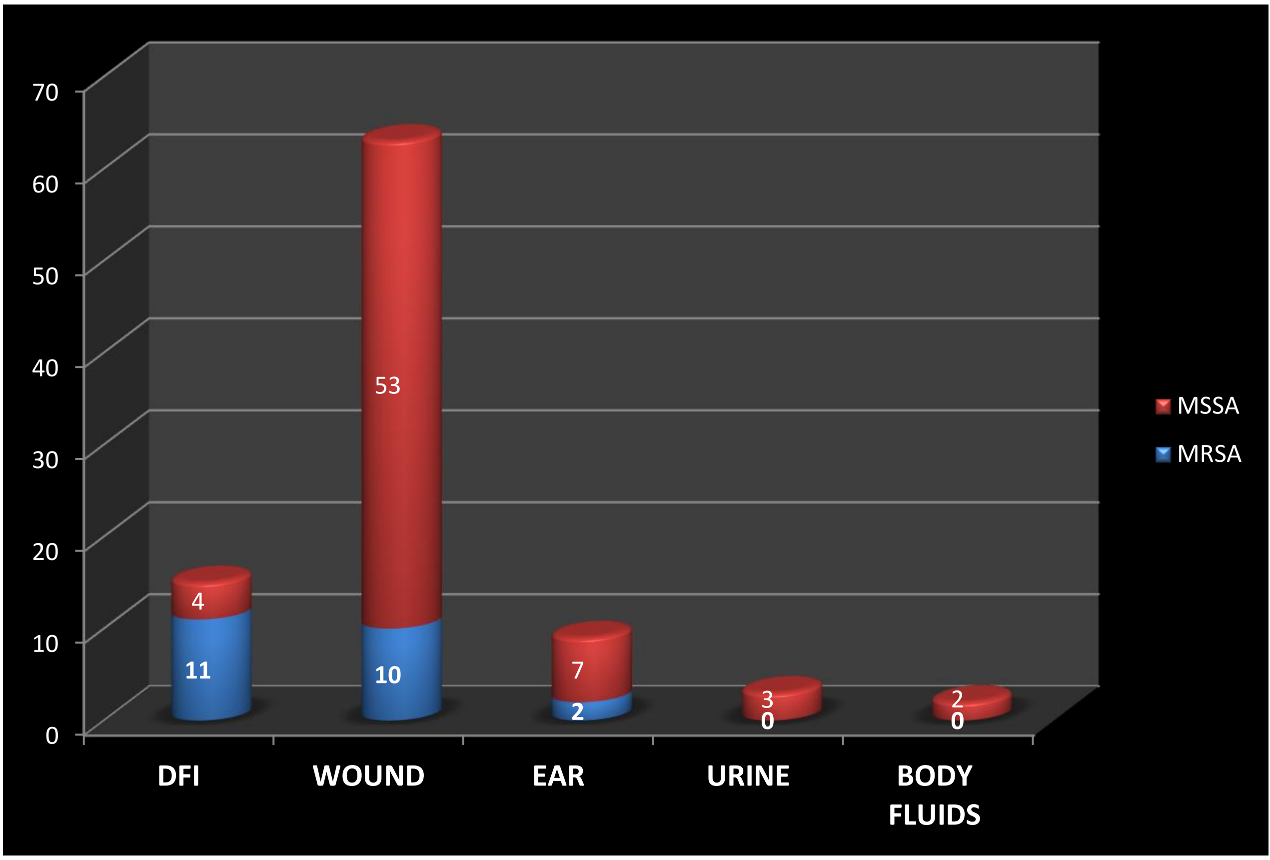


**Figure 3.8.** Proportion of MRSA isolates from the various clinical specimens, at TASH, CHS, AAU,2019.

## 3.6. Spa Typing of *S. aureus* isolates at TASH, CHS, AAU

A total of 307 *S.aureus* isolates were characterized for spa typing both from the isolates from the prospective study and stored isolates following amplification of spa gene ( Figure 3.9) and by analyzing the spa gene sequences ( Figure 3.10).

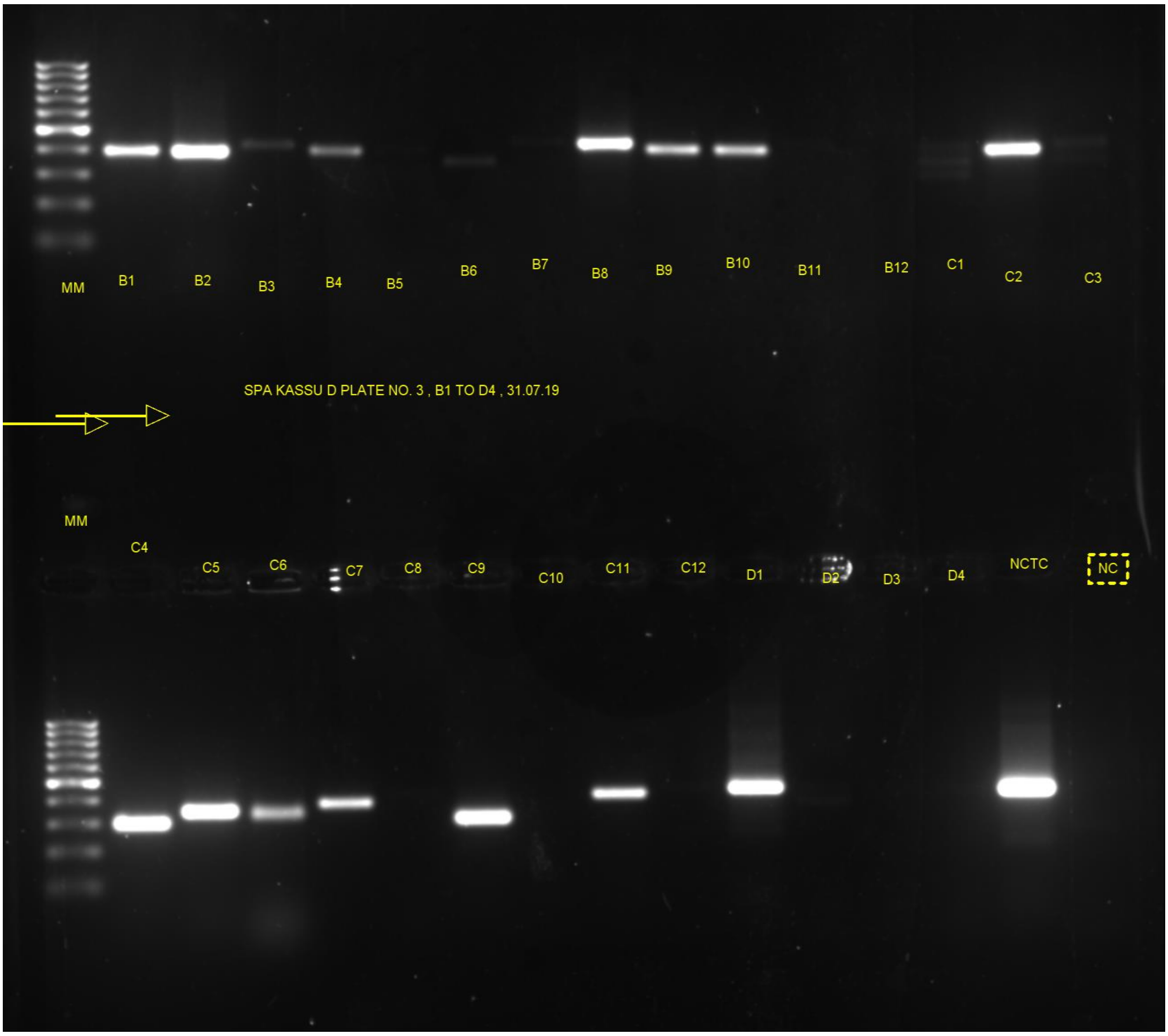


**Figure 3.9.** Spa gene bands after PCR amplification and electrophoresis of the amplified products . Image was taken by BIORAD imaging instrument. MM stands for molecular markers of 100 to 1000 bps, the letters from B 1 to D4 is PCR products for Spa genes of the *S. aureus* isolates, NCTC is a positive control with known Spa type and NC is a negative control where it contains supermix with $DDH_2O$.

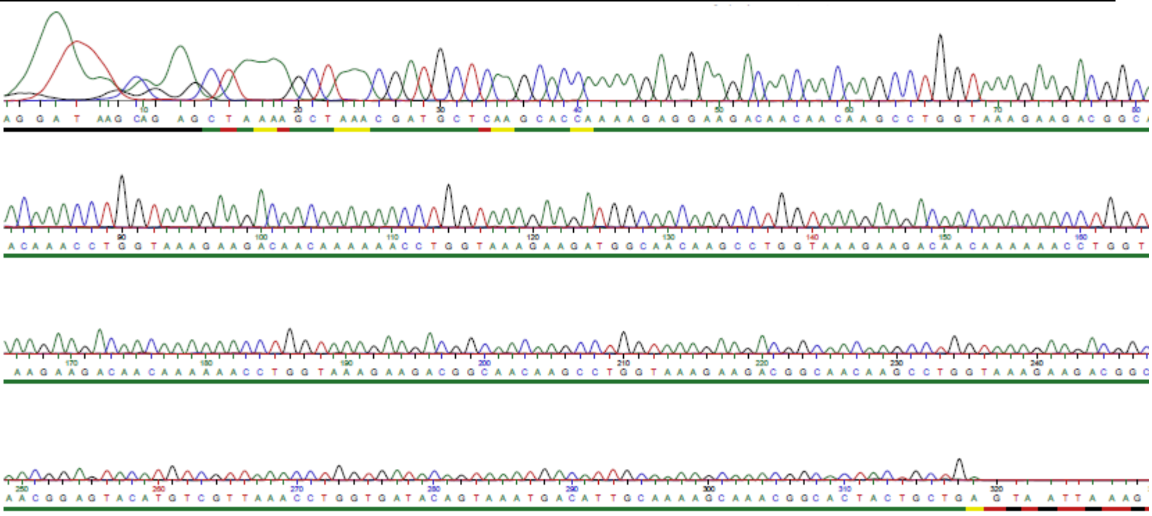

**Figure 3.10 .** Spa sequence obtained from an amplified Spa gene using conventional PCR protocol.

Among 189 *S.aureus* isolates from nasal swabs of HCWs and administrative staff, Mobile phones of HCWs, and administrative staff and gowns of HCWs, 131 spa types were produced based on the spa sequence. Among these, 42 spa types (32.06 %) were detected in two or more frequencies, while 89 of them (67.94 %) observed in singleton. Among 42 spa types again, 20 of them (47.61 %) occurred with a frequency of two, six of them (*t701, t1828, t080, t2235, t2302, and t14805*) occurred in three times each, another six spa types *(t314, t 380, t3841, t11375, t5338 and t14350*) occurred each with a frequency of four. Spa types t062, t318, and t937 happened five times each. Spa type 693 was observed seven times. The most frequent spa types in this study aret355 which are observed 23 times, followed by t223, t085, t131, and t003 which are observed with a frequency of , 20 times, 19 times, 13 times and 12 times respectively ( Table 3.19).

**Table 3.19. Summary of spa types for the various S, aureus isolates at TASH, CHS, AAU,2019**

| Spa type | No. | Spa type | No. | Spa type | No. | Spa type | No. |
|---|---|---|---|---|---|---|---|
| **t003** | **12** | t1511 | 1 | **t306** | **2** | t604 | 1 |
| **t018** | **2** | t152 | 1 | **t309** | **2** | **t605** | **2** |
| t045 | 1 | t15273 | 1 | t311 | 1 | t619 | 1 |
| **t062** | **5** | t15597 | 1 | t314 | 4 | **t6218** | **2** |
| **t064** | **2** | t1564 | 1 | t3160 | 1 | t643 | 1 |
| t065 | 1 | t15778 | 1 | **t318** | **5** | **t645** | **2** |
| **t080** | **3** | t1594 | 1 | t3343 | | t6762 | 1 |
| t084 | 1 | t16342 | 1 | **t355** | **23** | t6827 | 1 |
| **t085** | **19** | t164 | 1 | **t3638** | **2** | **t693** | **7** |
| t103 | 1 | t16489 | 1 | **t380** | **4** | **t701** | **3** |
| t10369 | 1 | t16999 | 1 | **t3841** | | t711 | 1 |
| t10397 | 1 | t1781 | 1 | t4019 | 1 | **t7692** | **2** |
| t1096 | 1 | t17939 | 1 | **t4038** | **2** | t7944 | 1 |
| t11219 | 1 | t18187 | 1 | t4326 | 1 | t7979 | 1 |
| **t11375** | **4** | **t1828** | **3** | t433 | 1 | t8221 | 1 |
| **t11602** | **2** | t18473 | 1 | t434 | 1 | **t8506** | **2** |
| t1172 | 1 | **t186** | **2** | t4393 | 1 | t8529 | 1 |
| **t1234** | **2** | t1916 | 1 | t454 | 1 | t8604 | 1 |
| t12531 | 1 | t2078 | 1 | **t458** | **2** | t8805 | 1 |
| t12913 | 1 | t2091 | 1 | t4822 | 1 | t8807 | 1 |
| t13078 | 1 | t21112 | 1 | t4949 | 1 | t903 | 1 |
| **t131** | **13** | t2163 | 1 | **t5045** | **2** | **t937** | **5** |
| t13166 | 1 | t2216 | 1 | t5224 | 1 | t9411 | 1 |
| t13377 | 1 | **t223** | **20** | t528 | 1 | **t9476** | **2** |
| t1339 | 1 | **t2235** | **3** | t5282 | 1 | t9606 | 1 |
| t13682 | 1 | **t2302** | **3** | **t5338** | **4** | **t995** | **2** |
| t13866 | 1 | t2343 | 1 | t538 | 1 | t604 | 1 |
| t1399 | 1 | t2376 | 1 | t5393 | 1 | | |
| t14193 | 1 | t2383 | 1 | **t5456** | **2** | | |
| t1421 | 1 | t2597 | 1 | t5469 | 1 | | |
| **t14350** | **4** | t272 | 1 | t5569 | 1 | | |
| t1476 | 1 | t2779 | 1 | t5636 | 1 | | |
| **t14805** | **3** | t2845 | 1 | **t5639** | **2** | | |
| t14879 | 1 | t2955 | 1 | t5804 | 1 | | |
| t1509 | 1 | t304 | 1 | t5999 | 1 | | |

From 131 spa types, 28 unique spa types were seen only from HCWs nasal swabs all except one ( t5045) found in single case. Similarly, 14 unique spa types from HCWs gowns, 9 from HCWs mobile phones,13 from nasal swabs of administrative staff, and 6 from administrative staff mobile phones were observed. The remaining spa is found in two or more frequencies at least in a combination of sources mentioned above (HCW nasal, gowns, mobile phone, administrative staff nasal, and mobile phones)

Likewise from 118 isolates from clinical samples (diabetic foot ulcer infection and other clinical sources), isolates from nasal swabs of mother-child pairs and diabetic patients, 74 spa types were deduced. Accordingly, 43 unique spa types were seen among clinical isolates in general. Seven unique spa types **( t11618, t1458, t3342,t5091,t548,t6076**, and **t8614**) seen from nasal swab of baby, six from maternal nasal swabs (t**2383, t3341, t359, t558, t704, t9606**), four from diabetic foot ulcers **(t11375, t1234, t619, t7944)** and only two spa types ( **t1997, t3608)** seen from nasal swabs of diabetic patients. The remaining 31 isolates were found in two or more frequencies.

Spa type t355 are the most frequently seen that that occurred 19 times, followed by t085, which occurred 7 times, t131 and t223 observed 6 times each; spa type t701 occurred 3 times and t062, t064, t084, t14350, t306, t314, t645, and t693 observed 2 times each ( Table 3.20).

**Table 3.20. Spa distribution of stored isolates (Clinical/ Diabetic foot ulcers, nasal swab of diabetic patients and mother-child pair ) of *S.aureus*.**

| Spa type | Frequency | Spa type | Frequency | Spa type | Frequency |
|---|---|---|---|---|---|
| t18187 | 1 | t2779 | 1 | t643 | 1 |
| t045 | 1 | t304 | 1 | t645 | 2 |
| t062 | 2 | t306 | 2 | t6827 | 1 |
| t064 | 2 | t309 | 1 | t693 | 2 |
| t084 | 2 | t314 | 2 | t701 | 3 |
| t085 | 7 | t318 | 1 | t704 | 1 |
| t10369 | 1 | t3341 | 1 | t7944 | 1 |
| t1096 | 1 | t3343 | 1 | t8221 | 1 |
| t11375 | 1 | t355 | 19 | t8506 | 1 |
| t11618 | 1 | t359 | 1 | t8614 | 1 |
| t1234 | 1 | t3608 | 1 | t8805 | 1 |
| t12913 | 1 | t3638 | 1 | t9606 | 1 |
| t131 | 6 | t3841 | 1 | | |
| t1339 | 1 | t019 | 1 | | |
| t13682 | 1 | t4028 | 1 | | |
| t1399 | 1 | t4038 | 1 | | |
| t14350 | 2 | t433 | 1 | | |
| t1458 | 1 | t434 | 1 | | |
| t1476 | 1 | t4393 | 1 | | |
| t1509 | 1 | t454 | 1 | | |
| t1564 | 1 | t458 | 1 | | |
| t5778 | 1 | t4805 | 1 | | |
| t1823 | 1 | t5091 | 1 | | |
| t1828 | 1 | t5338 | 1 | | |
| t186 | 1 | t5393 | 1 | | |
| t1997 | 1 | t5469 | 1 | | |
| t2091 | 1 | t548 | 1 | | |
| t223 | 6 | t605 | 1 | | |
| t2235 | 1 | t6076 | 1 | | |
| t2383 | 1 | t619 | 1 | | |
| t272 | 1 | t6218 | 1 | | |

Many spa types were reported in this study and significant proportion of them were also reported for the frist time in Ethiopia. Among theses, t131

(13x) ,t1234(2x),t318(5x),t6218(2x), t693(7x) ,t7692(2x) ,t8506(2x); t937(5x); t9476(2x) ,t995(2x) are presented from HCWs and administrative staff nasal sabs and mobile phones and gowns sources. While from ; t131( 6x), t14450(2x) and t,693(2x) were identified from clinical sources and nasal swabs of pateints. There are also many spa types which was seen in singleton and resported first time in Ethiopia.

### *3.6.1. S. aureus* Clonal Complex ( CC) deduced from spa software

The spa software can also estimate the clonal complex group of *S.aureus* with particular spa types. Hence 72 spa types data are generated and 12 clonal complexes (CCs) were also deduced (Figure 3.6). CC 15 and CC 22 group is the most frequent clonal complex each accounts for 20 *S. aureus* isolates. Clonal complex 5 (CC5) are the second most abundant clones (Figure 3.11).

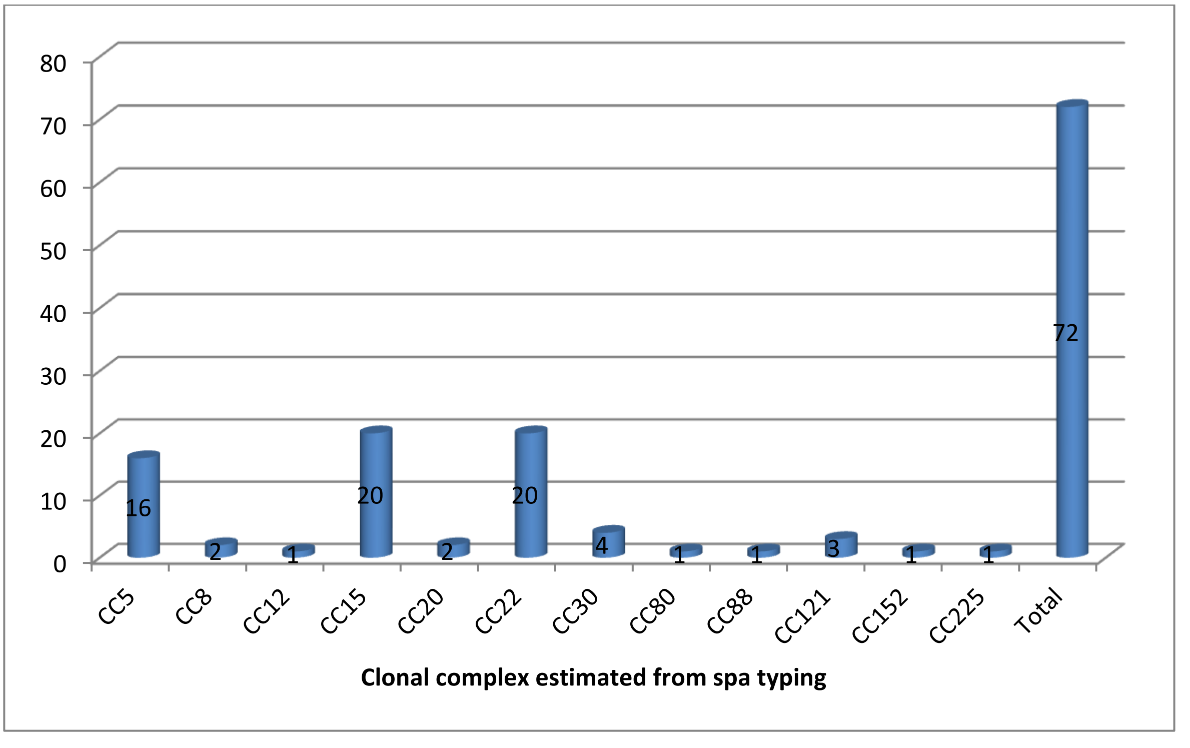


**Figure 3.11. Frequency of Clonal complex of *S.aureus* isolates deduced from spa typing, TASH, CHS, AAU, 2019**

Similarly, Spa CC is were also deduced from 74 spa types observed from various mixed clinical isolates and nasal swabs of mother-child pair and diabetic patients using spa software, and 11 spa CCs were generated the most frequent one is CC 15 observed 9 times, CC22 seen in 7 times, CC5 and CC8 seen 2 times each and CC12, CC30, CC80, CC88, CC 121, CC152 and CC1633 are observed one time each which is more or less similar from spa CC seen from nasal, mobile phones and gown isolates except CC225 is seen only in this group and CC 1633 seen among the clinical isolates.

## 3.7. Panton-Valentine leukocidin (PVL) Status of *S. aureus* Isolates

We have determined whether the isolated *Staphylococcus* strains are carrying PVL gene or not following amplification of pvl gene using the described PCR protocols ( Figure 3.12).

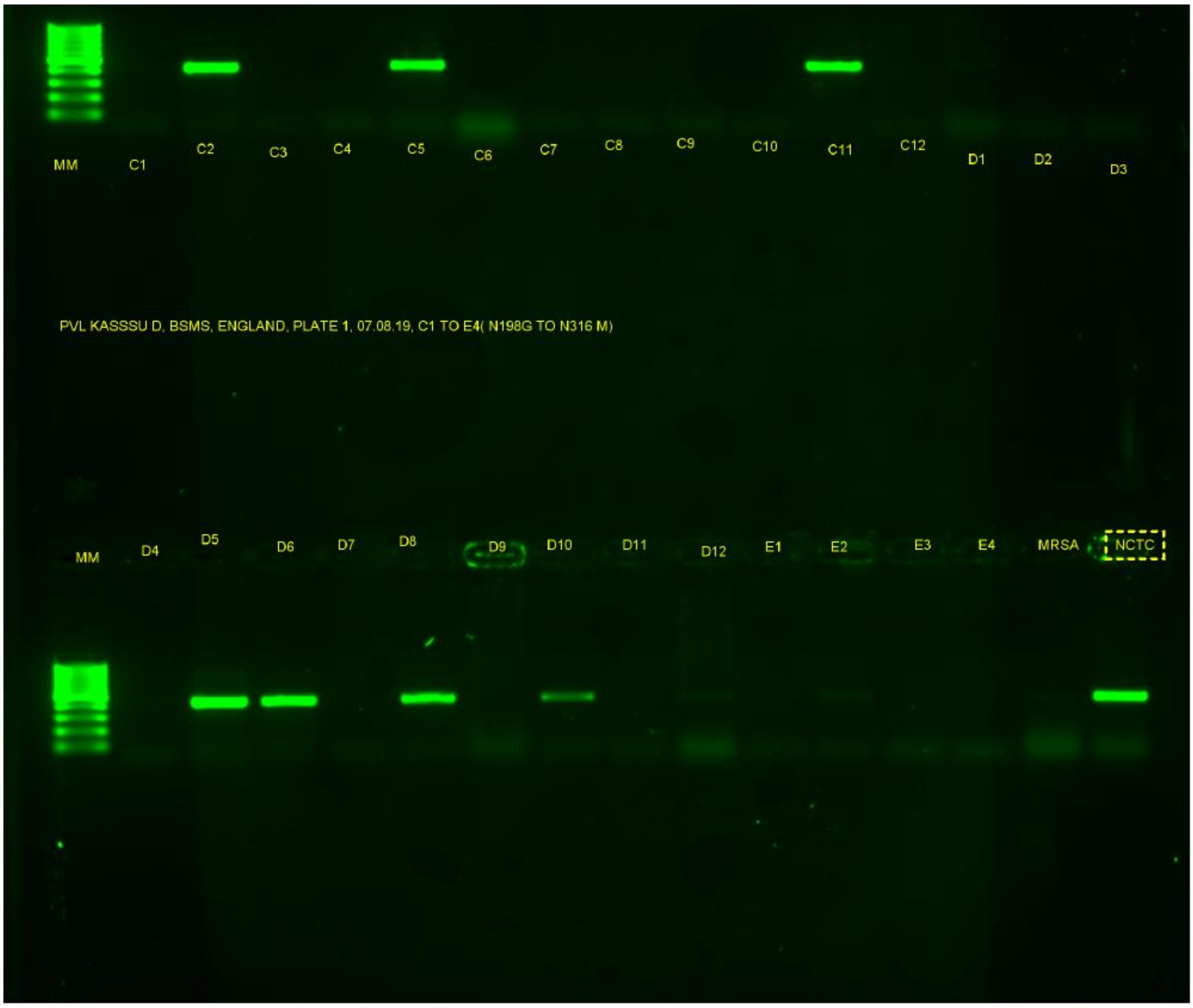


**Figure 3.12.** Gel band image for PCR result of PVL gene. Letter MM is for molecular markers , C1 to E4 is *S.aureus* tested for PVL gene, MRSA is a negative control and NCTC is positive control for pvl .

Accordingly, pvl data were available for 336 strains from different sources including 124 from nasal, 114 clinical, 51 mobile, and 47 from HCW's gowns (Figure 3.13).

One hundred seventy-eight isolates harbour PVL genes accounting for 53 % of the total isolates. The remaining are PVL negative.

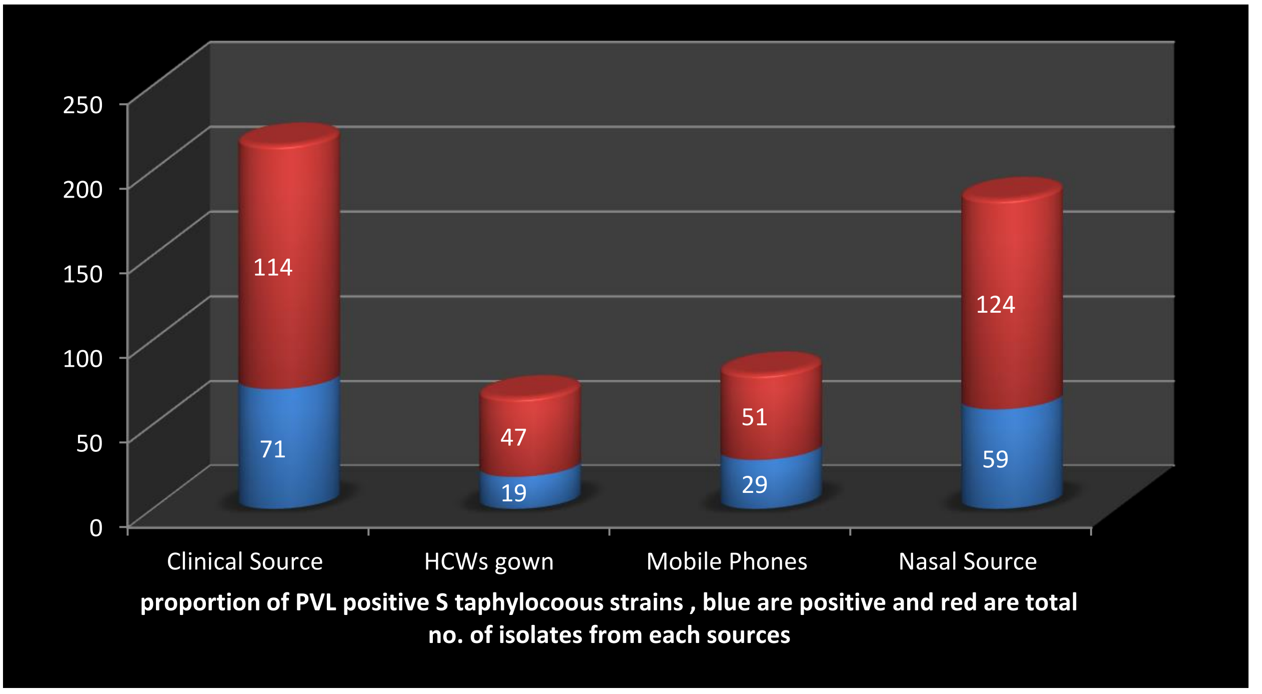


Figure 3.13, PVL status of *Staphylococcus* strains isolated from various sources, TASH, CHS, AAU

Statistically, significant differences have been observed concerning PVL status with the source of *S.aureus* isolates ( P-value < 0.05, data not shown). In addition, 78 strains were MRSA positive, and from this, 57 were PVL positive and out of 258 MSSA, 121 are PVL positive, there is also a significant difference between PVL gene carriage with MRSA status ( P-value < 0.05).SPAtypes, t355, t085, t131, t693, t318, t223 are the most frequent spa types which were PVL positive with PVL ratio of 13/ 23 (56.5%) , 9/19 (47.4 %) , 8/13 (61.5%), 5/7 (71.4 %) , 4/5 (80%), 5/20 (25 %) respectively .

**3.8. Multi Locus Sequence Typing (MLST) of *S. aureus* isolates**

A total of 52 *S. aureus* isolates were analyzed for Multi Locus Sequence Typing (MLST) using amplification and detection of all the seven housekeeping genes (**arcC, aroE, glpF, gmk, pta, tpi, yqi**) including one MRSA control strain (MRSA 258) ( Figure 3.14).

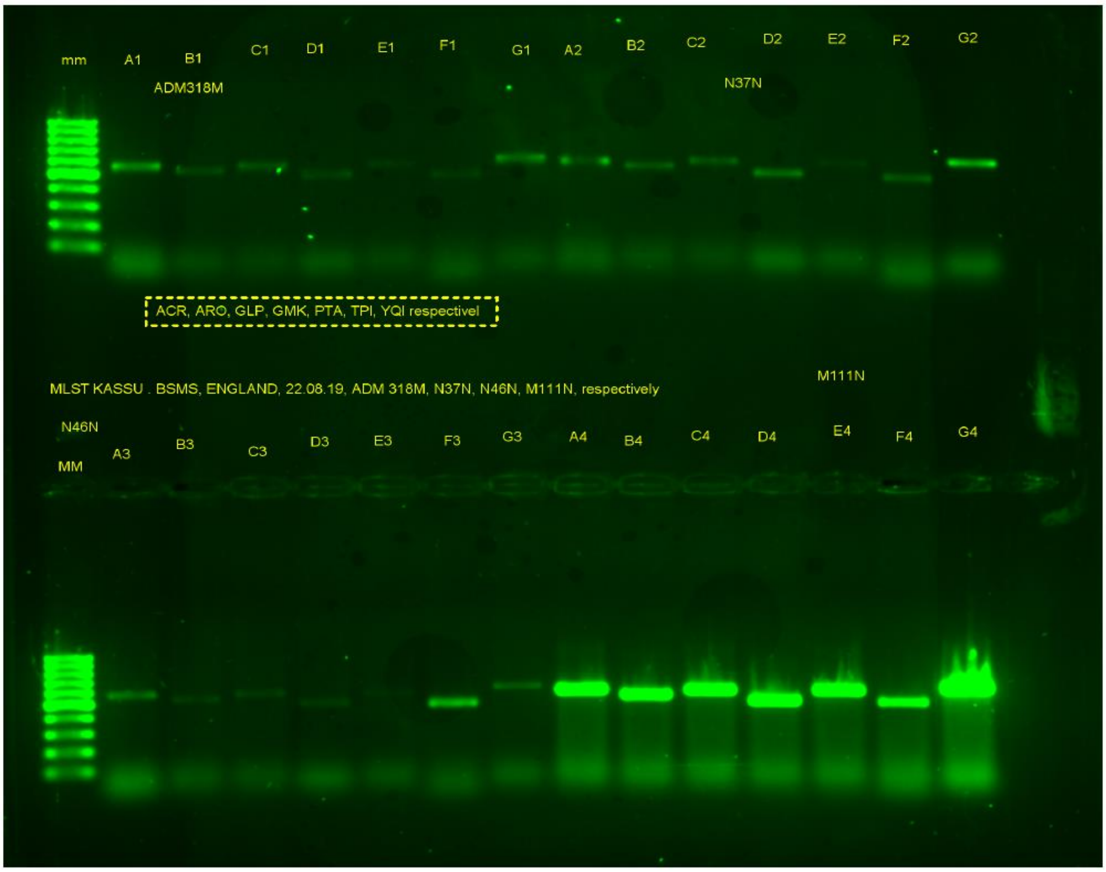


**Figure 3.14.** Representative gel image for the presence of the seven hose keeping genes of *S. aureus*. MM is for molecular marker, A1,B1,C1,D1,E1,F1,G1 is band showing the amplification of arcC, aroE, glpF, gmk, pta, tpi, and yqiL gene for *S.aureus* isolates tested for pvl genes  A2 to G2;, A3 to G3  and A4 to G4 represent one three different isolates for the seven MLST genes

Accordingly, the isolates were grouped into 31 sequence types (ST).  ST 152 is the most dominant ST accounting 20.3 % (11/ 54) followed by ST 4666, ST 5, and ST 744 each accounted 5.55 %  (3/54 for each three sequences) respectively (Table 3.21).

**Table 3.21.MLST Sequence types for the various *S. aureus* isolates at TASH, CHS, AAU, 2019**

| CODE | Source sample | Ssource persons | MRSA | arcC | AroE | glpF | Gmk | Pta | Tpi | Yqi | SST | CC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N174N + | Nasal | HCW | MRSA | 46 | 75 | 6 | 66 | 635 | 240 | 750 | 152 | 0 |
| M139N + | Nasal | HCW | MRSA | 263 | 75 | 49 | 66 | 289 | 170E | 60 | 152 | 0 |
| M11NDN+ | Nasal | HCW | MRSA | EM7 | 6 | 149 | 5 | EM8 | 8 | EM60 | 4225 | 2 |
| M111N | Nasal | HCW | MSSA | EM7 | 6 | EM1 | EM5 | EM8 | 352 | 6 | 22 | 2 |
| M156G | Gown | HCW | MSSA | 1EM | 601 | EM149 | 72 | EM12 | EM45 | EM10 | 744 | 5 |
| M159G | Gown | HCW | MSSA | 27 | 511 | 437 | 70 | 462 | 29 | 11 | 204 | 97 |
| M173G + | Gown | HCW | MRSA | EM1 | EM4 | EM1 | EM4 | EM12 | 495 | 10 | 5 | 5 |
| M106G | Gown | HCW | MSSA | EM13 | 13 | 744 | 354 | 206 | EM29 | 13 | 1886 | 5 |
| Ans 19G | Gown | HCW | MSSA | EM46 | 75 | 49 | 44 | EM13 | EM445 | EM60 | 152 | 0 |
| N157G | Gown | HCW | MSSA | EM6 | 5 | 6 | 281 | EM7 | CM240 | 368 | 51 | 0 |
| N88G | Gown | HCW | MSSA | EM46 | 75 | 49 | 44 | 661 | 349 | 60 | 152 | 0 |
| N295G | Gown | HCW | MSSA | EM1 | 123 | 149 | 23 | 289 | 445 | 11 | 12 | 0 |
| N303G DP | Gown | HCW | MSSA | 90 | 386 | 19 | 288 | 525 | 445 | 32 | 290 | 0 |
| M137M | Mobile | HCW | MSSA | 60 | 601 | EM149 | 72 | 12 | EM1 | EM10 | 744 | 5 |
| M36MDN | Mobile | HCW | MSSA | 584 | 854 | 721 | 48 | 195 | 218 | 37 | 190 | 0 |
| M36M | Mobile | HCW | MSSA | 556 | 75 | 49 | 44 | EM13 | 445 | EM60 | 4666 | 0 |
| M04M | Mobile | HCW | | EM1 | 822 | 391 | 169 | 12 | 445 | 755 | 5 | 5 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | MSSA | | | | | | | | | |
| M144M | Mobile | HCW | MSSA | EM7 | 6 | 149 | 5 | EM8 | EM8 | EM60 | 4225 | 2 |
| M129M | Mobile | HCW | MSSA | CM456 | 601 | 70 | 84 | CM437 | CM349 | EM10 | 874 | 5 |
| ADM318M | Mobile | ADMIN STAFF | MSSA | EM46 | 75 | 49 | 44 | 617 | 170 | EM60 | 5027 | 0 |
| ICL70DN | Clinical | Pateint | MSSA | EM3 | 696 | 149 | 155 | 64 | 497 | 3 | 449 | 8 |
| ICL76DN | Clinical | Pateint | MRSA | 64 | 532 | 149 | 279 | EM64 | EM4 | EM3 | 449 | 8 |
| ICL75DN | Clinical | Pateint | MSSA | 46 | 75 | 49 | 44 | 13 | 68 | EM60 | 152 | 0 |
| ICL72DN | Clinical | Pateint | MSSA | EM46 | 75 | 49 | 44 | EM13 | EM68 | 60 | 152 | 0 |
| ICL51DN | Clinical | Pateint | MSSA | 46 | 75 | 49 | 44 | EM13 | 68 | EM60 | 152 | 0 |
| IL35DN | Clinical | Pateint | MSSA | 698 | 75 | 212 | 44 | 13 | EM68 | EM60 | 1633 | 0 |
| ICL77DN | Clinical | Pateint | MSSA | 46 | 75 | 212 | 44 | EM13 | EM68 | 60 | 1633 | 0 |
| ICL69DN | Clinical | Pateint | MSSA | 606 | 13 | EM149 | 279 | 559 | 11 | 13 | 1972 | 5 |
| ICL69UP | Clinical | Pateint | MSSA | EM13 | EM13 | EM1 | 279 | 559 | 538 | 13 | 4744 | 0 |
| MRSA | Standards train | Standd strain | RSA | 2 | 518 | 2 | 281 | EM3 | EM3 | EM2 | 3674 | 0 |
| DFI27M | DFU | Patient | MRSA | EM46 | 75 | 49 | 44 | EM13 | EM68 | EM60 | 152 | 0 |
| DFI45M | DFU | Patient | MRSA | CM14 | EM1 | 532 | EM10 | EM11 | 51 | EM10 | 80 | 0 |
| DFI102 | DFU | Patient | MRSA | 9 | 1 | 4 | 279 | EM13 | EM5 | 4 | 25 | 97 |
| DFI78 | DFU | Patient | MSSA | 46 | 655 | 6 | 279 | 289 | 240 | 11 | 489 | 0 |
| DFI43M | DFU | Patient | MRSA | EM1 | 532 | 149 | 14 | EM11 | EM51 | EM10 | 80 | 0 |
| 197M | Nasal | Mother | MSSA | 314 | 3 | 299 | 279 | EM12 | EM11 | EM13 | 2434 | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 143MDN | Nasal | Mother | MRSA | EM1 | 601 | 149 | 4 | EM12 | 495 | 10 | 5 | 5 |
| 83B | Nasal | Baby | MSSA | 556 | 5 | 49 | 44 | EM13 | EM68 | EM60 | 4666 | 0 |
| 3M | Nasal | Mother | MSSA | EM3 | 1 | EM149 | 279 | EM1 | EM5 | EM60 | 97 | 97 |
| 30M | Nasal | Mother | MSSA | EM7 | 6 | 722 | 5 | EM8 | EM8 | EM60 | 21 | 2 |
| 139M | Nasal | Mother | MSSA | EM22 | 1 | 14 | 23 | 462 | EM4 | EM31 | 88 | 0 |
| 97M | Nasal | Mother | MSSA | 556 | 75 | 49 | 44 | 13 | EM68 | EM60 | 4666 | 0 |
| 114M | Nasal | Mother | MSSA | 598 | 75 | 49 | CM65 | 13 | EM68 | EM60 | 152 | 0 |
| Y50 | Clinical | Patient | MSSA | 3 | 532 | EM149 | 279 | EM4 | EM4 | 3 | 3781 | 8 |
| Y33 | Clinical | Patient | MSSA | 46 | 75 | 49 | 44 | EM13 | EM1 | EM60 | 152 | 0 |
| Y27+ | Clinical | Patient | MRSA | EM12 | 601 | EM149 | 72 | 12 | EM1 | EM10 | 6 | 5 |
| Y71 + | Clinical | Patient | MRSA | EM4 | 696 | EM149 | 279 | EM11 | 72 | EM11 | 672 | 0 |
| Y35 | Clinical | Patient | MSSA | EM13 | 13 | EM149 | 279 | EM12 | EM11 | EM13 | 1972 | 15 |
| Y73 | Clinical | Patient | MSSA | EM1 | 601 | 49 | 72 | EM12 | EM1 | EM10 | 744 | 5 |
| Y75 | Clinical | Patient | MSSA | EM13 | 3 | 348 | 279 | EM12 | EM11 | 524 | 15 | 5 |
| Y79 | Clinical | Patient | MSSA | EM22 | 1 | 14 | 23 | EM12 | EM4 | EM31 | 88 | 0 |
| 26D + | Clinical | Patient | MRSA | 4 | 75 | 49 | 44 | 289 | EM240 | EM60 | 152 | 0 |
| 224D | Clinical | Patient | MSSA | 435 | 75 | 49 | 4 | M | M283 | M60 | 722 | 0 |
| | | | | | | | | | | | | |

Among the 54 isolates, 24 % (13/ 54) were MRSA and 4 of them belonged to ST 152, two each belong to ST 5 and 80, one MRSA strain is MRSA 258 control strain that belongs to ST 3674. One MRSA strain each belongs to ST 6, ST 449, ST 672, and ST 4225 respectively.

The 54 MLST STs are characterized from 25 clinical isolates, 1 control strain, 9 from isolates of gowns, 7 of them were from isolates from a mobile phone, and 12 nasal swabs of HCWs and Mother-child pair nasal specimens.

There is a marked difference in the STs of the 52 isolates with in source sample. The clinical source consists of 24 strains while nasal, gown, and mobile source includes 12, 9, and 7 strains respectively (Table 3.22).

**Table 3. 22. Summary of MLST sequences according to the source of *S.aureus* , 2019**

| MLST ST summary with source sample, + denote MRSA | | | |
|---|---|---|---|
| **Clinical** | **Nasal** | **Gown** | **Mobile phone** |
| 6+ | 5+ | 5+ | 5 |
| 15 | 21 | 12 | 190 |
| 25+ | 22 | 51 | 744 |
| 80+ | 88 | 152 | 874 |
| 80+ | 97 | 152 | 4225 |
| 88 | 152+ | 204 | 4666 |
| 152 | 152 | 290 | 5027 |
| 152 | 152+ | 744 | |
| 152+ | 2434 | 1886 | |
| 152 | 4225+ | | |
| 152 | 4666 | | |
| 152+ | 4666 | | |
| 449+ | | | |
| 449 | | | |
| 489 | | | |
| 672+ | | | |
| 744 | | | |
| 1633 | | | |
| 1633 | | | |
| 1972 | | | |
| 1972 | | | |
| 3781 | | | |
| 3781 | | | |
| 4744 | | **NB:** + sign indicates the isolates are MRSA | |

Similar to the spa software, the MLST database generated the possible clonal complex of the sequence types (STs). Based on this fact, 24 CCs have been found and thirty of them are not generated for the respective CCs.

Clonal complex 5 (CC5) are the most frequent clones that account for 33.3 % (8/24) of the total CCs produced. CC 15 is the second most prevalent clones and CC 30 occurred the least in a single strain (Figure 3.15).

Seven CCs are MRSA and three of them belong to CC 5 and one each CC belongs to CC 22, CC8, CC 97, and CC30 where all are MRSA strains. Interestingly, all five *S.aureus* strains belongs to CC 15 are MSSA.

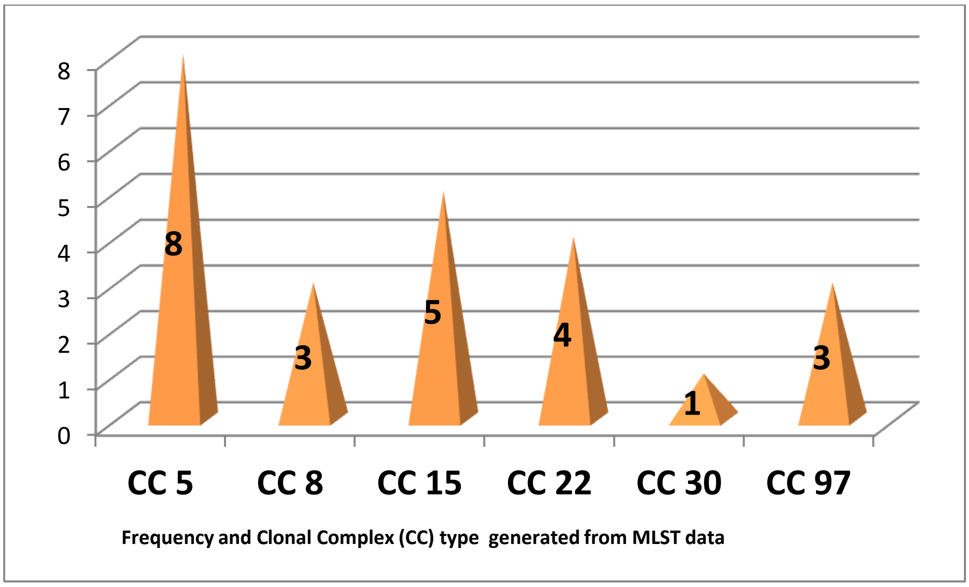


**Figure 3.15.** Clonal complex (CCs) of ***S aureus*** isolates generated from MLST sequence type

Clonal complex 5 comprises 8 sequence types (STs) namely ST5 (4 times), ST6, ST744 (2 times), and ST 874. Among CC5 three of them are MRSA strains isolated from a nasal swab of a mother, one clinical wound sample and more interestingly gown sample from a medical doctor.

CC 15 consists of 5 STs including ST15 itself, ST1886, ST 1972 (2 times), and ST 2434 where all do not contain MRSA strains. The strains are isolated from clinical samples, nasal samples of a mother, and gowns of HCW. CC 22 involves 4 STs ( ST 22, 4225 with frequency of 2 and ST 21 ) .One of ST 4225 contains MRSA strains isolated from the nasal swab of a medical doctor while the other one is MSSA but isolated from the mobile phone of a medical doctor.CC8 includes 3 STs including ST449 that happened 2 times and ST3781. All are isolated from clinical samples and one of the ST449 is MRSA strain. The last CC 97 has also 3 STs comprising ST 97 itself, ST204, and ST 25. Only ST 25 harbour MRSA strain isolated from diabetic foot ulcer sample.

In addition, 7 MRSA strains belong to 3 STs without having CC. ST 152 includes 4 MRSA isolates from two nasal isolates of a nurse and medical doctor, one isolate from diabetic foot ulcers, and one from a wound sample. ST 80 has 2 MRSA strains both were isolated from diabetic foot ulcer patients. While ST 672 has a single MRSA strain from the clinical sample.

The remaining isolates were MSSA that falls with different STs including 7 of them in ST152, 3 of them in ST 4666, 2 of each under ST 1633 and ST88. ST 12,51,190,290,489,3722,4744 and 5027 contains single MSSA strains from nasal, clinical, gowns and mobile phones.

**3.9. Comparison of the MLST allele sequences for up and downstream sequence**

We have compared the upstream and downstream alleles sequences of MLST genes. After PCR product cleaning of each MLST gene, sequencing was done for the two primers separately. Twelve *S. aureus* isolates were compared and 2 out of 12 isolates (16.67 %) have a difference in the overall MLST sequence type while 10 isolates ( 83.33%) have the same sequence types and clonal complex if it exists ( Table 3.23 ).

**Table 3.23.Comparison of allele sequence for down and upstream sequences for *S. aureus* isolates, 2019**

| Code | Source of isolate | MLST Alleles and sequence type / clonal complex | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Arc | AroE | glpF | Gmk | Pta | Tpi | Yqi | ST | CC |
| M173GDN | | 1 | 601 | 149 | 4 | EM12 | 495 | EM10 | 5 | 5 |
| M173G+UP | Gown | EM1 | EM4 | EM1 | EM4 | EM12 | 495 | 10 | 5 | 5 |
| M36MDN | | 584 | 854 | 721 | 48 | 195 | 218 | 37 | 90 | 0 |
| M36M UP | Mobile | 556 | 5 | 49 | 44 | EM13 | 445 | EM60 | 4666 | 0 |
| DFI43MUP | Foot ulcers | EM1 | 532 | 149 | 14 | EM11 | EM51 | EM10 | 80 | 0 |
| DFI43MDN | | 1 | 532 | 287 | 114 | EM11 | EM51 | EM10 | 80 | 0 |
| ICL70UP | | EM3 | EM3 | EM1 | EM1 | EM64 | 497 | 3 | 449 | 8 |
| ICL70DN | Clinical | EM3 | 696 | 149 | 155 | 64 | 497 | 3 | 449 | 8 |
| ICL76UP | | EM3 | EM3 | EM1 | EM1 | EM64 | 497 | 76 | 449 | 8 |
| ICL76DN | Clinical | 64 | 32 | 149 | 279 | EM64 | EM4 | EM3 | 449 | 8 |
| ICL75UP | Clinical | EM46 | EM5 | 49 | EM44 | 13 | 532 | 60 | 152 | |
| ICL75DN | | 46 | 5 | 49 | 44 | 13 | 68 | EM60 | 152 | 0 |
| ICL77UP | | EM46 | 75 | EM212 | 44 | EM13 | 68 | 60 | 1633 | 0 |
| ICL77DN | Clinical | 46 | 75 | 212 | 44 | EM13 | EM68 | 60 | 1633 | 0 |
| ICL72UP | | 46 | 75 | 49 | 44 | EM13 | 68 | 60 | 52 | 0 |
| ICL72DN | Clinical | EM46 | 75 | 49 | 44 | EM13 | EM68 | 60 | 152 | 0 |
| ICL69UP | Clinical | EM13 | EM13 | EM1 | 279 | 559 | 538 | 13 | 4744 | 0 |
| ICL69DN | | 606 | 13 | EM149 | 279 | 559 | 11 | 13 | 1972 | 5 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ICL51UP | Clinical | EM46 | EM75 | EM49 | EM44 | EM13 | 68 | 60 | 152 | 0 |
| ICL51DN | | 46 | 75 | 49 | 44 | EM13 | 68 | EM60 | 152 | 0 |
| ICL35UP | Clinical | 698 | EM75 | EM212 | 4 | 13 | 209 | 60 | 1633 | 0 |
| ICL35DN | | 698 | 75 | 212 | 44 | 13 | EM68 | EM60 | 1633 | 0 |
| 143MUP | Nasal Mother | EM1 | EM4 | EM1 | EM4 | EM12 | 495 | 10 | 5 | 5 |
| 143MDN | | EM1 | 601 | 149 | 4 | EM12 | 495 | 10 | 5 | 5 |

**EM :** Exact match

## 3.10. Evolutionary relationships of *S. aureus* strains using 16S rRNA based phylogenetics

### 3.10.1. Phylogenetic analysis

Evolutionary relationships among *S. aureus* strains were determined using 16S rRNA sequence comparisons, which represented the state of the art for the characterization of prokaryotes. Through the NCBI database, 22 closely related strains with the recommended sequence identity (≥ 98.7%) were retrieved from the BLASTn query. The evolutionary lineage is represented by a consensus tree inferred from 1000 replicates to increase the accuracy and reliability of the results (Fig 3.16). A bootstrapping percentage (BP) for each node was computed from the replicates. The final topology with the superior log likelihood value was selected. To improve visualization of the phylogenetic tree and clustering among lineages, the consensus tree was constructed using an outgroup. The final tree consisted of 41 nucleotide sequences.

Based on the phylogenetic tree, five operational clusters were identified. Cluster A comprised 16S rRNA partial sequences of strain LAH-K8 and LAH-29, isolated from the gown and a nasal swab of a health care worker along with *S. aureus* strain ATCC 12600 and several species of *Staphylococcus* spp. isolated mostly from human skin, nose, and specimens as well as animal skin, nose, and feces. Within the group, the monophylogy of LAH-K29 and *S. simiae* was well supported (BP=36).

Cluster B consisted of most strains identified from the current study along with *S. aureus* subsp. anaerobius and *S. aureus* strain NBRC 100910 previously isolated from an abscess in sheep and pleural fluid of a patient, respectively. The cluster was poorly supported (BP=6). One strain, DF43m-3 isolated from a foot ulcer of a diabetic patient was basally positioned. LAH-K60 and TASH-1 isolated from wound swab constituted cluster C (BP=22). The strains were closely related to cluster B. Partial sequence of LAH-K60 isolated from a nasal swab of a health care worker along with *S. aureus* strain S33R from pleural fluid of a tuberculosis patient were assigned to Cluster D (BP=34). Partial 16S rRNA sequence of LAH-K63 isolated from wound swab was grouped separately as cluster E. Generally, except LAH-K63, most strains were poorly separated in the phylogeny.

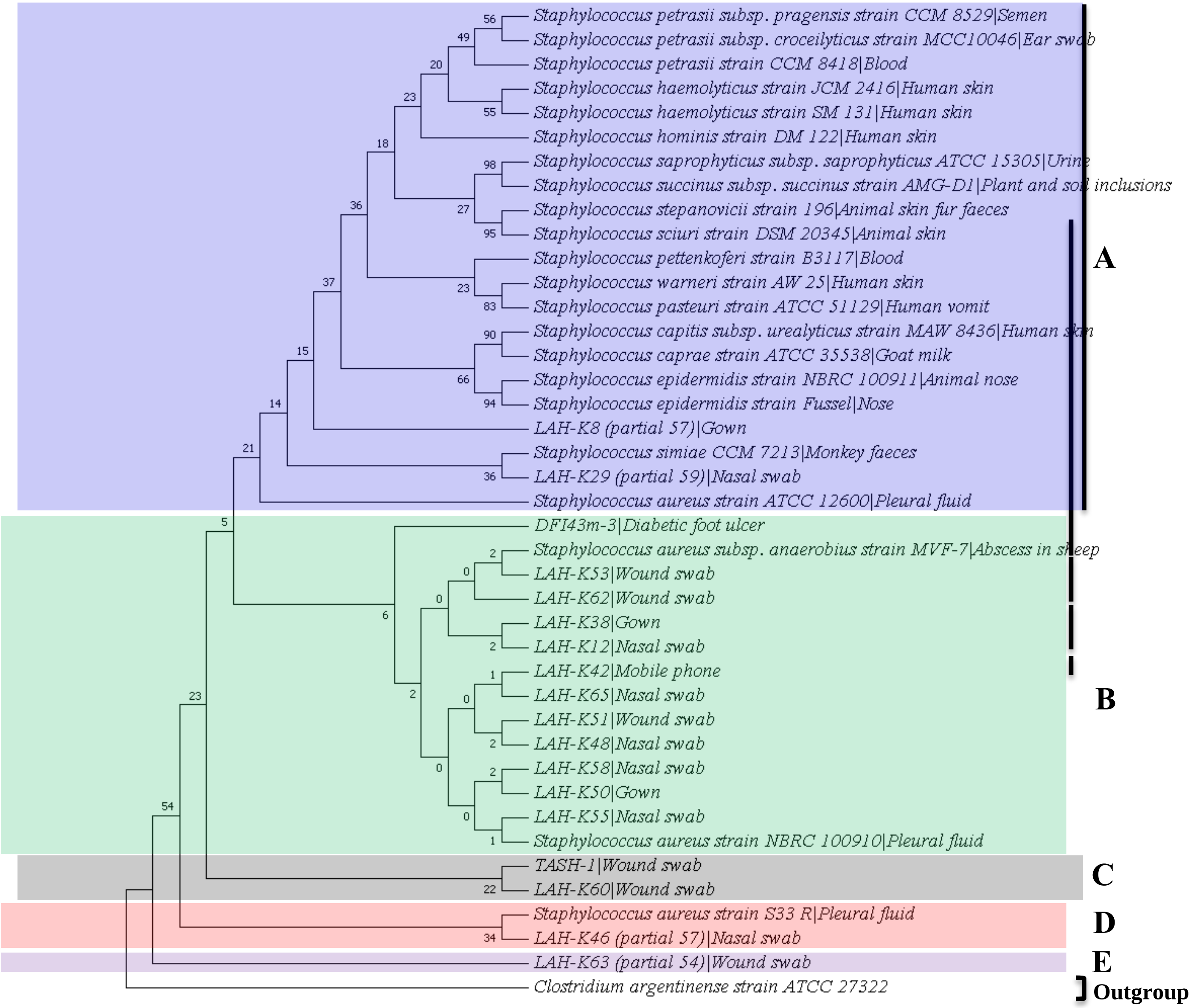


**Figure** 3.16. Evolutionary relationships among *S. aureus* strains that were determined using 16S rRNA sequence comparisons with sequences from NCBI database, 22 closely related strains with the recommended sequence identity (≥ 98.7%) were retrieved from BLASTn query.

### 3.10.2. Comparison of Whole Genome Sequence data (WGS)

Whole-genome comparison was also done using the average nucleotide identity (ANIb %) calculated with JSpeciesWS (Richter et al., 2016).

Accordingly, LAH-K12, K55, and K63 had a high sequence identity (99.9%) with *S. aureus* strain BB155 isolated from the nasal carriage (Table 3.24). Moreover, several showed high similarity with *S. aureus* strains identified from blood and wound infections.

**Table 3.24. Average nucleotide identity of genomes aligned with identified *S. aureus* strains**

| Strain | Aligned *S. aureus* strain | Source | ANIb [%] | Aligned [%] | Accession |
|---|---|---|---|---|---|
| **LAH-K8 NG** | GHA6 | Wound | 99.92 | 96.38 | CP043918.1 |
| **LAH-K12 NN** | BB155 | Nasal carriage | 99.95 | 97.39 | LN854556.1 |
| **LAH-K29 MN** | BB155 | Nasal carriage | 99.97 | 97.46 | LN854556.2 |
| **LAH-K38MG** | FORC_027* | Blood | 99.86 | 94.98 | CP012692.1 |
| **LAH-K42MM** | ITR-R | Blood-endocarditis | 99.92 | 96.51 | CP028470.1 |
| **LAH-K46NN*** | 45 | Blood-bacteremia | 99.87 | 94.46 | CP022718.1 |
| **LAH-K48ADN** | NRS271 | Wound | 99.76 | 96.24 | CP026064.1 |
| **LAH-K50PG** | GHA3 | Skin and soft tissue infections | 97.54 | 93.85 | CP043921.1 |
| **LAH-K51DFI*** | GR2 | Swab-colonization | 99.96 | 96.64 | CP010402.1 |
| **LAH-K53DFI*** | ATCC6538 | Lesion | 99.87 | 95.65 | CP020020.1 |
| **LAH-K55ADN** | BB155 | Nasal carriage | 99.96 | 97.61 | LN854556.1 |
| **LAH-K58ADN*** | NCTC13616* | Blood-septicemia | 99.82 | 97.15 | LR134193.1 |
| **LAH-K60 CL*W** | ATCC12600 | pleural fluid | 99.81 | 92.33 | CP035101.1 |
| **LAH-K62CL** | 55-99-44 | Blood-bacteremia | 99.96 | 96.6 | CP024998.1 |
| **LAH-K63CL** | BB155 | Nasal carriage | 99.9 | 95.89 | LN854556.1 |
| **LAH-K65MN*** | PS00001.3 | Environment | 99.82 | 96.75 | CP030452.1 |
| **TASH-1 CL*** | ST20130941 | hip prosthesis infection | 99.91 | 94.09 | CP012978.1 |
| **DFI43m-3DFI*** | 11819-97 | abscess on lower extremity | 99.93 | 97.04 | CP003194.1 |

*Methillin-resistant *Staphylococcus aureus*

## 3.11. Knowledge, Attitudes, and Practice of HCWs for control and Prevention of MRSA in TASH

### 3.11.1. Knowledge of HCWs towards MRSA prevention and control in TASH

During data collection, we had distributed 600 questionnaires and 586 HCWs responded. Among 586 HCWs in TASH, 58.6 % (343/586) have correctly responded that proper hand hygiene is the most important and effective measure for transmission of MRSA infection. However, only 44.3 % (258 /583) HCWs correctly responded that mupirocin is the available drug for the decolonization of patients with MRSA (Nasal) (Table 3.25).

**Table 3.25 Knowledge of HCWs at TASH towards MRSA prevention and control, 2019**

| Knowledge Question with response | Frequency | Percent |
|---|---|---|
| **1** The most important and effective measure to prevent transmission of MRSA infection is (n=586) | | |
| 1.Use of prophylactic drugs | 36 | 6.1 |
| 2.Environmental Hygiene | 207 | 35.3 |
| 3.Proper Hand hygiene | 343 | 58.6 |
| **2.** What is MRSA ( Methicillin resistant S*taphylococcus aureus*) n=585 | | |
| *A. Staphylococcus aureus s*which is resistant to Methicilin drug alone | 229 | 39.1 |
| B. *Staphylococcus aureus* which is resistant at least to penicillin &cephalosporions groups | 299 | 51.1 |
| *C. Staphylococcus aureus* which is sensitive to all drugs | 57 | 9.8 |
| **3.** MRSA could be transmitted from health care personnel to patients (n=583) | | |
| 1.Correct | 532 | 91.3 |
| 2.Incorrect | 51 | 8.7 |
| **4.** Clean hands protect MRSA related illness in health care institutions (n=587 ) | | |
| A.Correct | 521 | 88.8 |
| B. Incorrect | 66 | 11.2 |
| **5.** Hand washing practice does not help to interrupt transmission of MRSA in hospital set up (n= 584) | | |
| A.Correct | 65 | 11.1 |
| B. Incorrect | 519 | 88.9 |
| **6.** It is not fair to decolonize MRSA carrying patients before Surgical procedures (n= 581) | | |
| A. Correct | 135 | 23.2 |
| B. Incorrect | 446 | 76.8 |
| **7.** Contaminated hands , cell phone and gowns can carry MRSA and or other germs and be transmitted to patients (n= 581) | | |
| A.Correct | 546 | 94 |
| B. Incorrect | 35 | 6.0 |
| **8.** If TASH plan decolonization of patients with MRSA ( Nasal) which drugs is more appropriate (n= 583) | | |
| A.Hot water bath | 238 | 40.3 |
| B. Mupirocin | 258 | 44.3 |
| C.TTC Ointment | 87 | 14.9 |

The average knowledge score of HCWs was calculated from 8 knowledge questions and considering a cut of 60 % and above as having good knowledge. With this regard, more than 85.5 % (503/587) had good knowledge while 14.3 % (84/587) had poor knowledge about MRSA prevention and control.

Our data also showed that there is a significant difference between sex, HCW's professional category, and level of education with overall knowledge score, p-value < 0.05) (Table 3.26 ).

**Table 3.26. Overall Knowledge score of HCWs with Socio-demographic data at TASH, 2019, Addis Ababa, Ethiopia, 2019**

| Variables | Overall Knowledge status of HCWs | | |
|---|---|---|---|
| | **Good knowledge no. ( %)** | **Poor knowledge no. ( %)** | **$X^2$ p value OR ( CI)** |
| **Sex (n= 579)** | | | |
| Male | 222 (38.34 %) | 19 (3.28 %) | 12.2, 0.000, 2.57(1.49-4.43) |
| Female | 277 (47.84 %) | 61 (10.53 %) | |
| **Age group (n=573)** | | | |
| 20-26 Years | 387 (67.53 %) | 54 (9.42 %) | |
| 27-33 Years | 68 ( 11.86 %) | 16 (2.79 %) | 2.96; 0.397 |
| 34-40 Years | 18 (3.14 %) | 3 (0,52 %) | |
| > 41 Years | 24 (4.18 %) | 3 (0.52 %) | |
| **Marital status ( 585)** | | | |
| Married | 297 ( 50.76 %) | 47 (8.03 %) | 0.419; 0,811 |
| Single | 200(34.18 %) | 36 (6.15 %) | |
| Divorced | 4( 0.68 %) | 1 ( 0.17 %) | |
| **Profession (n= 587)** | | | |
| Medical doctor | 161( 27.42 %) | 6 (1.02 %) | |
| Nurse | 230 (39.18 %) | 59 (10.05 %) | 28.29; 0.000 |
| MedicalLaboratory personnel | 29 (4.94 %) | 7( 1.19 %) | |
| Pharmacy personnel | 28 (4.77 %) | 1(0.17 %) | |
| Others | 55 (9.36 %) | 11 (1.87 %) | |
| **Educational category(n=585)** | | | |
| Diploma | 6 ( 1.02 %) | 0 ( 0 %) | 30.05; 0.000 |
| BSc Degree | 293 ( 50.08 %) | 75 (12.82 %) | |
| Medical doctor | 53 (9.05 %) | 3 (0.51 %) | |
| MSc | 36 (6.15 %) | 3 ( 0.51 %) | |
| Specialty certificate | 109 ( 18.63 %) | 3 (0.51 %) | |
| Others | 4 ( 0.68 %) | 0 (0%) | |
| **Work experience (n= 586)** | | | |
| 1-2 Years | 172 ( 29.35 %) | 25 ( 4.26 %) | 4.66; 0.323 |
| 3-4 Years | 134 ( 22.86 %) | 24 ( 4.09 %) | |
| 5-7 Years | 96 ( 16.38 %) | 15 ( 2.55 %) | |
| 8-10 Years | 34 (5.80 %) | 11( 1.87 %) | |
| More than 10 Years | 66 (11.26 %) | 9 ( 1.53 %) | |

In addition, HCWs mobile phone types, use of the mobile phone during actual clinical practice, and frequency of changing gown are significantly associated with the overall knowledge score of HCWs (p-value < 0.05 ( Table 3.27, Appendix XII).

The overall knowledge score of HCWs in TASH is significantly associated with the source of information for MRSA, whether HCWs took MRSA-related training, availability of hand hygiene materials in TASH, and other variables as depicted in the table below ( Table 3.28, Appendix XII), all with a p-value of < 0.05.

There is also a significant difference between the overall knowledge score of HCWs in TASH with a history of surgical intervention for the previous one year and the current working department in TASH p-value < 0.05 (data not shown).

**3.11.2. Attitude of HCWs towards MRSA prevention and control at TASH, 2019**

The majority of HCWs in TASH have agreed generally on the importance of a system-wide approach to preventing MRSA in the hospital (95.2 %, 542/ 582). More than 91.9 % of HCWs (559/ 585) agreed that HCWs can carry MRSA and could transmit the infection to patients. A high number of HCWs in TASH, 28.8 % (168 / 583) agreed that frequent hand hygiene causes irritation of hands and does not halt MRSA transmission (Table 3.29).

Over 72.9 % of HCWs (426/ 584) have agreed ( strongly and agree) that safety measures taken by HCWs can minimize the MRSA rate in a hospital ( n= 426/584). Table 3.29, below shows a summary of the attitude of HCWs towards MRSA prevention and control.

**Table 3.29. Attitude of HCWs towards MRSA prevention and control at TASH, 201 9**

| Attitudes questions | Strongly agree, N, ( %) | Agree N,( %) | Neutral N, (%) | Disagree N, ( %) | Very strongly agree N, (% ) |
|---|---|---|---|---|---|
| 1. System wide approach about MRSA prevention is important in TASH (n= 582) | 438 (75.3 | 104 (17.9) | 13 (2.2) | 14 (2.4) | 13 (2.2) |
| 2. Health care workers can carry MRSA and they could transmit it to patients during handling in the hospitals? (n= 585) | 372 (63.6) | 187 (32.0) | 15 (2.6) | 10 (1.7) | 1 (0.2) |
| 3. Adherence of health care workers on personal protective devices (PPD) is key factors for control of MRSA related illness / infection? (n=581) | 300 (51.6) | 234 (40.3) | 25 (4.3) | 14 (2.4) | 8 (1.4) |
| 4. Use of frequent hand hygiene could causes irritation on hands and does not halt MRSA transmission (n=583) | 71(12.2) | 97(16.6) | 55 (9.4) | 223 ( 38.3) | 137 ( 23.5) |
| 5. All health care workers should be screened for MRSA colonization during outbreak condition (n= 581) | 282 (48.5) | 224 (38.6) | 36 (6.2) | 26 (4.5) | 13 (2.2) |
| 6. Cell phone and gown could carry MRSA and it can be a source of infection (n= 583) | 312 (53.5) | 231 (39.6) | 17(2.9) | 15 (2.6) | 8( 1.4) |
| 7. Health care personnel who is colonized with MRSA should be decolonized before Patient contact (n=584) | 261 (44.7) | 239 (40.9) | 46 (7.9) | 26 (4.5) | 12 ( 2.1) |
| 8. . If health care workers had MRSA in the nares the person can involve in minor Surgical intervention (n= 581) | 81 (13.9) | 210 (36.1) | 109 (18.8) | 115 (19.8) | 66 (11.4) |
| 9 When “you feel ill” , you should not be isolated from work place. (n= 584) | 64 (11.0) | 154 (26.4) | 76 (13) | 174 (29.8) | 116 (19.9) |
| 10. Safety measures taken by health care personnel does not minimize MRSA rate in a hospital ( n= 584) | 44 (7.5) | 89 (15.2) | 25 (4.3) | 194 ( 33.2) | 232 (39.7) |

We have summarized the overall attitudes scores with socio-demographic and other factors in table 3.30, below and 54.68 % of HCWs in TASH have a good attitude. There is a significant difference between overall attitude score and sex, professional category, and educational level (p-value < 0.05) (Table 3.30).

**Table3. 30. Overall attitude score of HCWS with Socio-demographic data at TASH, 2019, Addis Ababa, Ethiopia, 2019**

| Variables | Overall attitude score of HCWs | | |
|---|---|---|---|
| | **Good attitude no. ( %)** | **Poor attitude no. ( %)** | **$X^2$ ; p value** |
| **Sex ( n= 576)** | | | |
| Male | 150 ( 26.04 %) | 89 ( 15.45 %) | 10.74, 0.001 |
| Female | 165 ( 28.64 %) | 172 ( 29.86 %) | |
| **Age group ( 580)** | | | |
| 20-26 Years | 237 ( 40.86 %) | 202 ( 34.82 %) | |
| 27-33 Years | 50 ( 8.62 %) | 33 ( 5.68 %) | 1,79; 0.616 |
| 34-40 Years | 12 ( 2.06 %) | 19 ( 3.27 %) | |
| > 41 Years | 17 ( 2.93 %) | 10 ( 1.72 %) | |
| **Marital status (n= 582)** | | | |
| Married | 191 ( 32.81 %) | 151 ( 25.94 %) | 1.1; 0.575 |
| Single | 123 ( 21.13 %) | 112 ( 19.24 %) | |
| Divorced | 2 ( 0.34 %) | 3 ( 0.51 %) | |
| **Profession (n= 584)** | | | |
| Medical doctor | 119 ( 20.37 %) | 47 ( 8.04 %) | |
| Nurse | 122 ( 20.89 %) | 166 ( 28.42 %) | 42,49; 0.000 |
| Medical Laboratory personnel | 22 ( 3.76 %) | 14 ( 2.39 %) | |
| Pharmacy personnel | 21 ( 3.59 %) | 7 ( 1.19 %) | |
| Others | 34 ( 5.82 %) | 32 ( 5.47 %) | |
| **Educational category (n=582)** | | | |
| Diploma | 5 ( 0.85 %) | 1 ( 0.17 %) | |
| BSc Degree | 164 ( 28.17 %) | 202 ( 34.70 %) | |
| Medical doctor | 39 ( 6.70 %) | 17 ( 2.92 %) | 39.65; 0.000 |
| MSc | 25 ( 4.29 %) | 14 ( 2.40 %) | |
| Specialty certificate | 80 ( 13.74 %) | 31( 5.32 %) | |
| Others | 4 ( 0.68 %) | 0 ( 0%) | |
| **Work experience (n= 583)** | | | |
| 1-2 Years | 105 ( 18.01 %) | 92 ( 15.78 %) | 4.03; 0.401 |
| 3-4 Years | 86 ( 14.75 %) | 71 ( 12.17 %) | |
| 5-7 Years | 58 ( 9.94 %) | 51 ( 8.74 %) | |
| 8-10 Years | 21 ( 3.60 %) | 24 ( 4.11 %) | |
| More than 10 Years | 48 ( 8.23 %) | 27 ( 4.63 %) | |

Nasal colonization of HCW by MRSA was statistically different between attitudes question on whether cell phone and gowns could carry MRSA and could be a source of infection and practice question concerning how frequently wear a mask during caring and examining patients, (P-value < 0.05).

There is also a significant difference between overall attitude scores with cell phone types, cell phone cover, frequency of changing gown, use of the mobile phone during actual clinical practice, p-value,< 0.05 (Table 3.31, Appendix XII).

Similarly, the source of information about MRSA used by HCWs is significantly associated with overall attitude score with Pearson $X^2$ of 30.17 and a p-value of 0.00. Availability of sufficient hand hygiene materials, guidelines, and leaflets about MRSA with a p-value below 0.05 ( Table 3.32 Appendix XII).

Moreover, there is a significant difference between HCW's current working department and overall attitude score about MRSA control and prevention, Pearson Chi-Square value of 19.71 and p-value of 0.049.

### 3.11.3. Practice of HCWs towards MRSA prevention and control at TASH

The overall practice of HCWs is summarized in table 3.33 below. Accordingly, only 17.8 % of HCWs (104/583) wash hands before and after patient contact. Likewise, 53.3 % (310/ 582) HCWs very often and or often wear aprons and gowns during contact with patients. Surprisingly 41.1. % (234/ 581) of HCWs in TASH do not clean their cell phone and or cell phone cover.

**Table 3.33. Practice of HCWs towards MRSA prevention and control at TASH, 2019**

| Practice related questions | Never, n (%) | Seldom n,(%) | Sometimes n, (%) | Often n, (%) | Very often n, (% ) |
|---|---|---|---|---|---|
| 1. Wash hands before and after patient contact (n= 583) | 45 (7.7) | 49 (8.4) | 262 (44.9) | 123 (21.1) | 104 (17.8) |
| 2. Wear aprons and gowns during contacts with patients (n=582). | 82 (14.1) | 51 (8.8) | 139 (23.9) | 150 (25.8) | 160 (27.5) |
| 3. Wear mask during caring / examining patients (n=581) | 115 (19.8) | 114(19.6) | 223 (38.4) | 79 (13.6) | 50 (8.6) |
| 4. Trained about hand hygiene and other personal protective devices (PPDs) methods to prevent MRSA transmission (n=582) | 204 (35.1) | 74 (12.7) | 143 (24.6) | 94 (16.2) | 67 ( 11.5) |
| 5. Clean gowns and other personal protective devices (n=581) | 26 (4.5) | 38 (6.5) | 160 (27.5) | 230 (39.6) | 127 (21.9) |
| 6. Does not clean cell phone and or cell phone cover (n=581) | 154 (26.5) | 85 (14.6) | 218(37.5) | 79 (13.6) | 45 (7.7) |
| 7. Encourage other staffs to adhere on cleaning of gowns and cell phone/cover (n= 581). | 149 (25.6) | 97 (16.7) | 220 (37.9) | 70 (12.0) | 45 (7.7) |
| 8. Consult appropriate personnel about MRSA transmission while you had infection (n=580). | 220 (37.9) | 82 (14.1) | 140 (24.1) | 91 (15.7) | 47 (8.1) |
| 9. Perform / Request regular medical checkup (n= 582) | 254 (43.6) | 82 (14.1) | 163 (28.0) | 58 (10.0) | 25 (4.3) |
| 10. Teach families and other close friends that you might transmit MRSA and other related infection to them (n=581) | 208 (35.8) | 89 (15.3) | 171 (29.4) | 68 (11.7) | 45 (7.7) |

The overall practice score has been summarized by considering 50 % score and above from the total 10 practice-related questions. Therefore, 68.4 % (398/ 582) had good practice while 31.6 % (184/ 582) of HCWs had poor practice about MRSA prevention and control measures in general (Table 3.34).

**Table 3.34. Overall practice of HCWS with Socio-demographic data at TASH, 2019.**

| Variables | Overall practice score of HCWs | | |
|---|---|---|---|
| | **Good practice, n ( %)** | **Poor practicen. (%)** | **$X^2$ p value** |
| **Sex (n= 574)** | | | |
| Male | 153 ( 26.65 %) | 87 ( 15.15 %) | 3.93 0.047 |
| Female | 239 ( 41.63 %) | 95 ( 16.55 %) | |
| **Marital status (n=580)** | | | |
| Single | 231( 39.82 %) | 109 (18.79 %) | 0.29; 0.86 |
| Married | 163( 28.10 %) | 72 (12.41 %) | |
| Divorced | 3 (0.51 %) | 2 (0.34 %) | |
| **Age group( n= 568)** | | | |
| 20-26 Years | 284 ( 50.0 %) | 153 (26.93 %) | |
| 27- 33 Years | 68 (11.97 %) | 15 (2.64 %) | 10.35, 0.016 |
| 34-40 Years | 16 (2.81 %) | 5 (0.88 %) | |
| >/= 41 Years | 20 (3.52 %) | 7 (1.23 %) | |
| **Profession (n= 582)** | | | |
| Medical doctors | 87 (14.94 %) | 79 (13.57 %) | |
| Nurses | 216 ( 37.11 %) | 70 (12.02 %) | 30.23, 0.000 |
| Medical Laboratory Personnel | 29 (4.98 %) | 7 (1.20 %) | |
| Pharmacy personnel | 22 (3.78 %) | 6(1.03 %) | |
| Others | 44 (7.56 %) | 22 (3.78 %) | |
| **Education Category (n=580)** | | | |
| Diploma | 5 (0.86 %) | 1( 0.17%) | |
| Degree | 273 (47.06 %) | 92 (15.86 %) | 26.96, 0.000 |
| Medical Doctor | 27 (4.65 %) | 28 ( 4.82 %) | |
| MSc | 28 ( 4.82 %) | 10 ( 1.72 %) | |
| Specialty certificate | 62 (10.68 %) | 50 (8.62 %) | |
| Others | 2 (0.34 %) | 2 (0.34 %) | |
| **Work experience (n= 581)** | | | |
| 1-2 Years | 136 (23.40 %) | 60 ( 10.32 %) | |
| 3-4 Years | 101 (17.38 %) | 55 (9.46 %) | 5.1; 0.74 |
| 5-7 Years | 70 (12.04 %) | 39 ( 6.71 %) | |
| 8-10 Years | 35 (6.02 %) | 10 ( 1.72 %) | |
| > 10 Years | 56 (9.63 %) | 19 ( 3.27 %) | |

Except for current working departments, others variables are not statistically different from overall practice scores ( Table 3.35, under annex XII) .

Moreover, there is no significant difference between the overall practice of HCWs and MRSA training status, type of mobile phone, presence of mobile phone cover or not, availability of guideline, /leaflets, history of hospital admission, and surgical intervention .

The overall practice level and overall knowledge level of HCWs tabulated and there is no significant difference between knowledge score and overall practice score (P-value > 0.05). However, there is a significant difference concerning a specific question about handwashing practice does not help to interrupt transmission of MRSA in the hospital set up and " If TASH plan decolonization of patients with MRSA ( Nasal) which drugs is more appropriate " response is significantly different with overall practice score ( p value < 0.05).

Cross-tabulation of HCW's overall knowledge score and attitude score showed there is a significant association ( Pearson Chi-Square value of 51.6 , p-value 0.00, OR of 6.9 ; 95 % CI : 3.8-12.4). However, there is no significant association between overall knowledge score and practice score and overall attitude and practice score among HCWs in TASH P-value > 0.05.

# 4.0. CHAPTER FOUR: DISCUSSION

The emergence and dissemination of MRSA across the world underscore that it is one of the public health concerns (Grundmann et al., 2006, Gould , 2013; Muto et al., 2003).

Colonized HCWs with MRSA could play a role in the spread of this bug to patients in the hospital settings and the community at large. HCWs are on the boundary between the community and hospital visitors hence they could be at increased risk of MRSA colonization and or infection (Albrich and Harbarth ., 2008, Sax et al., 2007 ). Accordingly, the carriage rate of MRSA among HCWs, administrative staff, patients, and inanimate objects, the molecular types, and their antibiotic susceptibility patterns, in TASH was determined.

## 4.1. Isolation and Antimicrobial susceptibility of *S. aureus* isolates

We have seen a higher rate of *S.aureus* isolates from nasal swabs of HCWs compared to administrative staff ( 16.32 % versus 8.97 % respectively) and the same pattern for *S.aureus* rate among HCWs mobile phones than administrative staff (6.46 % versus 3.84 % respectively) indicating the HCWs exposure to patients and the hospital environment. Previous studies in Ethiopia had a higher rate of nasal colonization with *S.aureus* 28.8 % and 20.3 % as reported among HCWs in Dessie referral hospital and Mekele hospital respectively ( Shibabaw et al., 2014; Gebreyesus et al., 2013). Another study from Uganda showed 28.8 % *S .aureus* from nasal swabs of HCWs in

Kampala international hospital (Abimana et al., 2019). Like wise in Namazi hospital, Shiraz, Iran *S.aureus* isolation rate among HCWs nasal swab was 28.7 % ( Askarian et al., 2008), and 31 % among 200 HCWs of Al Shifa hospital in Gaza strip ( El Aila et al., 2017). A higher rate of 74.6 % *S.aureus* colonization was reported from HCWs group from Brazilian primary health care units ( Goes et al., 2021). Our *S. aureus* isolation rate from nasal swabs of HCWs is a bit higher than previous Ethiopian study at Adigrat and Wukro hospitals, Tigray that reported an *S aureus* rate of 12 % (29/242) ( Legesse et al., 2018). Such difference in the isolation rate of *S aureus* could be explained by the difference in sample size, in the laboratory methods used for isolation and identification of *S aureus* and the overall context of the study sites.

Overall in, our study 31 strains of *S.aureus* were sensitive to all tested antibiotics and 237 *S.aureus* isolates were resistant to at least one drug tested which is higher than the previous study done in Dessie Referal hospital Ethiopia where all the 34 *S.aureus* isolates were resistant at least to penicillin ( Shibabw et al., 2014). While in terms of multidrug-resistant similar patterns were seen from Dessie referral hospital and Jimma medical students, Ethiopia ( Shibabw et al., 2014; Efa et al., 2019). The current study included a larger *S.aureus* collection mainly from nasal colonization and isolates from mobile phones and gowns of HCWs and administrative staff unlike the two previous studies mentioned which include only nasal swabs that could have contributed to the presence of higher susceptible strains in the current study.

Resistance of the isolates to tetracycline in this study which was 75.2 % was comparable with the Netherlands study where 71 % penicillin resistance was seen among nasal isolates of *S. aureus* from the general practitioners though they did not have MRSA in their report (Rijnders et al., 2010) but lower than the previous study done in Dessie referral hospital that reported 100 % resistant to penicillin ( Shibabaw et al., 2014). This difference could be due to the mix of the source of samples in our study because we included isolates from colonization of HCWs, administrative staff plus from gowns and mobile phones.

Higher resistance rate for penicillin was also reported from nasal isolates of all the 14 MRSA isolates from HCWs of Adigrat and Wukro Hospital Ethiopia that reported 100 % resistance to Penicillin (Legesse et al., 2018), In Southern Brazilian HCW that reported resistance level of 90.6% ( Danelli et al., 2020); in Gaza strip, they reported a 100 % penicillin resistance for all MRSA and MSSA nasal isolates which is a serious challenge for rational antibiotic use ( El Aila et al., 2017) .

Likewise, the rate of MRSA, that is resistant to cefoxitin (oxacillin) in the current study was 28.6 % and this is higher compared to a previous study done in Adigrat and Wukro Hospitals, Ethiopia that reported 5.8 % ( Legesse et al., 2018). On the other hand, Shibabw from Dessie referral hospital reported oxacillin resistance of 12.7 % ( Shibabw 2014). The difference in the number of *S aureus* isolates and antibiotic disc used for MRSA determination could be one factor.

Erythromycin resistance in this study was 15.1 % which is a bit lower than a report from Abrha that report 18.2 % and 18. 7 % for nasal sources from dairy farmers. The difference in the use of erythromycin among humans and animals could result in this difference. Higher resistance of 57 % and 29 % for MRSA and MSSA group were also noted from an Indian study (Agarwal et al., 2015) and erythromycin-resistant rate of 98.7 % from nasal sources and 100 % for hand isolates of HCWs was reported among Nigerian HCWs ( Fadeyi, ., et al 2010) . In Libya Erythromycin resistance of 74 % was reported among HCWs ( *Ahmed et al.,2012)*. Geographical differences and antibiotics use differences in the different nations or settings may bring also a difference in specific antibiotic-resistant patterns.

The resistance rate to clindamycin in this study was 8.8 % (21 isolates) and in the previous study in North Ethiopia higher resistance of 17.2 % for clindamycin was reported ( Legesse et al., 2018). In our study, we have detected a higher level of resistance for clindamycin in isolates from HCW than isolates from administrative staff, indicating that exposure to resistant strains for clindamycin in the hospital setting is higher than in the community. Higher resistance was also reported from a Libyan study (*Ahmed et al., 2012)* with a rate of 20 % and an Indian study where 64 % for MRSA and 15 % for MSSA isolates were also noted (Agarwal et al., 2015) , including astudy from Iran reporting resistant rate of 69 % for clindamycin (Askarian , et al., 2009).

Resistant to tetracycline and trimethoprim-sulfamethoxazole in our study was 62.6 % and 68.9 % respectively which is higher than Legesse ‘s finding which reported tetracycline and Trimethoprim-sulfamethoxazole resistance of 55.2 % and 51.7 % respectively among nasal isolates of HCWs in North Ethiopia and another study from Mekele , North Ethiopia also showed tetracycline-resistance of 86.1 % ( Gebreyesus et al., 2013). Probably the isolates from gowns and mobile phones in our study could contain susceptible strains. Moreover, the presence of *S.aureus* isolates from administrative staff in our case could contribute to the low level of resistance to this drug. In the Tanzanian study trimethoprim-sulphamethoxazole resistant rate of 46.8% were noted for MRSA isolates (Joachim et al., 2019) and in the Indian study tetracycline-resistant rate of 43 % and 23 % for MRSA and MSSA isolates respectively and for Cotriomoxazole resistant rate of 57 % and 29 % for MRSA and MSSA isolates were reported (Agarwal et al., 2015). Higher resistance to cotrimoxazole was also reported from a Nigerian study that reported a 69.9 % rate for nasal sources and 94.2 % for hand isolates ( Fadeyi , et al 2010) , from Libyan study

50 % resistance for trimethoprim sulfamethoxazole, and reports from Nepal, rate of 84.2 % among MRSA group were noted ( Khatri et al., 2017). One study from Jordan reported a resistance rate of 67 % to 100 % (Alzoubi et al., 2020) for various groups of study participants. The difference in source population and sample size among all these studies could also bring the difference in the rate of resistance.

Our MRSA and MSSA isolates were sensitive to rifampicin and all MRSA tested and 10 % of tested MSSA strains were sensitive to vancomycin. Similar findings have been seen from previous reports in Egypt (Salem et al., 2015), in Nepal (Khatri et al., 2017), a study from Iran (Askarian et al., 2009) and isolates from HCWs of Argentina (Boncompain et al., 2017), in southern Brazil, ( Danelli et al., 2020), suggesting these two drugs are still useful and effective for managing *S.aureus* and MRSA related infections. Although the source population is different from the current study, a recent study in North Ethiopia also showed the absence of resistance to Rifampicin and vancomycin (Abrha 2019). Resistance to Rifampin was reported among 50 % of MRSA and 6 % of MSSA in the Indian study (Agarwal et al., 2015). Since rifampicin is not routinely used for the treatment of *S.aureus* infection in Ethiopia, this might be the reason for the sensitivity of all the isolates to this drug. However, resistance to vancomycin have been reported from one study in Mekele, North part of Ethiopia, where out of the isolates from 177 HCWs 36 (20.3%) were MRSA, and 2 (5.6%) of the nasal isolates were vancomycin-resistant ( Gebreyesus et al., 2013) and from a Nigerian study where 1.3 % of the HCWs have resistant strain for vancomycin ( Fadeyi, et al., 2010) and another report of 6 % from Nigeria Hair court teaching hospital staff isolates (Nwokah et al., 2017) and 12 % vancomycin-resistant from a Libyan study ( Ahmed et al., 2012), which is a matter of concern. Probably the use of a disc diffusion test for vancomycin by these studies could be one reason for their findings, which have not been confirmed by other methods such as molecular methods. In the current study, we have used additional methods including PCR.

There is a statistically significant difference between MRSA among administrative staff and HCWs p-value <0.05. Similarly, one out of 60 administrative staff and 20 out of 178 HCWs *S. aureus* isolates have resistance to clindamycin and the difference is statistically significant ( p-value < 0.05) OR 95 % CI , 1.10 (1.04-1.17). Likewise out of 60 *S.aureus* isolates from administrative staff only 9 were resistant to tetracycline compared to 80 of the 178 *S.aureus* sources from HCWs again the difference was statistically significant ( p-value < 0.05, OR 1.54 ,95 % CI: 1.3-1.83). The MRSA burden is also higher for HCWs group than administrative staff. This is expected as the HCWs group are exposed to patients in their day-today activities that increase the higher rate of MRSA and carriage of drug resistance *S .aureus*. Though, our study population is different from a study conducted among the clinical and non-clinical students of Tanzanian university who reported only a single MRSA from the exposed clinical students, which somewhat substantiates our finding ( Okamo et al., 2016).

Fifty-five out of 71 MRSA are tetracycline resistant compared to 34 of 167 MSSA the difference is statistically significant ( p-value <0.05) OR 13.44 ( 95 CI: 6.86-26.33), similar trends were also seen for Trimethoprim-sulfamethoxazole drugs, where out of 71 MRSA 44 of them were resistant to Trimethoprim-sulfamethoxazole compared to 30 MSSA out of 167 and the difference was statistically significant ( p-value <0.05) OR 7.4 ( 95 CI 3.99-13.84).

Resistance to erythromycin and trimethoprim-sulfamethoxazole was common and higher among *S.aureus* isolates from HCWs group than the administrative staffs but the difference is not statistically significant with about 16 out of 60 administrative staff and 58 of the 178 HCWs have resistance to trimethoprim and sulfamethoxazole (P-value > 0.05**) .** Similarly, 9 out of 71 MRSA are resistant to Erythromycin compared to 27 of MSSA and the difference is not statistically significant ( p-value > 0.05) . The absence of MRSA among caretakers compared to HCWs and patients in the Ghanain study also underscore HCWs are more exposed than other groups of people ( Walna et al., 2020). All the aforementioned differences in our study also underscored that HCWs are exposed to more resistant strains compared to administrative staff who had no or very limited contact with patients. A similar finding was also noted among hospital exposed medical students and non-exposed groups in the Indian study ( Sharma et al., 2020).

An interesting finding in the current study was the significant difference between penicillin resistance among MRSA and MSSA isolates. Almost 97.1 % of MRSA were resistant to penicillin while 65.8 % of MSSA strains were resistant to this antibiotic. Similar findings have been observed from one study in India where 100% MRSA were resistant to penicillin compared to 76 % of the MSSA isolates taken from

HCWs nasal swabs. The same trend has been observed for tetracycline and cotrimoxazole in the current study , as well as the study from India (Agarwal et al., 2015). This is expected since MRSA strains are multidrug-resistant and result in a high-level of resistance to other drugs.

There is also a significant difference between penicillin resistance among MRSA and MSSA isolates. Almost 97.1 % of MRSA were resistant to penicillin while 65.8 % of MSSA strains were resistant to this antibiotic. Similar findings have been observed from the study in India where 100% MRSA were resistant to penicillin compared to 76 % of the MSSA isolates from nasal swabs of HCWs. The same trend has been seen for tetracycline and cotrimoxazole in the current study and the study from India (Agarwal et al., 2015).

### 4.2. MRSA Burden

In this study, we determined the burden of MRSA from different groups of participants, that is nasal colonization of HCWs, administrative staff, and patients. We have also determined the MRSA contamination rate of HCWs gowns, mobile phones of HCWs, and administrative staff. Moreover, the proportion of MRSA was estimated from different clinical samples.

Accordingly, the nasal colonization rate of 4.8 % (28 / 580) for MRSA was observed among HCWs of TASH which is similar to MRSA carriage rate of 4.6 %, reported in a meta-analysis of 127 studies around the world involving screening of 33318 HCWs (Albrich and Harbarth, 2008); and another systematic review in Europe and the US during a non-outbreak setting that showed a pooled prevalence of MRSA carriage of 1.8 – 4.4 % (Dulon M et al., 2014). Similar MRSA nasal carriage of 4.6 % is also reported in a non-outbreak setting in nine German acute care hospitals among medical staff in an European region (Sassmannshausen et al., 2016).

The MRSA burden in this study is lower than previous two studies done in Ethiopia, namely HCWs of Dessie referral hospital that reported 12.7 %, (Shibabaw A et al., 2013), 5.8 % in Adigrat and Wukro hospitals, Tigray, Northern Ethiopia (Atsebaha HL et al., 2018),14.1 % among HCWs of Mekelle hospital ( Gebreyesus et al., 2013) and 8.4 % of MRSA carriage among medical students of Jimma University, Ethiopia ( Efa et al., 2019). Recently, Reta and colleagues performed a meta-analysis on nasal carriage of MRSA in Ethiopia and the pooled prevalence of MRSA was 10.9 % (Reta et al., 2019) which is higher than the current report. The the low MRSA rate observed in the current study might be explained by the laboratory methods used for detection.

In the current study, we have used cefoxitin disc diffusion test and Mec A gene detection using PCR, while most of the other studies in Ethiopia have used only oxacillin or cefoxitin disc test alone. In addition, our sample size is relatively larger and includes diverse HCWs.

Previous work in Ethiopia reported that the overall prevalence of nasal carriage of MRSA among hospital exposed and non-exposed janitors in Gondar University was 4.8 % and MRSA carriage was higher among hospital exposed group than non-exposed group (8.1 % versus 1.4 %) (Abie et al., 2020). Likewise 6.25 % MRSA carriage rate was also reported among janitors (cleaners) of Mekelle University, North Ethiopia (Kahsay et al., 2018). In the current study lower rate of MRSA carriage among the administrative staff of TASH/ CHS (0.21 %) was detected. Among 468 administrative staff involved in our study, 117 of them were cleaners and laundry workers. We did not consider janitors as HCWs, though they are working in health care facilities, and included among administrative staff category. The single MRSA carriage observed in our study belongs to cleaners that result in MRSA rate of 0.85 % (1/177). This sample size is lower than from both previous studies mentioned above in Ethiopia and it contributes to the low prevalence of MRSA among cleaners in the current study. More importantly, the cleaner with MRSA carriage in our study was working in the hospital ward which exposes her more for MRSA. Despite they did not use both cefoxitin and mecA gene testing to rule out MRSA.

All the Ethiopian studies on nasal carriage of MRSA reported higher prevalence than ours including MRSA carriage among Medical students of Jimma University (8.4%) , except one study done in preschool children in Debremakos town that reported 0 % MRSA (Reta et al., 2017**)**, (Efa et al., 2019). Most previous studies used the coagulase test alone for identification compared to the current study in which we used a combination of coagulase and or DNAse test which brought a difference in the overall MRSA rate. These observations need further investigation using better microbiological and molecular approaches.

We have seen a discrepancy in *S.aureus* identification in some of our observations that could potentially result in an inflated rate of MRSA in routine clinical microbiology services and or research reports. Similar observation and concern have been reported by Beker and colleagues ( Beker et al., 2006) where co-colonization of

methicillin-resistant coagulase-negative *Staphylococcus* and Methicillin sensitive *S. aureus* which results in a wrong interpretation of the isolate as MRSA. This is much expected in developing nations like ours where the microbiology laboratory infrastructure is not well developed . We feel that there might be over-reporting of MRSA in most of the previous studies done in Ethiopia and other similar settings around the globe. We have seen discrepancy interms of MRSA detection reported by the primary data collector ( the M.Sc. study) and the current study from two MRSA isolates from mother-child pair which tell us the routine MRSA testing could result in exaggeration and as much as possible phenotypic methods have to be supported by molecular methods.

Higher rates of MRSA have been reported by different authors in various parts of the world and in Africa, such as 11.7 % among HCWs in Sudan (BabikerIdris et al., 2017), 13.4 % among HCWs in Uganda using Cefoxitin test and 8.24 % using Mec A detection method ( Abimana JB et al., 2019) ; 15.6 % (59/379) MRSA among HCWs in four tertiary and regional hospitals in Dares Salam, Tanzania (Joachim A et al., 2018), 8.5 % Ghanaian HCWs ( Walana et al., 2020 ), 19 % in Libyan doctors and nurses ( Ahmed et al., 2012) , 34.7 % among HCWs nasal carriage in one hospital of Nigeria (Taiwo et al., 2004), and 52.5 % MRSA nasal and hand carriage among nasal sources (Fadeyi A. et al., 2010) of HCWs working in the ICU of

Nigerian hospital. From other continents, 25 % (51/200) MRSA was reported in Al Shifa hospital in Gaza street using oxacillin disc diffusion test, and the rate was reduced to 20 % when they used MecA gene detection (El Aila et al., 2017); 7.5 % of MRSA from HCWs of tertiary care Hospital in Kathmandu, Nepal ( Khatri et al., 2017), 5.3 % in Namazi hospital Shiraz , Iran (Askarian et al., 2009) , 6.1 % in central Taiwan ( Wu et al., 2019), 32.8 % pooled MRSA prevalence among HCWs of Iran between 2000 to 2016 is also reported (Emaneini et al., 2017 ) and 6.3 % in Argentina (Boncompain et al., 2017 ) and 12.3 % among HCWs and students in Southern Brazil Hospital (Danelli et al., 2020).

The higher MRSA burden of (19 % ) (Ahmed et al., 2012) reported from Libya could be due to the fact that HCWs were included from four hospitals in contrast to ours where we did in one single large tertiary teaching and referral university hospital setting. This by itself could bring a marked difference in MRSA colonization, because it could differ from one Hospital to another. Only nurses and doctors were included in their study, while we have included almost all HCW categories that may not have equal exposure to patients and risky procedures.

A study done among 60 HCWs from burn and surgical critical care units of Menoufia University Hospital, Egypt, MRSA nasal colonization rate of 36.7 % was reported ( Salem et al., 2015), much higher than ours, though our sample size is much higher. The proportion of MRSA among HCWs, patients, and caretakers in Ghana was 8 % ( 4/50), 13.8 % ( 5/36), and 0 % (0/20) respectively (Walana et al., 2020). The difference with our study could be explained by higher sample size we used compared to only 50 participants in their study, which will not give a precise estimate of the burden of MRSA.

Slightly higher MRSA (6 %) is reported among HCWs practicing in long-term care facility settings of Taiwan (Shih et al., 2021). In a French rehabilitation centre, MRSA burden of 10 % (8-10%) has been reported which is more than double from our finding (Legrand et al., 2015). Likewise, MRSA burden of 8.7 %, 7.8 %, and 12.3 % have been reported among HCWs practicing in a tertiary health care hospital in Peru, Taiwan and among HCWs and students attending a University Hospital in Southern Brazil respectively [University Hospital of Universidade Estadual de Londrina ] ( Garcia et al., 2016; Wu et al., 2019; Danelli et al., 2020 ). In Ecuador, nasal and pharyngeal carriage of 6.1 % has been reported among medical students which is higher than our study (Bastidas et al., 2019). The differences with our findings could be due to our use of nasal swabs only, while some of the studies mentioned above have also used both nasal and pharyngeal swabs which have likely increased the higher MRSA isolation including the difference in study settings.

A study from Duhok city, Kurdistan Region of Northern Iraq where HCWs have higher MRSA nasal carriage (13.7 %) compared to non HCWs (4.0%) in the same hospital with a statistically significant difference in carriage rate (Hussein et al., 2017). In our study, similar trends have been observed where HCWs have higher MRSA carriage than administrative staff (4.8 % versus 0.21 %).

On the other hand, our MRSA rate from nasal swabs of HCWs is higher than the MRSA rate of 1.4 % of nasal swabs from dairy workers in Mekele, North Ethiopia (Abrha et al., 2019) , which might indicate the significance of the Hospital environment in nasal colonization with MRSA. No MRSA was isolated among 246 Kenyan HCWs (Omuse et al., 2012) , 0.37 % reported in Turkey (Genc and Arikan 2020) , 0.7 % in Queensland Australia adults study ( Munckhof et al., 2009) ; 2.3 % nasal carriage of MRSA among HCW of Port Hair court hospital in Nigeria (Nwokah et al., 2017). Similarly in Tromso, Norway, the Tromsø Staph and Skin Study comprising 2277 participants with 405 HCWs revealed that no MRSA detection between 2007-2008 (Olsen et al., 2013). A nasal colonization study from 395 general practitioners in the Netherlands also showed the absence of MRSA and 0 % MRSA among 128 medical students in Thailand (Rijnders et al., 2010; Treesirichod et al., 2014). In a German cardiac centre, out of 149 staff that had contact with MRSA patients only one staff had MRSA (0.7 %) (Schubert et al., 2019) . The good infection prevention practice in Europe and involvement of HCWs who had very low or limited contact with patients in the Kenyan and Thailand studies could explain the very low or zero levels of MRSA.

MRSA burden of 1.9 % and 2.9 % is also reported among basic science and clinical students in Jordan university hospital respectively ( Alzoubi et al, 2020). Similarly, MRSA rates of 1.5 % and 0.9 % were found among HCWs and medical students respectively in Madagascar (Hogan et al., 2016). A comparative study in India also showed low MRSA nasal colonization among hospital exposed medical students (0.9 %, 1/107) and 0 % among non-exposed Medical students (Sharma et al., 2020).This further strengthened that hospital exposed individuals be it HCWs and students could harbour MRSA and other medically important pathogens.

In Western Nepal MRSA prevalence of 3.4 % has been reported among HCWs of a tertiary care hospital (Khanal et al., 2015). In our study we did not include medical students of any category that may contribute to the difference with these studies. . Moreover, our sample size is nearly three times more than the Jordanian group and it could contribute to the different burden of MRSA between these studies. Moreover, different host factors, bacterial and environmental factors , antibiotic use, and infection prevention practices among countries and hospitals could also determine the rate of MRSA colonization among HCWs and or patients across the world (Sakr et al., 2018 The difference in MRSA burden among different studies described above is expected as different researchers applied different sampling techniques , isolation, and identification of MRSA, and interpretation of MRSA. Moreover, the infection control practice could differ from hospital to hospital in the same country and between countries which result in different MRSA carriage rate among HCWs ( Vonberg et al., 2006) . The use of oxacillin as a surrogate marker for MRSA is discouraged and evidence showed that the cefoxitin diffusion test is the preferred marker for MRSA (Broekema et al., 2009; Cauwelier et al., 2004; Swenson et al., 2005). Hence, some of the large differences in MRSA observed could be because some of the investigators used oxacillin rather than cefoxitin. It has been also noted that co-colonization of methicillin-resistant Coagulase-negative *Staphylococcus* species could result in false-positive MRSA ( Beker et al., 2006).

In a Tanzanian study 37 (42.5%) HCWs with more than five years of working experience in health care services were colonized with MRSA strains and there was no association between MRSA carriage and gender distribution, ( Joachim et al., 2019). Similar findings have been reported in a Southern Brazilian study for MRSA but a significant difference was observed for *S. aureus* carriage with sex and work category (being male and students respectively ( Danelli et al., 2020). Since the number of female HCWs in TASH is higher and being a nurse could have more contact with patients, and could associated with more exposure, therefore the rate of MRSA is slightly higher than males though the difference is not statistically significant. Concordant reports were also found from a Nigerian study **(**Nwokah et al., 2017) among an equal number of male and females HCWs which could strengthen our hypothesis.

Similar findings were also found in a Madagascar study where, nasal carriage of *S. aureus* and MRSA was more prevalent in women than in men (Hogan et al., 2016). In the Tromsø Staph and Skin Study, where being a woman and those women who are living with children have a higher tendency of *S. aureus* colonization, though no MRSA was reported. (Olsen et al., 2013). On the contrary, another study from southern Brazil reported significantly higher *S.aureus* carriers in males and students but not for MRSA (Danelli et al., 2020)

Many studies indicated that the burden of MRSA is higher among nursing staff than other health workers, in agreement with the current study (Shibabaw et al., 2013,Atsebaha et al., 2018, Gebreyesus et al., 2013, Joachim et al., 2019. Khanal et al., 2015, El Aila et al., 2017, Abimana et al., 2019, Dulon et al., 2014,Askarian , et al., 2009, Agarwal . et al., 2015,). In our case nurses had significantly higher MRSA than other HCWs though, their number is much higher than other HCW practicing in TASH.There is an overall assumption that, nurses and doctors have more and frequent contact with patients and there is a possibility of acquiring MRSA and other pathogens (Fadeyi, et al., 2010). It has been indicated that colonization of both patients and HCWs in hospital settings have been associated with the occurrence of surgical site infection in admitted patients in Tanzania (Moremi et al., 2019, and Price et al. ,2017) verified that out of 25 instances of patient acquisition of *S. aureus*, seven of them were transmitted from HCWs.

HCWs with the age category of 20-26 years have 92.8 % (26/28) of MRSA in the current study which is somewhat similar to the Nigerian study (Nwokah et al., 2017). This might be explained as this age group of HCWs are active and they do have more interaction with patients and other colleagues which tends to expose them to more risk for MRSA colonization. Moreover, as HCWs become being senior and older there might have less contact with patients due to other responsibilities other than patient management. Other study variables like educational, marital, work experience, number, and type of gowns, type of cell phone and presence of mobile phone cover, use of cell phone during clinical practice, history of hospital exposure like admission and surgical procedure are not statistically associated with MRSA burden. A similar finding is also reported from Iran (Askarian et al., 2009).

Interestingly 12/28 MRSA nasal carriage was detected among HCWs who change their gown every other day (Fisher exact test 13.24, P-value $<0.05$) and the contamination level was not also different among HCWs who uses long and short sleeves gowns A recent review by Lena ( Lena et al., 2021) described that MRSA contamination rate is higher among those who are wearing long sleeves than a shorter one. The way gowns are handled, washed, ironed could justify the variation of MRSA contamination in HCWs gowns. Our finding with this regard is a good indicator that gown could be a vehicle for microbes including MRSA that needs serious attention by HCWs themselves and the management of TASH. Some hospitals have guidelines and policies for the use of gowns and other personal protective devices ( FDA ,2015).

MRSA nasal colonization is not linked with a specific department of the HCWs in our study unlike one study conducted in the Gaza Strip that showed, where the majority of MRSA colonization was seen among HCWs who were practicing in the department of surgical wards and internal medicine with a carriage rate of 35 % and 41.5 % respectively (El Aila et al., 2017). Similarly, high MRSA carriage was (36.7 %) observed out of 60 doctors and nurses that are practicing only in the surgical intensive care units of an Egyptian hospital (Salem et al., 2015). In our case, we did not see any significant difference concerning MRSA carriage and the department where the HCW is working. This is also expected as sometimes nurses and residents could work in two or more departments of TASH on a rotation basis.

Surprisingly, no MRSA colonization is seen among medical laboratory personnel in our study. Similar findings were reported among technicians and nursing students at Mayo Institute of Medical Sciences, Barabanki, Uttar Pradesh, India (Agarwal . et al., 2015). On the other hand, 10.5 % MRSA carriage was reported among laboratory personnel working at Kathmandu Medical College and Teaching Hospital, Nepal, and these personnel harbour the highest frequency of MRSA than other HCWs including nurses and doctors (Khatri et al., 2017). Similarly, the study conducted in Argentina showed that the carriage was 30 % for physicians (30 %) and higher for laboratory technicians (57 %) (Boncompain et al., 2017). The possible explanation for such difference could be the geographic difference between ours and the Asian or Latin American settings, and the difference in MRSA burden among countries. Moreover, we used both phenotypic and Mec A gene detection using PCR methods unlike the other studies who used only phenotypic disc diffusion and minimum inhibitory concentration methods that may overestimate their MRSA rate. MRSA nasal colonization has been also reported among the medical laboratory personnel in the previous study done in Ethiopia which used only the disc diffusion method *(Shibabaw* *et al., 2013)*. Further investigations is needed to understand why some profession has high MRSA carriage than others.

To see whether staff other than HCWs has similar nasal colonization, we have recruited administrative staff of the College of Health Sciences. Accordingly, the burden of MRSA was found to be 0.2 % (1/468). This very low nasal colonization of MRSA is in line with some other studies like the one reported in Australia in the Queensland adult community which reported MRSA prevalence of 0.7 % (5/699) (Munckhof et al., 2009). As described above MRSA burden among HCWs in the same compound have higher colonization than the administrative staff. Such difference is expected as HCWs are exposed to patients and the hospital environment that predisposed them to MRSA and other pathogens.

Though the study population is different from our administrative staff previous studies in Ethiopia showed a different prevalence of MRSA. MRSA carriage among food handlers of Gondar University cafeteria, Ethiopia was 9.8 % (4/200). Two of the MRSA carriage was observed among food handlers working in the college of medicine and health sciences while one each from two other campuses (Dagnew et al., 2012). This is higher than our report (0.2 %). Higher prevalence of MRSA was also reported among school children in different parts of Ethiopia, the overall prevalence of MRSA among primary school children and prisoners in Jimma , Southwest Ethiopia was 23.08 % which is higher among prisoners than school children (48 % versus 18.8 % respectively) ( Kejela et al., 2013). Though the proportion of MRSA was calculated among *S.aureus* isolates from school children and prisoners separately, the burden looks exaggerated. If the rate is calculated from the total school children and prisoners the aforementioned prevalence could be reduced. Still, even in that case, their finding is higher than our report. As mentioned earlier, we used both cefoxitin and mec A gene to identify MRSA compared to the other studeis which used only cefoxitin disc diffusion test. That brought change in the rate of MRSA.

Our finding is still lower than a prevalence report among urban and rural school children in Gondar, North West Ethiopia that found MRSA prevalence of 9.79 % which is among urban school children than rural school children (13.3 % and 9.6 % respectively) ( Tigabu et al.,2018). The high prevalence of MRSA could be the different study population and laboratory methods used . A higher MRSA burden, 10 % was also reported among community members in Sudan (20/200) (BabikerIdris , et al., 2017). The small sample size they used and the use of Oxacillin disc to rule out MRSA could contribute to high prevalence in the Sudanese study.

From a previous study, the prevalence of *S. aureus* among pre-School children in Debre Markos town, Ethiopia was 13% (52/400) and no MRSA was detected

indicating that prevalence in children or preschool children could be very low or absent (Reta et al., 2017). Although they used both oxacillin and cefoxitin discs, MRSA was not detected like our report from children of the mother-child pair (Reta et al., 2017). Both ours and their sample size are low and the way nasal swabs were collected, transported and the microbiological processes could also contribute for the absence of MRSA which require further investigation.

On the other hand, a high prevalence of MRSA was reported among healthy children less than 5 years in Eastern Uganda that reported a prevalence of 6.1 % ( 45/ 742) ( Kateete et al., 2019). The use of nasopharyngeal swabs could contribute to favouring the isolation of MRSA/ MSSA over nasal swabs (Hamdan-Partida et al., 2010; Senn et al., 2012). Moreover the large sample sizes they include also have an impact on the high prevalence of MRSA in the Ugandan study. The use of multiple swabs at different body sites of the child has been also used in some investigators and the magnitude of MRSA was higher ( Benito et al., 2015).

A study from Brazil showed that the nasal colonization of MRSA among infants in daycare centers was 1.2 % which is in contrast with the 0 % prevalence among children in our case. (Lamaro-Cardoso et al., 2009). Our sample size is only 212 compared to 1192 of the Brazilian study that contributes to the difference in MRSA burden. Moreover, our mother-child pair were recruited while they are visiting hospitals mainly for vaccination purposes, unlike the Brazilian study which followed 62-daycare centers that further could describe the difference in the MRSA carriage.

MRSA nasal colonization from under five children visiting an outpatient clinic of a Brazilian hospital showed that out of 2034 children only 0.2 % of them have MRSA and 701 neonates admitted in four neonatal ICUs also showed MRSA carriage of 0.6 % (Vieira et al., 2014). This is in contrast with zero MRSA carriage among under-five children from 212 mother-child pair swabs samples in this study. The large sample size used in the Brazilian study , being a daycare center and collection of multiple swabs during admission and discharge could increase the chance of isolating MRSA.

It is known that, among patient groups, diabetes mellitus (diabetics) , HIV-infected people and end stage renal diseases with haemodialysis and patients who have a history of recent hospitalization and antimicrobial consumption have a higher risk of MRSA colonization and or infection (Hidron et al., 2005; Zacharioudakis et al., 2014; Zervou et al., 2014). The magnitude of MRSA among nasal swabs taken from diabetic patients in the current study was also low, 0.236 (1/420). This is much lower than the pooled prevalence of 9.2 % that was generated from 23 data sets comprising 11577 diabetic patients. Obviously, the large sample size they used and the difference in the prevalence of diabetic patients in different countries could be a possible reason for such discrepancy ( Stacey et al., 2019).

A study conducted in Chang Gung University, a hospital in Taiwan comprising diabetic patients with and without foot ulcers revealed that the MRSA nasal carriage rate of MRSA was similar between these two groups of diabetic patients, 5.4% vs. 1.7% for diabetic patients with and without foot ulcer infection (Lin et al., 2020). In another report from the same country, hospital MRSA nasal colonization among 245 type 1 diabetes patients was found to be 5.3 % (13/245) (Kang et al., 2021). Like wise the burden of MRSA among diabetic patients in China was 4.1 % compared to 2.81 % among non diabetic persons ( Lin et al., 2017). The difference in the burden of diabetes in each country, duration of diabetes , presence of other comorbidities among diabetic patients could justify the difference in MRSA nasal colonization with the current and other studies mentioned.

The prevalence of MRSA carriage among admitted patients or patients with different morbidity vary from case to case. For instance MRSA prevalence among HIV patients in Mekele hospital, Northern Ethiopia was 2.4 % (6/249) (Gebremedhin et al., 2016) . MRSA prevalence of 28.9% is reported among nasal swabs in patients at Jimma Hospital, South West Ethiopia (Balta et al., 2003). Likewise, MRSA burden among 204 admitted patients in two regional hospitals in Changhua, Taiwan was 7.8% and patients on haemodialysis have a significantly higher MRSA colonization rate ( Wu et al., 2019).

The diabetic patient with MRSA in the current study was a 67 years old male patient with type II diabetes with a duration of 19 years. The person has uncontrolled blood sugar and diabetic foot ulcer infection, though the sample from the foot ulcer was not analyzed for MRSA. The risk of infection, in general, is higher among diabetic adults receiving insulin than apparently healthy persons (Tamer et al., 2006, Muller et al., 2005). On the other hand, the single MRSA seen from a mother noted that she was a 36-year-old person with no history of diabetes, renal or liver diseases indicating additional risk factors or exposure could also play for MRSA acquisition.

Finally, the burden of MRSA was also estimated for stored isolates collected from several different clinical specimens at TASH. Overall, the MRSA burden among the clinical *S.aureus* isolate was 25 % (23/92). The proportion of MRSA was high among diabetic foot ulcer infections that accounts 73.3 % (11/15) making the actual MRSA burden 13.5 % (11/80), followed by ear discharge that accounts MRSA rate of 22.2 % (2/9) and wound samples have MRSA rate of 15.8 % (15/63). Previous reports in Ethiopia showed relatively comparable findings including on MRSA burden or prevalence among wound samples in a referral hospital that reported 28.3 % (Tsige et al., 2020) . The pooled prevalence of MRSA was found to be 16.78 % (Stacey et al., 2019) where the data is generated from a pool of 10994 diabetic patients with foot infection . The small sample size we used could contribute to the difference in MRSA burden.

Higher MRSA burden was also reported from isolates recovered from pus, wound, blood, sputum and cerebrospinal fluid in two tertiary hospitals, Namazi and Faghihi, in Shiraz ,Iran between December 2017 to September 2018 showed that MRSA prevalence of 31.4 (50) based on disc diffusion and Mec A PCR methods **(**Hashemizadeh et al., 2019) .

The absence of MRSA from ear discharge and urine sample is most probably the small sample size we had, only 3 *S.aureus* isolates from urine and 2 isolates from ear discharge which might have contributed to the absence of MRSA from these specimens**.**

A low prevalence of 1.2 % MRSA was also reported from Gamo rural hospital in Southern Ethiopia (Verdu-Exposito et al., 2020). They reported only one MRSA among 80 *S.aureus* isolates mainly from wound samples. Probably MRSA burden could vary from region to region within the same country. Our isolate collection time was for four months time, unlike the Gamo hospital where the *S.aureus* isolates were collected over five years. The time difference could also create some bias on the burden of MRSA between these two studies.

Uncontrolled use of mobile phones by HCWs may have consequences like the carriage of multidrug-resistant microbes including MRSA, and this will, in turn, have a risk of transmission to patients and the community ( Ramesh et al., 2008, Manning et al., 2013 ; Sondhi et al., 2013; Nerminathan et al., 2017; Al-Harmoosh et al., 2017, Simmonds et al., 2020 ).

In a hospital like TASH, the source of MRSA and other drug-resistant pathogens could be patients, HCWs, and inanimate objects including gowns and mobile phones. With this regard, we have attempted to look at whether the mobile phones of HCWs in TASH harbour MRSA. and whether this incidence is different from non HCWs, and thus included the administrative staff of CHS, including those working at TASH. Accordingly, the MRSA contamination level of HCWs mobile and that of administrative staff was found to be 2.7 % (16/588) and 1.3 % (6/468) respectively. `

The MRSA burden reported here is different from previous work in Ethiopia. For instance, In Jimma University specialized hospital Ethiopia,71.2 % of mobile phones of HCWs and non-HCWs had bacterial contamination and MRSA burden was 9.8 % and interestingly they do have three Vancomycin-resistant *S.aureus* among the MRSA group. The degree of bacterial contamination was higher among health care workers' mobile phones and all the participants do not wash their hands after touching their mobile phones and many drug resistance is reported from HCWs group than non HCWs (Misgana et al., 2015). The finding of a high MRSA mobile phone contamination in Jimma's study could be attributed to use of only disc diffusion test, unlike ours where we have confirmed with MecA gene detection along with disc diffusion method.

A previous study among HCWs in Eastern Ethiopia showed that mostly 94.2 % of mobile phones of HCWs were contaminated CONS, and *S. aureus* and

*Klebsiella* species were the most commonly isolated bacteria ( Bodena et al., 2019). A report from Gondar also showed that out of 50 students and employees mobile phone tested, *E. coli, E. aerogenes, Streptococcus* spp. and *S. aureus* were detected with frequency of 23.53%, 23.53%, 17.65%, and 35.30% respectively (Verma et al., 2015). Very recently a report from Debrebirhan, Ethiopia revealed that among 65 HCWs mobile phones 46.4 % and 53.6 % of mobile phones were contaminated with gram positive and gram negative bacteria respectively most contamination was seen among

HCWs practicing in ICU , surgical and laboratory departments ( Asfaw and Genetu ., 2021) None of these studies mentioned in Ethiopia reported MRSA, probably it might be linked with the small sample size they included and the laboratory methods used could have an impact on the detection of MRSA.

Our MRSA contamination rate is comparable with the MRSA contamination rate of 2.1 % of HCWs mobile phones and lower than 6.5 % from non-healthcare workers mobile phones reported out of 55 participants from Tamil Nadu, India (Anupriya et al., 2018). Our finding correlates with the result from Japan, where among 221nurses that use hospital use only mobile phones used by nurses at Japanese University hospital 2.3 % of mobiles had MRSA contamination (Kanayama et al., 2017), and importantly the isolates from mobile phones and hands of nurses are genetically similar (Kaiki et al., 2020).

A study conducted by Heyba and colleagues revealed that the level of MRSA contamination of mobile phones of clinicians working in the pediatric intensive care and neonatal care units of Kuwait hospital was 1.4 % out of 213 participants ( Heyba et al., 2015). In this study, they found that those clinicians who failed to disinfect their mobile had 2.42 times the risk of contaminating their mobile phones compared to those who disinfect.

Our Mobile phone contamination level is much lower than metadata done by Ulger F and colleagues which reviewed 39 articles published between 2005 and 2013 and they reported that 19 (48.7 %) and 26 (66.7 %) (Ulger et al., 2015) articles identified *Coagulase-negative Staphylococus species* (CONS) and *S.aureus* respectively in the mobile phone of HCWs and the average MRSA contamination level of mobile phones was 17.9 %. This is much higher than MRSA contamination level of 2.7 % (16/588) among mobile phones of HCWs, and 1.3 % (6/468) among administrative staff that is reported in the current study. MRSA contamination level of 4.4 % was reported in the tertiary health care teaching hospital of Uttarakhand state, India (Pal et al., 215).

From our neighbouring countries high MRSA mobile phone and hand contamination was reported from Sudanese study 98 % ( Osman et al., 2018) and evidence from Egypt shows that out of 40 mobile phones of HCWs and patients at the Alexandria University Students' Hospital found that MRSA and CONS prevalence of 53 % and 50 % respectively (Selim , et al., 2015). Out of 21, MRSA detected in the mobile phones, 43.7 % (7/16) were from nurses, 62.5 % (5/8 ) from patients, 71.4 % (5/7) from workers, 60 % (3/5) from laboratory technicians and 25 % (1/4) were from doctors mobile phones. Obviously the small sample size they include in both countries and the inclusion of MRSA from HCWs hand and ear in the Sudan study could inflate the burden of mobile phone contamination. Similar findings were seen in a study in Pune India, out of 30 HCWs hands 24 (80 %) of them contain MRSA, and 30 mobile phones 16 (53.3%) were harbouring MRSA, and application of ethyl alcohol both for hands and mobile phone was 100 % effective (Angadi et al., 2014).

Higher MRSA mobile phone contamination (10 %) was also seen from a study from Japan. Out of 50 hospitals use mobile phones used by doctors (Kaiki et al., 2020), and at the same time use of 222-nm UV disinfection resulted in a reduction in the microbial agents including MRSA is well noted. HCWs could have different knowledge and skill about hand hygiene and infection prevention in general and how frequently they used their mobile phone during clinical practice which could bring a difference in the level of mobile phone contamination. Moreover, sample size difference and difference in overall MRSA burden in the country will bring a difference in MRSA rate of mobile phone contamination.

The MRSA contamination level of HCWs in our finding is twice that of the administrative staff and this is expected where the former have very close contacts with patients and risky environments for microbial exposure. Though the administrative staff is working in the same compounds, very close contact of HCWs with patients and body fluids of the patients could expose them to a higher load of microbes and MRSA in particular.

Many studies include CONS and other gram-negative bacteria from the mobile phones of HCWs . Except for very few mobile phones, the majority of them had growth of CONS which is not reported in this work. Parallel microbiologic analysis of the same specimen of our study, also shows a significant number of HCWs mobile phones at TASH harbored ESBL and other pathogenic gram-negative bacteria (Araya et al., 2021).

In this study, the level of MRSA mobile phone contamination of 2.72 % (16/ 588) among TASH HCWs is somewhat higher than the one reported from the Kuwait study which showed, MRSA contamination level of 1.4 % but comparable with the contamination from administrative staff in our case (Heyba et al., 2015). Similarly, in Pakistan, out of 200 HCWs and 100 university students mobile phones , MRSA was found in 1.6 % of mobile phones of HCWs, and no MRSA was seen among university students (Qadi et al., 2021). Our sample size ( 588 versus 213) is more than double of the Kuwait study and comprises all types of HCWs across our hospital that could explain the high rate of MRSA in our settings. In the Kuwait study, samples from mobile phones were taken from clinicians working in the intensive care units only, unlike ours where we took from all wards of TASH. This might indicate that there might be a difference in the infection prevention measures in the two hospitals. We have seen that a significant number. of HCWs in TASH feel that there are inadequate infection prevention materials in the hospital that could result in a higher rate of MRSA.

Interestingly in the Kuwait study, only 33.5 % of clinicians cleaned their mobile phones regularly and those who fail to clean their mobile phones have a 2.4 times risk of mobile phone contamination (Heyba et al., 2015) and in Saudi Arabia 67.6 % of HCWs did not clean their cell phone **(**Zakaia et al., 2016). Moreover, the majority (12/16) of mobile phone contamination by MRSA seen in our study is among HCWs who have only two gowns and change their gowns on weekly basis. Unexpectedly, all mobile phone contamination was observed among HCWs who do not have MRSA-related training and among HCWs who perceived that there is no guideline or leaflet about MRSA in TASH.

In another study , female sex (OR 0.651, p-value=0.039) and service year (OR 0.468, p-value=0.038) of HCWs were found to be the most significant factors associated with healthcare professionals' mobile phone and bacterial contamination (Araya et al., 2021). This is not true in the current report, specific to MRSA contamination. There was a difference in the number of MRSA and gram-negative bacteria that did not have association with service year and gender.

The 16 HCWs who admitted that they are using their mobile phone during any clinical practice, as expected, had their mobile phones contaminated with MRSA (16/ 588). In a situation where hand hygiene compliance is minimal and the existence of an unhygienic environment including surfaces, equipment, clothing, and possibly infected patients can easily facilitate the contamination of mobile phones of HCWs (Scheithauer et al., 2010; Pittet et al., 2009; Boyce et al., 2007; Loh et al., 2000). Moreover, a previous study in Jimma also showed all HCWs did not wash their hands after using their mobile phone (Misgana, et al., 2015) and from an Eastern Ethiopia study, the absence of regular phone cleaning was associated with mobile phone contamination (Bodena et al., 2019) . Being a male and absence of regular phone cleaning were associated with mobile phone contamination with, CONS (58.8%), *Klebsella* spp 6.9 %, and MDR was 69.9 % (Bodena et al., 2019) that underscores the role of hand hygiene in preventing contamination of mobile phones.

A review work by Olsen and collegues showed that from 56 studies done in 24 countries, revealed that between 2005 to 2019 all studies except 2 (54/56) reported that HCWs and community members' mobile phones harbour bacteria and *S.aureus* , *MRSA*, and CONS are the most dominant bacteria observed and 16 articles showed the presence of fungi on the mobile phone ( Olsen et al., 2020).

In this study, out of 16 MRSA contamination, 7 each was observed among nurses and doctors, one from medical laboratory personnel and other HCW. This underscores that these two groups of HCWs are more exposed to patients that could be infected or colonized with MRSA. A similar explanation is given for the high rate of MRSA in the nasal cavity. It has been said that HCWs especially doctors could use their mobile phone apparatus to take vital signs of patients such as pulse rate in turn their mobile could be contaminated. Moreover, their mobile phone could be a source of infection to other patients (Morris et al., 2012, Amadi et al., 2013). Though we did not record such incidences, we have observed such actions during our data collection time. Sometimes nurses also used their mobile phones as a torchlight while examining admitted patients.

The type of mobile phone apparatus could determine the level of contamination and it has been reported that keypad mobile phones have been linked with increased risk of microbial contamination compared to touch screen phones ( Pal et al., 2013) and others reported smartphones could have a high level of microbial contamination (Lee et al., 2013). In this study, the majority of the HCW's mobile is a touch screen or mobilephone, and a high level of MRSA contamination is seen from this group. While half of the contaminated mobile phones of administrative staff are smartphones, the remaining have ordinary or keypad mobile phones and no significant difference was observed. The small number of keypad-type mobile phones in this study and hygiene measures taken by HCWs and administrative staff could determine the level of contamination besides the type of mobile phone. Concordantly, very high levels of gram-negative ESBL and other gram-negative bacteria also contaminate smartphones ( Araya et al.,, 2021). Whether bacterial strains and nature or type of mobile device is related or not has not been explored well.

The highest MRSA mobile phone contamination in the Indian study was seen among medical students (9%) and hospital doctors and staff (3.5 %) and college faculty and staff ( 5.5%) and only one MRSA is detected from the hands of medical students and no MRSA is seen from the mobile phones and hands of the public group (Pal et al., 215). Unlike this study in our findings, high number of MRSA mobile phone contamination is seen from doctors and nurses who are practicing in the hospital as they do have very close contact with patients. Moreover, the large sample size of our HCWs (580 versus 132) group and the absence of medical students and control group outside TASH could explain why we get higher MRSA mobile phone contamination among nurses and doctors in our case. Interestingly, we have got MRSA mobile phone contamination from the administrative staff of CHS which calls for proper investigation whether they got it from the workplace or the community.

In the Indian study, 76 % of hospital doctors and staffs use their mobile phones while attending patients and only 3 % of them cleaned their mobile phones (Pal et al., 215) which is similar to our findings where 80.96 % (472 / 583) of HCWs use their mobile phones while they are doing different clinical work. Such practice has to be properly reviewed and mechanisms should be set to limit contamination and transmission of MRSA in and outside the hospital settings. Proper hand hygiene practices and decontamination of mobile phones with alcohol wipes have been linked with a reduction of healthcare-associated infection that might be transmitted by mobile phone contamination (Jeske et al., 2007).

In general, there is evidence showing mobile phones of HCWs could serve as a reservoir of microbial pathogens and in the absence of proper hygiene, it plays a pivotal role in the transmission of these dangerous bugs to the hospital community and or the general public (Jayalakshmi et al., 2008; Singh et al., 2010; Ustun et al., 2012). Keeping this in mind we can show that the mobile phone of HCWs of TASH and a few administrative staff harbors important pathogens including MRSA and or ESBL producing gram-negative bacteria and could be a risk for themselves, patients, and their families.

Personal protective device (PPE) are items including gowns, aprons, masks or respirators and goggles which is used by HCWs to create a barrier between the body part and the microbes. They can prevent not only the HCWs but also reduce transmission of the pathogen to susceptible hosts. Hospitals should provide sufficient gowns to their employees and regulate the proper use of PPEs by HCWs in a regular manner (FDA, 2021).

One of the major means of transmission of pathogenic microorganisms is through the interaction of HCWs and patients mainly through hands. However, other means of transmission include hospital environments and HCW's clothing such as gowns and uniforms (Scheithauer et al., 2010; Pittet et al., 2009; Boyce et al., 2007, Pineles et al., 2017).

An observational study in nursing homes of Maryland and Michgan revealed that MRSA contaminated HCWs gowns and gloves has been linked with the transmission. The level of contamination is 14 % for gowns and 24 % for gloves. More importantly, the spa types *S. aureus* isolates from gowns/ gloves and resident isolates were similar in 89 % of the cases, and in 8 % it was closely related. ( Roghmann et al.,2015). This is good evidence that the existence of MRSA on HCWs gowns at TASH could be an additional source of MRSA for the HCWs themselves, patients,colleagues , students, and the society at large and needs timely remedial action.

Keeping this in mind, we determined the level of gown contamination by MRSA at TASH. The overall level of gowns and uniform contamination was found to be 2.89 % (17 / 588) and all MRSA contamination is found among the age group of 20- 33 years, Slightly higher among female than male HCWs (12 vs 5). No significant difference is seen between sex, age, marital status, educational status , and educational level (P value > 0.05).

Slightly more females (12) HCWs gowns are contaminated compared to 5 gowns of male HCWs. About 76.4 % (13/17) gowns contamination was found among HCWs who have work experience of 1- 4 years. In the same way, 16 out of 17 MRSA is found among HCWs who have 1-3 gowns, 10 of 17 gowns MRSA contamination seen among HCWs who change their gowns on weekly basis. More interestingly, 13 out of 17 (76.47%) mobile phone MRSA contaminations are observed among HCWs who use their mobile phone during any of their clinical practice. However, none of these differences is statistically significant (P-value > 0.05). Our finding goes in line with a recent meta-analysis result that includes studies between 2000 to 2020 which showed, the overall MRSA presence in HCWs attire ranged from 1.3 to 79 %. MRSA rate found in 1.3- 14 % of gowns,4-79 % among white coats, 0-19.1 % among scrubs,3-5-19.1 % among studies that consider long and short sleeve uniforms, and 2.5-32 % among studies that consider ties of HCWs (Lena et al., 2021). The contamination of HCWs gowns and uniforms in the current study is underestimated as we took a pooled swab sample of Gowns and uniforms of HCWs mainly from the pocket area and on the sleeves. If we have included samples from attire and other attire in TASH the contamination rate will be higher than what we have reported.

All MRSA gowns contamination was observed among HCWs whose age is between 20-33 years old which indicated that this age group is so young and actively interacting with patients and other colleagues with little infection prevention practice. The presence of MRSA in gowns of healthy university students in Brazil has been noted and 53.3 % of the 300 isolates were MRSA which could also show these age groups are active in their daily activities in the hospital (Batista et al., 2019). In fact , this is higher than our finding as they used oxacilin disc as a marker of MRSA which could exaggerate their findings. Differences in isolation and identification of *S.aureus* in the two countries could also contribute to the change in the level of contamination gowns with MRSA.

Out of 17 gowns, 10 MRSA contamination was seen from nurses and 4 from medical doctors, and the remaining from other HCWs. This could happen as nurses and medical doctors could have more active and repeated interaction with patients and the surrounding environment which leads to their hands and gowns being contaminated. A study conducted, at a tertiary-care center in Maryland ,the United States has been described that transmission of MRSA through gloves and gowns of HCWs seen in 5.4 % of the case during caring patients in the non-intensive care settings and those who care patients very closely (Nadimpalli . et al., 2020).

More importantly, 13 of the 17 gowns contamination were observed from HCWs who have served TASH for 1-4 years, and 16 of the 17 HCWs gowns contamination seen in those who possess 1-3 gowns and change their gown every week. Among HCWs, 92 of them said that they do have four or more gowns and we have seen only single gown contamination of MRSA which underscores that having sufficient and frequent change of gown could minimize the level of gown contamination by MRSA and other pathogens. This information is essential for the hospital administrators and infection prevention committee to review the availability of adequate gowns and develop a guideline on the use of gowns in TASH. It is known that the hands of HCWs have frequent contacts with patients and their clothes including gowns and uniforms which in turn spread the pathogens to themselves, to patients, and their families. This is more pronounced in a situation where hand hygiene adherence is minimal (Scheithauer et al., 2010; Pittet et al.,2009; Loh et al., 2000).

Interestingly, 15 HCWs admitted (two of them said sometimes) that they used their mobile phones during clinical work and it seems there is frequent contact with their mobile phones and gowns, as a result, their mobile phone is contaminated with MRSA. Nine of HCWs gowns have long sleeves and 8 HCWs have short sleeves gowns and their gowns are contaminated with MRSA. There are controversies with the use of short versus long sleeves gowns. Long sleeves gowns may be easily contaminated during clinical practice as it has direct contact with patients and the surrounding environment. However, a randomized controlled trial revealed that both long-sleeved physician white coats and newly laundered standardized short-sleeved uniforms were contaminated within hours of donning and after 8 hours of wearing these clothes, and no significant difference was seen both in terms of bacterial contamination and MRSA contamination ( Burden et al., 2011) .

We have collected swabs samples from HCWs including gown samples at any time of the regular working hours mostly and sometimes on weekends and during evening time , and as a result, we can not comment on the peak time for contamination of HCW's gowns and this has to be further investigated in future works.

The use of personal protective equipment including gowns for the prevention of infection in hospital settings is a well-known fact and HCWs are using it in their routine work moreover, the frequency of hand hygiene and its compliance is linked with reduction of infection in the hospital(CDC,2019, WHO, 2009, WHO,2020 Wilson et al., 2019). With this regard a significant number of HCWs in TASH ( N=88, 15%) have only a single gown, this is a concern from an infection prevention point of view and staff should have a fairly adequate number of gowns so that they could take hygiene measures as much they can. Interestingly, during data collection, we have observed that there are instances where HCWs washed their gowns and uniforms in the hospital by themselves or they wash their gowns and uniforms at home. This calls the hospital management to avail laundry service at the hospital settings as it will minimize transmission of MRSA and other important pathogens to their family members and the public at large. If HCWs are taking their clothes home it means that they could import and or export these pathogens to the hospital or family members.

HCW's gowns contamination is higher among those who did not take MRSA training and said guidelines and leaflet about MRSA are absent in the hospital. More than 50 % MRSA gown contamination is seen among HCWs who feel that there are inadequate hand hygiene materials in TASH. This highlights that in the absence of hand hygiene materials it is hard to control MRSA and other microbes in hospital settings. Although we did not measure the hand hygiene compliance in this study, previous studies in other hospitals in Ethiopia and other developing countries hand hygiene compliance is very minimal (Engdaw et al., 2019, Bayleyegn et al., 2021; Eljedi et al., 2014; Daniel and Mir ,2015 ).

In this study, 12 of the 17 gown, MRSA contamination was seen among HCWs who do not have previous hospital admission. While there is a significant difference between MRSA gown contamination concerning the history of surgical intervention in the past year (P < 0.05). Likewise, there is a statistically significant difference between the knowledge level of HCWs with the level of gowns contamination (p-value <0.05) but not with that of attitude and practice of HCWs. Unfortunately having good knowledge does not mean that they do have a positive attitude and practice. How HCWs gowns are washed, ironed, and strictly used only in the patient-specific area have been mentioned a determinant factors for the level of gown contamination and transmission of MRSA and other microbes in the hospital (Pilonetto et al., 2004; Uneke et al., 2010; Gaspard et al., 2009).

Unlike MRSA nasal colonization which happened more among nurses than other health cadres, no significant difference was seen between the current working department and source of information about MRSA with the level of gowns contamination (P-value > 0.05; data not shown). Other behavioural factors could be involved in the occurrence of high MRSA in HCWs gown across the department. In a simulation model, Harris and colleagues showed that reduction of MRSA acquisition is seen due to the barrier effects of gowns and gloves, along with improved hand hygiene and reduction of HCW patient contact rates as observed in 20 US intensive care units ( Harris , et al., 2017).

### 4.3. PVL genes

Among the versatile virulence factors produced by *S. aureus* are Panton-Valentine leukocidin (PVL), which is a cytotoxin that forms pores in the membrane, and has been associated with several skin infections and diseases severity (Labandeira-Rey et al., 2007, Etienne ,2005; del Giudice , et al., 2009; ). In this study, from a diverse strain collection, a high level of pvl gene is recorded (48.63 %; 178/366) both from MSSA and MRSA isolates and with significant difference between the two groups (P-value < 0.05). A previous study in Ethiopia reported pvl positivity rate of 72.84 % from *S.aureus* isolates that were collected from clinical sources of Gamo rural hospital, South Ethiopia (Verdu´-Expo´sito et al., 2020). However, our isolates are large and composed of both clinical and non-clinical sources hence our pvl rate is lower. Moreover, they have collected their isolates over five years unlike ours, which is a cross-sectional study.

Though there is a difference in the source of *S. aureus* isolates with Abrha study in Ethiopia, who reported a pvl positivity rate of 36.8 % (71/193), somewhat lower than our report ( 48.63%) (Abrha A, 2018). This is expected as his source of isolates were from SSTI, nasal swabs of dairy farms, and cow's milk. However, isolates from SSTI have the highest pvl gene than nasal sources and milk sources (53.7 %, 13.6 %, and 4.2 % respectively). While in our case, the proportion of pvl gene is consistently high, 49.3 % , 40.4 %, 56.8 % and 47.5 % for clinical, HCWs gown, mobile phones of HCWs and few administrative staffs and nasal source of HCWs and administrative staffs respectively but without a significant difference among the source of isolates (P-value > 0.05) unlike Abrha's findings (Abrha A, 2019). On the other hand, pvl gene status is different between MSSA and MRSA isolates (p-value < 0.05) compared to only 4 MRSA isolates reported by Abrha (Abrha A, 2019). The few numbers of MRSA in Abrha's report can not be comparable with the current study.

From an epidemiologic point of view, PVL is present mainly in community-acquired *S.aureus* isolates, unlike in most developed nations, a high level of PVL positive *S.aureus* was very common from African studies (Schaumburg [a] et al., 2014). Pvl rate between 17- 74 % was reported among 5 African countries, namely Cameron, Madagascar , Morocco , Niger, and Senegal, and studies from Nigeria and Egypt (Breurec et al., 2011; Okon et al., 2009,Soliman et al., 2020).

Likewise in Lahore Pakistan pvl gene was positive among 51.2 % (21 /41) of MRSA and 36.0 % ( 31/86) of MSSA strains that are originally taken from SSTI patients (Iqbal et al., 2018). In Nigeria, among the 96 isolates, 41 (42.7%) were PVL positive, but the MRSA isolates were PVL negative (Okon et al., 2009). In Germany, Saxony region, out of 100 S.aureus, 30 (30 %) were pvl positive (Monecke et al., 2011).

Low level of pvl have been reported in many countries including 0.6 % (1/155) in Saxony, Germany's from asymptomatic carriers of *S. aureus* isolates (Monecke et al., 2009), 11.7 % from HCWs and students nasal isolates in Madagascar (Hogan et al ., 2016), 7.2 % among nasal swabs of HCWs and other healthy individuals in Jordan (Aqela et al., 2015). 3.2 % of *S. aureus* isolates from medical students of Ecuador (Bastidas et al., 2019), 0 % in Portuguese study from nasal and hand carriage isolates ( Castro et al., 2016), All the 63 *S.aureus* isolates that include 48 % MRSA that were collected between 2006 –to 2007 in Vladivostok, Russia showed pvl gene was not detected (Baranovich et al., 2010), 0 % from a study in Brazil isolates from mothers and neonates (Vieira et al., 2014), 6.5 % (9/139) pvl rate among 324 nasal isolates of HCWs and students in a university hospital, Southern Brazil (Danelli et al., 2020).

The low prevalence or absence of pvl reported in the aforementioned countries could be the large sample size we have and the inclusion of different sources of isolates in our study, which could justify the difference. In addition, there are thoughts that Africa could be the origin of pvl genes and gradual dissemination of the pvl gene occur to Europe and other nations (Ruimy et al., 2008). This is substantiated by a Belgian study where pvl genes have been linked with the importation of these strains after travel to North Africa and South America. All the 16 MRSA cases were CMRSA except one case (Denis , et al., 2005). However, whether this assumption or fact needs a well-controlled observation and historical investigation of the isolates or the putative genes from representative countries.

On the other hand, there is difference in the study period between the current and other studies mentioned above. *S.aureus* strains which were pvl negative could acquire the genes and pvl encoding strains could emerge. For instance recent work in Germany showed that among 740 MRSA isolates taken from patients hospitalized > 48 hrs in Heidelberg University Hospital, between 2015 and 2018 6.2 % (n = 46) of the isolates were PVL-positive and 32.6 % of PVL-positive MRSA were linked with hospital-acquired MRSA (Klein et al., 2020) This observation could support the emergence or increase in pvl genes in the *S. aureus* pool in countries.

In a systematic review of data from Africa, pvl gene positivity rate varies from country to country, such as in Algeria pvl rate of 75% (46/61), 30%,(19/64), and 77% (94/122); In Kenya 20 % (14/69), in Libya 29 % (10/35), and in Nigeria 47 % ( 33/70). In South Africa, 0.3 % (1/320), 4% (4/97) and 9% (5/56); in Tunisia 94 % ( 68/72), 100 % (64/64), and 62(43/69) ; in Uganda 73 % (30/41 and in one multicenter study, 23 % (20/86) have been reported (Abdulgader et al., 2015), and all pvl reports were as high as ours or higher than our findings. The number of *S.aureus* isolates included in our study is much higher than all the above African studies, which might be explained by the diversity of the source of specimen in our study. .

Other countries in Africa reported a high pvl rate as ours even up to 100 % pvl positivity. One of the Malian studies showed a high proportion of pvl even reaching up to 100 % specially for ST152 isolates that were taken from nasal swabs (Ruimy et al., 2008). This is true also in our findings where 4/11 ST 152 were pvl positive and mecA positive. Likewise, Out of 91 clinical isolates of *S. aureus* from a regional Hospital in Arkhangelsk, Russia 44 % of them were pvl positive both from MSSA and MRSA (Vorobieva et al., 2008) . A study from Spain also reported pvl rate of 36.4 % among 113 MSSA invasive *S. aureus* collected from 21 Spanish hospitals showed pvl rate of 36.4% (Pérez-Vázquez et al., 2008), in Pakistan also out of 45 *S. aureus* isolates taken from skin and soft-tissue infections showed pvl rate of 49 % and 31 % were both pvl and mecA genes positive ( Madzgalla et al., 2016). The high incidence of pvl in these countries could be attributed to emerging pvl containing strains in these countries.

In Iraq , Duhok, Kurdistan region among 109 *S.aureus* isolates from HCW , 55 (50.4%) were MRSA carrying the mecA gene and 3.7 % (4/55) were PVL positive while 54/109 (49.5%) isolates were MSSA and 3.3% were MSSA-PVL positive. While from 23/103 (22.3%) isolates from community students 5/23 (21.7%) and 17/23 (73.9%) isolates were MSSA-PVL positive and MSSA-PVL negative, respectively and only one isolate was MRSA and pvl positive ( Hussein N et al., 2019). This is in contrast with our finding where we have a high pvl rate both from MRSA and MSSA group. It could be attributed due to the large isolates we have from both groups.

We are not able to test our *S.aureus* isolates for different toxins except pvl like the previous studies (Abrha 2019, Monecke et al., 2009) ). It is essential to include different type of toxins and SCCmec typing to understand the detailed characteristics of both MSSA and MRSA during carriage and infection.

In earlier times, the presence of pvl positive MSSA/ MRSA have been used as a marker for community-acquired infection but later the existence of these genes from hospital-acquired MSSA/MRSA strains explains that the community-acquired strains are expanding to the hospital settings (Alkharsah, et al., 2018; Etienne ,2005).

The existence of pvl genes in the *S.aureus* isolates obtained from HCWs gown in the current study is an indicator for how much these strains are ubiquitous in the hospital environments. This finding is in contrast with one report from Northern Brazil that reported the absence of pvl gene from the nasal and laboratory coats of HCWs (da

Silva et al., 2020). The large sample size we included (588 HCWs) compared to only 80 HCWs in Brazil may contribute to the absence of pvl in the Brazilian study. Similarly, the presence of pvl encoding *S.aureus* from mobile phones of our HCWs and few administrative staff could call for further investigation of the pvl and other virulence genes from various sources in TASH and other settings in Ethiopia.

With this regard, a high level of pvl genes from MSSA/ MRSA isolates of HCWs in TASH is a matter of concern. We did not collect epidemiologic and other important data except the history of hospital admission and previous surgical procedure. Only few HCWs had this risk and it is difficult to conclude the source of the *S. aureus* isolates in the hospital environment or the community. Until this fact is ruled out, one can say that our HCWs could play a double role in the acquisition and or transmission of both health care and community-associated MSSA OR MRSA.

Being pvl is a virulent factor for *S aureus* (Labandeira-Rey et al., 2007, Etienne ,2005), the presence of a high level of pvl genes in our findings underscores that these isolates are potential pathogen that can be easily transmitted among HCWs themselves, contaminate gowns and mobile phones of HCWs , transmitted to immune-compromised patients and to the family members of the HCWs during their daily clinical practice or social life. **T**ransmission of pvl–producing *S. aureus* to a physician during the resuscitation of an infant with fatal pneumonia has been described elsewhere and the physician exhibited numerous furuncles and the isolates from the physician and the infant were genetically the same ( Chalumeau et al., 2005 ).

More importantly one can assume that colonization of HCWs with pvl positive *S.aureus* / MRSA could be potential risk factors for acquiring clinical infection and or they could act as a source of outbreaks.

**4.4. Molecular typing**

Spa typing is one of the several molecular genotyping tools that are used to trace the origin of *S. aureus* strain its distribution and whether there is a common origin of CC of both MRSA and MSSA in a particular hospital or community setting ( Mairi et al., 2020).

In the current study, we have reported 131 spa types out of 189 *S.aureus* isolates indicating the spa types are very diverse. Importantly 67.9 4% of the *S.aureus* isolates are found in single spa type meaning only 32.06 % of the 189 *S.aureus* were found in two or more frequencies. Spa t355 is the most frequent spa among the various isolates of *S.aureus* in this study that occurred 23 times. This is in line with a previous study done in North Ethiopia (Abrha A 2019). In this study t042 was the most frequent spa type that was detected 31 times out of 190 *S.aureus* total isolates, followed by t355 which is the leading spa type in the current study. Similarly, t306, t084, t085,t306, t314, were commonly found in the current and the study done by Abrha ( Abrha A , 2019), but with different frequencies. More importantly, t355 was the most frequently reported spa-type among the clinical isolates in the case of Abrha et al study, and the first also in our case underscoring this spa type is very common and probably the leading one among clinical and carriage sources.

On the other hand spa type 042 which was the most frequent spa type in Abrha study (Abrha 2019) it was not found in the current study along with t4206, t458, t002, and other spa types. Remarkably, out of 31 t042 strains in Abrha report, 29 of them were found among milk sources and only one each from SSTI and nasal source , and its absence in the current study may be the fact that spa type 042 is a rare spa type among nasal and clinical isolates. Since our sample source comprises of nasal, gown, mobile phones, and clinical samples the presence of diverse spa type is expected. This observation is supported by a previous study from dairy cows of smallholder farms in North West Ethiopia (Mekonnen et al., 2018). About 79 *S.aureus* isolates from intramammary infections were categorized in to 20 spa types (t355, t409, t17185, t10018, t14061, t2085, t17834, t4206, t9300, t17835, t17184, t306, t223, t2801, t273, t1376, t448, t4701, t042, t15786) and t042 was the leading spa types followed by t15786 which is absent in the current study. Moreover, t223 (17 times) ,t306, and t355 were also reported in Mekonens' work as well as in the current study (Mekonnen et al., 2018). The existence of additional spa types that is reported for first time in Ethiopia indicated that we have collected large number of *S.aureus* isolates from different sources which increase the diversity of the spa types. However, there are untypable isoaltes that might be due to mutation of the spa gene that may not be expressed.

In the current study, we have got spa type t085,t306, t085.5338 which were also found in the milk, nasal, and or SSTI isolates in Abrha study (Abrha 2019) also indicating these spa types could be ubiquitous. The presence of these spa types could tell us there might be a transmission of these strains from humans to animals or from animals to humans through milk consumption but needs to be confirmed by future studies.

Moreover, spa type t223, t085, t131, and t003 were found with a frequency of 20 times, 19 times, 13 times, and 12 times respectively and these spa types are the most frequent ones in our study next to spa type 355 which is different in terms of frequency from previous Ethiopian study. Moreover, there are many spa types that are not reported in the previous studies in Ethiopia that indicated the large *S.aureus* isolates we include in the current research work compared to others studies so far which allows us to obtain many spa types that are reported for the first time in Ethiopia. Spa type 003 was one of the dominant MRSA spa types among MRSA isolates found in Heidelberg, Germany between 2015 to 2018 ( Klein et al., 2018) along with t002 ( 44/734), t127 ( 41/734), t008 (36/734) and t063 (20/734) were also found to be the dominant spa types which most are not reported in our case. This is mainly attributed to their isolates being taken from hospitalized patients unlike ours which are from mixed sources.

Among the listed spa type in a Kenyan study, t131, t064, t318, t223, t355, t314, t318 are also obtained in our study showing that these two countries could share these *S.aureus* strains (Omuse et al., 2016). However, our isolates are much higher than the Kenyan collection (93 *S.aureus* isolates ), and difference in the study period might contribute to the large difference in the spa types between the current Ethiopian and Kenyan study. More interestingly, spa type 037 is the dominant spa type reported from Kenya which is absent in our report. The previous report from Mali also underscore that *spa* type t355 was noted in 7 out of 21 ST152 isolates (33.3%), t084, noted in two of the ST152 isolates (9.5%), and spa types t311, t701, each observed one ST152 isolate (4.8%) (Ruimy et al., 2008), where all these spa types were also found in the current study , which shows that these spa types are common in African countries.

In Europe, a total of 28320 MRSA and MSSA isolates were characterized by spa typing and t 032 was the most frequent type followed by t008, t002, t044, t003, t067, t018, and t004 (Asadollahi et al., 2018). While in our analysis although we included different *S.aureus* isolates, only t003, t067, and t018 are observed. Spa type t003 is found more frequently from HCWs gown (7 times) while 2 strains from each from HCWs nasal source and mobile sources. An isolate from the nasal cavity of one administrative staff contained t003. From 8615 Asian isolates, t030, t037,t002, t437,,t1081, t004, t001, and t2460 are the commonly seen spa types. No similar spa types are found in our case at least from the commonly reported spa in Asia (Asadollahi et al., 2018).

While from 8141 American *S .aureus* collection, t008, t002, t242,t012,t084, t003, t311, and t0149 are the most frequent ones (Asadollahi et al., 2018). In our case, we have also seen t084 and t003 as one of the most frequent spa types, and t311 at least in one isolate. From 2126 *S.aureus* African collection, t037, t084, t064, t1257, t045, t012, t1443, and t314 are more frequently identified spa types; therefore we expected more similar spa types in our investigation. However, we have got only t084, t045 and t314 spa types found also with very low frequency. We were able to see t314 from a nasal swab of administrative staff working in the hospital, mobile phones of HCWs, and administrative staff and gown of HCW, and one case of t045 from a clinical isolate. t084 seen in one isolate of a nasal swab of administrative staff. Surprisingly, we did not get t037 which is the predominant African spa type and has been previously reported in Ethiopia by Abrha only from 2 cases of SSTI in the Northern part of Ethiopia (Abrha ,2019).

We may say that spa t037 is not the most frequent spa type in Ethiopia, rather it can happen in very limited hosts. This hypothesis should be tested in multiple studies in the wider geography of Ethiopia. From 192, *S. aureus* Australian collection, t202, t037,t437,t172, and t011 are the most frequent spa types summarized in the review (Asadollahi et al., 2018). None of these commonly reported spa types are observed in our study. This might be the low number of *S.aureus* characterized in Australia compared to ours and other continents. Moreover, Ethiopia is relatively far from Australia**.**

Spa type t6218 strain singly found in the mobile phones of an HCW at TASH. This spa type has been reported from SSTI cases in the Mekele area , Ethiopia ( Abrha 2019) in three cases. This implies that HCW's mobile phone at TASH could acquire this clone from patients during clinical practice and there is a possibility of transmitting this strain to other patients and family members unless there are hygienic measures. We have got only a single case and whether it was acquired it at TASH or not it needs further investigation. Similarly, t084 is observed in a single case of administrative staff nasal cavity and t 085 found in four nasal swabs of HCWs and 2 each from HCWs mobile phones, HCWs gowns, nasal swabs of administrative staffs and mobile phones indicating these strains are also found ubiquitously.

Spa typing from the nasal carriage of MRSA from Ashanti region , Ghana taken from children result in 35 different spa types and t355 (n = 25), t84 (n = 18), t939 (n = 13) are the leading spa types (Eibach et al., 2017). Similarly, study from Mali ,spa type t355 was one of the dominant spa types isolated from the nasal carriage of patients along with t084, t1149, and t1476 ,t024, t127, t279, t311, t701, t774, t861, and t1215 which were detected from nasal carriage study (Ruimy et al., 2008). The presence of a high number of spa type t355 and t084 and ST 152 and 15 in the current study makes these two spas are more common in the aforementioned countries though we have few isolates from children. It is possible to share some spa types at different continents like the case of Thromso skin survey; HCWs vs. non-HCWs had a 2. 17 and 3. 16 times higher risk of colonization by spa types t012 and t015, respectively which are absent totally in our study (Olsen et al., 2013) but Spa type t084, t065, t018, are also detected in this study and ours which further indicate spa types like t084, t065, t018 are common spa types even at the continental level. However, the frequencies are different in each of the studies.

Isolates from HCWs and students in Madagascar (Hogan et al ., 2016) showed that MRSA carriage with spa type t186 is the leading spa type from the 20 MRSA nasal isolates along with spa type t2393, t5562, t5772 and t13653 were also detected . In our study 2 spa type t186 one from the nasal carriage and the other is from clinical isolate is observed indicating that t186 are commonly circulating in Africa.

Characterization of 159 *S.aureus* isolates recovered from pus, wound, blood, sputum , and cerebrospinal fluid in two tertiary hospitals ,Namazi and Faghihi, in Shiraz ,Iran between December 2017 to September 2018 showed that t021, t030, t037, t081, t325, t386, t790, t345, t816, t1877, t314, t186, t304, t003, and t018 are the commonest spa types described in this study (Hashemizadeh et al.,2019) and spa type

314,t304,t003,t018, and t186 were also detected in the current study either from clinical sources or nasal swab of HCWs or gown of HCW. Interestingly 2 spa t018 and, 1 out of 3 t314 and 2 out of 12 t003 isolates were MRSA. This further strengthens the hypothesis that some spa types have a wide distribution across countries and associated with severe infections. Moreover, spa type t223 ,t131,t937, t085 are the most frequent spa observed in our study from different source samples including nasal , mobile phone and gowns of HCWs. Substantially 2/17, t223, 4/7 , t131, 2/5 , t937 and 4/12 , t085 spa types are MRSA.

The existence of unique spa types in each sample source may indicate that there might be specific spa types that are common in each source, be it in colonization, contamination of mobile phones and gowns of HCWs , and or clinical sample. However, the number of spa types under these categories is very small in number in the majority of the cases and we need to have further investigation to see whether this association is true or not.

In a study from China, among 127 MRSA and 143 MSSA isolates taken from different sample sources collected between May 2011 to June 2012, showed that, t030 is the leading type isolated from patients with blood stream infection, drainage, body fluids, and sputum sources and t309 is the second most frequent spa that is linked with MRSA associated infection. t309 (15.9%), t4371 (11.4 %), and t796 (6.8 %) are the leading spa types for causing skin and soft tissue infection (Chen et al., 2014). We had also a single strain of t309 from clinical isolates and three t701 from colonization and clinical sources which can be a source of infection for immune-compromised patients and the carrier themselves.

Spa CCs have been deduced from spa types and CC15, CC22, CC5, CC30, CC121, CC8, CC20, CC12, CC88, CC152, and CC225 are found in our *S.aureus* strains. This is more or less similar with the spa CCs reported by Abrha in 2019 (Abrha ,2019) that includes CC80, CC15 ,CC152,CC5, CC25,CC121,CC88,CC1,CC22,CC8, and CC239. Except for CC225 present only in our case and only CC 239 present from the previous study, all others CCs are common though there are differences in their frequency. This is also expected since our isolates were from diverse sources.

Genotyping of the MRSA in In Dutch-German EUREGIO study reported spa types t032 (CC22, mecA positive) and t223 (CC22, mecA positive) for three isolates (Sassmannshausen , et al., 2016). In our study t223 is one of the dominant spa types both MRSA and MSSA harbouring strains. One strain with spa t223 CC22 MRSA from diabetic foot ulcer case and another spa t223 CC22 MRSA positive was also isolated from nasal swab of a medical doctor. Moreover about 18 spa t223 with or without spa CC 22 is seen from clinical isolates, nasal swabs of different HCWs, a nasal swab from babies, and isolates from HCW gown. However, no spa t032 is identified in our analysis. Similarly, we have many spa types which are reported only in our study. Nasal swabs isolates from HCWs and other healthy individuals in Jordan also revealed 17 different spa types and spa type t223 was the dominant type among these groups which goes in line with our findings (Aqela et al., 2015).

The availability of different molecular typing for *S.aureus* allows us to compare previous and current *S. aureus* strains, differentiate whether there are outbreaks or not. Among the various molecular methods spa typing and MLST typing are currently used in many places around the world ( Stefani et al., 2012).

We have selected randomly 60 *S.aureus* isolates from different sample source and 52 MLST sequence were generated for 52 *S. aureus* isolates and 29 STs are found .These are ST 152 (11), ST5 (3), ST4666(3), ST744(3), ST80(2) ,ST88 (2), ST449(2),ST1633(2),ST1972(2),ST3781(2),ST4225(2),ST6(1),ST12(1),ST15(1),ST2 1(1),ST22(1),ST25(1),ST51(1),ST97(1),ST190(1),ST204(1),ST290(1),ST489(1),ST67 2(1),ST874(1),ST1886(1),ST2434(1),ST4744(1) and ST5027(1) . It seems that the STs described here are as diverse as the source of the *S.aureus* isolates are different. Meaning, we have included nasal, clinical, gowns of HCWs and isolates from mobile phones of HCWs and a few administrative staff.

Our findings include many STs that are commonly reported from one previous study done in Ethiopia. ST 152 is the dominant MLST type (37.9 %) in our study followed by ST 5, which is in concordance with MLST finding from Southern Ethiopia Gamo rural hospital where ST 152 is the leading ST though only one MRSA is reported from 80 total strains over 5 years. (Verdu´-Expo´sito et al., 2018). However, ST 1, ST 30, ST121, ST727, ST3199, ST2066, ST3224, ST2431, ST1698, ST772, ST4997, ST676, ST3887 that are isolated from patients at Gamo hospital Ethiopia is not found in our study. Moreover, there is a new STs in this report. This is mainly the fact that our *S.aureus* isolates includes clinical , nasal sources and isolates from gowns and mobile phones which result in a diverse type of STs compared to a single source of sample mainly from patients with skin infection seen in the Gamo study (Verdu´-Expo´sito et al., 2018). Moreover, our *S.aureus* isolates are relatively small in number and we cannot make any generalization.

Data on MLST of *S.aureus* is lacking in Ethiopia from clinical sources other than those mentioned above. In one study ST 4550, ST1, ST22, and ST 848 were reported from *S.aureus* isolates in milk samples of dairy cows in North Ethiopia which is generated from representative six *S.aureus* isolates (Mekonnen et al., 2018). Though our sample source does not contain milk samples, only ST 22 is found to be a common MLST both from milk samples and isolates from a nasal swab of HCW at TASH. This is important as these strains could be transmitted from cows to humans or vise versa. Interestingly, Mekonen and his colleagues also reported ST 4550 (Mekonnen et al., 2018) which is a double locus variant of ST 97 that is commonly reported from bovine mastitis (Smith et al., 2005; Delgado et al., 2011 ).

The existence of one ST 97 (CC97) from carriage source in our study needs a query where this is a livestock-associated *S.aureus* strain is dominant or not in Ethiopia. Probably this is the first report from Ethiopia concerning clinical sources. Ethiopia is a nation with high livestock resources and very close contacts of humans and animals, the existence of ST 97 and CC 97 (three isolates) harbouring *S.aureus* strains is not unexpected.

A recent review on genotype distribution across the five continents, mentioned that ST 239 is the most frequent ST reported among Asian and African source countries (Asadollahi et al., 2018). In this review data from Ethiopia is not included, most probably the absence of MLST data. Comparatively, our MLST data consists of about 52 *S. aureus* isolates that includes clinical, carriage and isolates from inanimate objects like HCWs mobile phone and gowns. We can assume that this is the first MLST report from Ethiopia in terms of carriage and strains from inanimate objects like gowns and mobile phones. We may claim that ST 152 is the most frequent MLST ST type in the Ethiopian context as we observed in our study and a report from a clinical sample in a rural hospital in southern Ethiopia also indicated this ST is common (Verdu´-Expo´sito et al., 2018).

Data from meta-analysis mentioned (Asadollahi et al., 2018), ST 8, ST247, and ST 5 are the most frequently seen sequences seen in America (both North and South America), ST 22, ST8, ST 247 and ST 5 are more frequent in Europe, ST239, ST22 and ST5 are more common in Asia and ST 239, ST15 and ST8 are seen in African countries. In our investigation, ST 5 are seen in three cases one each from nasal, HCW gown, and mobile phone. The nasal and gown strains are MRSA. Moreover, we have got one ST 15 and ST 22 from clinical and nasal isolates respectively. Thus, we need to analyse more data to understand the MLST distribution of *S. aureus* isolates in Ethiopia and other developing nations to expand our understanding of the MLST patterns of *S. aureus*. As technology advances countries like Ethiopia and other developing nations should build their capacity in order to characterize *S. aureus* strains and other microbes.

Report from Kenya, Tanzania, Egypt, Algeria , Ghana and MLST report from five African cities also mentioned ST 5.15,22,25, 152, and some other STs are commonly found in their analysis. The source of the isolates are mainly clinical and few nasal swabs (Omuse et al., 2016; Moremi et al., 2019; Soliman et al.,2020 , Eibach et al., 2017, Djoudi et al., 2015 ,Breurec et al., 2011). Likewise, different countries in Asia and Europe also reported ST5, ST15, and ST 22 are commonly distributed in the *S.aureus* isolates (Sassmannshausen , et al., 2016; Wu et al., 2019,Goudarzi et al., 2017). MLST type from remote Gabonese Babongo Pygmies region showed that *S. aureus* carriage rate of 33% and 10 STs predominated by ST15, ST30, ST72, ST80, and ST88. (Schaumburg [a] et al., 2011) which underscore ST 15, ST80 , and ST88 are potentially common STs across Africa and other world.

In Turkey, out of 488 MRSA isolated collected ,ST239 (85.1%) was the most common MLST type followed by ST 737 (4%), and ST97 (2.8%), both SCCmec type IV. Two isolates were ST80 with SCCmec type IV (Bozdoğan et al., 2013). ST 97 and ST80 are also observed in our study where both ST80 are MRSA and one ST 97 is

MSSA. Indicating that ST80 are one of the frequent MLST ST in Ethiopia and Turkey. However, we did not get ST 239 among Ethiopian isolates which is the most ST in Turkey , rather ST 152 are the most frequent ST seen among clinical ,carriage and isolates from gowns of HCWs.

The presence of the same ST alone in different countries does mean that the isolates are 100 % identical. For instance, we have got MLST ST5 and this ST is also reported from a study from Lima Peru where the isolate was collected from bloodstream infection. However, the spa type they mentioned is not present in the ST5 groups of *S.aureus* in either of these countries (Garcia et al., 2016). This implies that we need to use multiple genotyping procedures to compare and contrast different isolates in different countries with better precision.

In Lima Peru they reported that ST72 is one of the commonest ST and in Taiwan study (Garcia C et al., 2016), ST59, ST45, and ST239 were the commonest STs of MRSA both from HCWs and patient group which is undetected in our report (Wu et al., 2019). Rather 36 .4 % of our ST152 contains MRSA strains. This implies that there might be specific STs distribution in specific countries and the source of our *S.aureus* isolates are mixed one, unlike these two countries which took the isolates from bloodstream infection.

Similarly, ST 152 and ST5 were dominant clones from 13 acquired clones and ST 5 was also one of the dominant sequences from admitted patients seen in the Tanzanian study (Moremi et al., 2019). Moreover isolates from SSI in Tanzanian finding, these two sequences were common. Likewise, we have got one ST22, ST 88 (2), in our study which is similar to the report from Tanzania ( Moremi et al., 2019) and isolates from five major African towns ( Breurec et al., 2011). However, we have got different sequences other than the one reported in Tanzania and other African cities and we did not have ST 612, 72, 3118, 4266 in our study indicating MLST sequences vary from place to place even with in the same country not alone in different nations and specific MLST strains could circulate in a particular places.

In China, out of 78 community-associated *S.aureus* collected from bloodstream infection of paediatrics patients ST 188 and ST 7AND ST59 are the commonest

MSSA and MRSA STs. In addition ST5, ST6, ST20, ST22, ST25, ST26, ST30, ST121, and ST487 are also seen ( Wang et al., 2012). Only ST 5, ST6, ST 22 are seen in our finding, and ST152, ST5, ST 15 are dominant though our *S.aureus* isolates are small in number compared to the 78 strains in this Chinese study.

What makes our finding more interesting ,though we did not get ST 5 from clinical isolates, , we have got ST 5 with CC5 from nasal isolates and isolate from gowns of HCW (doctor) which are MRSA, and one isolate from a mobile phone of a medical doctor, suggesting these STs are likely the commonest one circulating in patients, HCWs and the general hospital environment and underscore the strengthening of the infection prevention system in the hospital. The absence of ST 5 from clinical isolates is small number of isolates we run for MLST typing which need confirmation large *S.aureus* collection.

In an Egyptian study, out of 18 MRSA strains collected for molecular characterization, two ST 5 VI were identified and these clones were also isolated from ICUs. Interestingly, out of the 9 HA-MRSA isolates ST80-IV and ST 5-VI were mentioned (Soliman et al., 2020). In the current MLST analysis, out of 13 MRSA isolates, two of them were ST 5 (one from a nasal isolate of a mother and one isolate from the gown of a medical doctor). Moreover, ST 88 MRSA with spa type 690 was reported among hospitalized patients in Bugando Medical Centre and Sekou Toure hospital in Mwanza, Tanzania (Moremi et al., 2019). Although we did not get MRSA strains of ST 88, we have got two MSSA $ST_{88}$ sequences, one from colonization and one from wound sample isolate. The occurrence of these STs in our study is a good sign where this strain is circulating in Ethiopia and it can acquire the MRSA genes through time or this strains harbouring mec A gene is already circulating in the country.

ST $_{6}$,$ST_{15}$,$ST_{25}$,$AT_{80}$,$AT_{449}$,$ST_{489}$,$ST_{672}$,ST1633,ST1972,ST3781,ST4744 are found only from clinical sources ,ST21,ST22,ST97 and ST2434 found only in nasal samples ,ST12,ST51,ST204,ST290 and ST1886 is seen only from gowns of HCWs, while ST 190, ST874, and ST5027 are found only in mobile phones of HCWs. The presence of such unique STs in each source should be investigated further.

ST 5 and ST774 are found in clinical, gowns and mobile phones of HCWs. ST152 is the most abundant one found in all source types except mobile phones of HCWs. ST88 and ST 4225 found in clinical and nasal sources and in nasal and mobile phones respectively. Literature is scarce to compare these important findings. The presence of same STs in two or more sources in the same hospital guides us, these strains are ubiquitous and precaution should be taken during infection prevention measures. Two ST 1633 starins and one ST672 MRSA was detected in the current study which was also reported Kenyan (Kyany'a et al.,2019) and Indian study (Sunagara et al., 2016) respectively .

In resource-limited cases, MLST typing is done using sequence data generated from one of the downstream or upstream sequences and one has to use the same primers for sequencing. Considering there might be a difference in MLST ST whenever we use only single sequence data, we compared the MLST STs of few isolates using the upward or the downward sequence. There is concordance in the final MLST ST except in one isolate and the MLST ST generated here in our work looks acceptable.

In terms of clonal, five types of MLST CCs are seen and CCs 5 is the most frequent CC that consists of 8STs (ST 5 (3), ST744 (3),ST 874 and ST6) . CC15 consists of 5 STs (ST 1886, ST1972(2), ST2434 and ST 15). Similarly, CC22 has 4 STs ( ST22,ST4225(2) ST21). CC8 has 3STs (ST449 (2) and ST3781) and CC97 has 3 STs ( ST97, ST25, and ST 204). All these are very common MLST CCs around the world and associated with life-threatening infections (Monecke et al., 2009) CCs CC1,CC15,CC30,CC121, and CC152 were the major clones of *S.aureus* which have been detected among representative *S.aureus* (MSSA) isolates from five African towns in Cameroon, Madagascar, Morocco, Niger, and Senegal (Breurecet al., 2010) suggesting the clonal complex structure of *S.aureus* could be diverse among different countries.

We have CC8, from three clinical isolates and one of it MRSA clones, and CC 15 from nasal, clinical, and HCW's gowns. A study in Germany also reported that CC8 and 15 are the two dominant clones among 155 *S.aureus* isolates collected from an asymptomatic carrier in Germany (Monecke et al., 2009). Interestingly in the other German study, CC22 was found to be common clone of *S. aureus* among nasal l isolates (Sassmannshausen , et al., 2016) . However, both CCs are MSSA in

Germany's case unlike we have one MRSA from CC8 suggesting this CC could contain both MRSA and MSSA which could exist in the two countries.

The existence of CC5 ST5 MRSA from HCW gown and a nasal swab of a mother is one of the important findings in the current study. Likewise we have got CC22 ST 22 MSSA from nasal swab of a medical doctor and CC22 ST 4225 MRSA from a nasal swab of a medical doctor and the same ST MSSA from a mobile phone of a HCW is a good indicator these clones are a potential cause for outbreaks or a concern for the health system of Ethiopia in general and TASH in particular. Both CC5 and CC22 MRSA have been described in various parts of Africa indicating the continental presence of these clones (Abdulgader et al., 2015). Hence concerted efforts are required across the country and continents to trace the common clones/ STs of both MSSA and MRSA and plan appropriate intervention measures accordingly.

WGS data could provide the best discrimination with better quality compared to spa typing, MLST, and other genotypes methods in general. We have tried to have WGS data for 18 isolates from nasal sources of HCWs, administrative swabs, gowns of HCWs, and mobile phones. Moreover, few *S aureus* isolates of clinical source were also included and a phylogeny tree was constructed along with known strains of the organism from the NCBI database. Accordingly, there are five clusters A to A to E , and most of our *S. aureus* isolates are poorly separated from the phylogeny and this is mainly because our isolates are from different sources and most likely the clustering is far apart. The 16 SrRNA based classification and phylogeny have been used for long for comparison and classification of prokaroytes as it is stable (Kumar et al., 2020; Saleh et al., 2018).

In addition, we have also compared our WGSs with known strains of *S. aureus* from the database based on the average nucleotide identity (ANIb %). Consequently, LAH-K12, K55, and K63 had high sequence identity (99.9%) with *S. aureus* strain BB155 isolated from the nasal carriage.

Like wise, LAH-K12, LAH K55, and LAH K63 had high sequence identity (99.9%) with *S. aureus* strain BB155 isolated from the nasal carriage. Interestingly the first two of our isolates ( LAK12, and LAHK55 ) were isolated from the nasal swab of a medical doctor and a nurse practicing in TASH. While the last strain (LAHK63) was a clinical isolate. Suggesting colonization strains are capable of causing invasive and non-invasive infections (Turne et al., 2019).

LAHK8 is a strain isolated from the gown of a nurse and it matches with isolates from a wound at ANI% 99.92 % which also gives us evidence that gowns of HCWs could carry potentially pathogenic strain and cause infection of it could be transmitted to susceptible hosts during practice. Strains LAH K38 , LAHK42, LAHK46 LAHK481 are isolated from the gown of a medical doctor, mobile phone of another medical doctor, a nasal swab of a nurse that is also MRSA, and nasal source of administrative staff respectively. All these WGS matches at 99.76 % and above with isolates taken from blood, blood endocarditis, bacteremia, and wound sample. This is a lot for infection prevention personnel at TASH strict adherence to hand hygiene and optimal use of gowns and mobile phones and proper disinfection is crucial to minimize and or avoid contamination and or infectious conditions.

All in all, as a snapshot, the WGS data is essential to trace the source of infection and or if there are transmission events in hospital settings especially if our *S.aureus* isolates are the same type. Recently in England, longitudinal WGS data indicated that there were several transmission events of *S. aureus* among patients and HCWs ( Coll et al., 2017).

### 4.5. Knowledge, attitude, and practice HCWs about MRSA prevention and control

Knowledge, attitude, and practice HCWs about MRSA prevention and control are important to minimize MRSA burden and transmission in the hospital setting. Moreover knowing the burden of MRSA from patients, HCWs and environmental perspectives alone is not enough rather there should be a concerted effort from microbiological, managerial, and behavioural perspectives of HCWs ( CDC, 2020; Henderson and Nimmo , 2018)

Hence we have determined the Knowledge attitude and practice of HCWs practicing at TASH about MRSA prevention and control in general. Overall, 85.6 % of HCWs had good knowledge. However, knowledge of the score related to hand hygiene, 58.6 % (343/586) has responded correctly as hand hygiene is the most important and effective measure to control MRSA transmission and the remaining 41.4 % of HCWs at TASH had this knowledge gap and this is a matter of concern. This is not unique to the current finding, as a recent study in West Arisi zone , South East Ethiopia also showed that out of 648 HCWs enrolled only 53.7 % of them have good knowledge (Geberemariyam, et al., 2018). While knowledge score of 60.4 % was reported among 226 HCWs of Alnasar Hospital, Saudi Arabia which is comparable with our finding (Hamid et al., 2019).

More than 55.7 % of HCWs at TASH did not know or remember mupirocin is the drug of choice for the decolonization of patients or HCWs MRSA. The low level of knowledge in this regard is partly; the composition of our HCWs is diverse including doctors, residents, nurses, laboratory personnel, pharmacy personnel, and others. As different health cadres have different knowledge about treatment, this will reduce the overall knowledge score. However, we have seen the knowledge gap across all disciplines. This result also goes with the fact that a significant number of HCWs at TASH did not take any training about MRSA. Likewise among 585 HCWs, only 51.1 % of them properly define the meaning of methicillin-resistant *S.aureus* ( MRSA) which strengthens the previous knowledge. This was also true among nurse graduates of the University of Southern Mississippi who have an overt lack of knowledge regarding antibiotic treatment for MRSA (Suss ,2017).

On the other hand HCWs of TASH have a very good knowledge score that MRSA could be transmitted from HCWs to patients and that clean hands protect MRSA related illness in the hospital settings, more than 88.8 % of the HCWs and more than 94 % of HCWs also know contaminated hands, cell phone , and gowns could transmit MRSA in the hospital. This is good news for TASH and continuous updates on MRSA and other multidrug-resistant microbes should be given. A similar knowledge score is also reported in a study from Brazil (d Silva et al., 2010)

More than 76.8 % of HCWs knows the benefit of the decolonization of patients with MRSA before surgery which could reduce MRSA related infection and overall 85.5 % (503/587) of HCWs at TASH had good knowledge while 14.3 % (84/587) had poor knowledge about MRSA prevention and control. This is a bit exaggerated from other reports as we took 60 % and above as a cut-off for good knowledge. If we increase the cutoff, definitely the knowledge score will be reduced.

Overall knowledge score was significantly different among the sex of HCWs, professional category, and level of education (p-value < 0.05). This is expected as nurses and doctors, doctors and pharmacy personnel, and other health care cadres could have different educational experiences and exposure to MRSA-related topics. Similar findings were also noted among HCWs in Alnasar Hospital Saudi Arabia (Hamid et al., 2019).

Interestingly, those HCWs who use their mobile phone during actual clinical practice and frequency of changing gown and mobile phone types, source of information about MRSA, and availability of hand hygiene materials are significantly associated with the overall knowledge score of HCWs (p-value < 0.05). This means that if HCWs frequently change their gowns the likely hood of acquiring MRSA could be minimal.

Similarly, the attitude of HCWs towards MRSA prevention and control at TASH has been assessed and more than 95 % of HCWs had a positive attitude towards the importance of a system-wide approach to prevent MRSA in TASH and 91.9 % of HCWs had also a positive attitude where HCWs can carry MRSA and could transmit the infection to patients. Importantly 28.8 % of HCWs at TASH believed that (168 / 583) frequent hand hygiene causes irritation of hands and does not halt MRSA transmission. A similar finding was also reported from very recent work in Jimma Ethiopia where, 28.1 % of the HCWs have a concern about skin irritation following the use of alcohol-based hand sanitizer (Assefa D et al., 2021). During the recent pandemic of COVID 19, a significant number of health care workers in China reported hand hygiene-induced skin damage (74 %, 321/434). Those who did hand hygiene 10 times per day had dermatitis-like symptoms (Lan J et al., 2020). This underscores the need to provide the best and safest hand sanitizer for HCWs to maintain compliance.

Over 72.9 % of HCWs (426/ 584) have a positive attitude on the fact that safety measures taken by health care personnel can minimize MRSA rate in a hospital. Similarly, a study in Brazil also mentioned that majority of HCWs (94.6 %-98.1 %)

acknowledged, preventive measures on MRSA benefit both HCWs and patients in general (da Silva et al., 2010).

About 87.1 % of HCWs in TASH agreed to the screening of all HCWs at TASH and 37 .4 % of HCWs agreed that if they feel ill they should not be isolated from the work place. About 72.9 % of HCWs at TASH agreed that safety measures taken by HCWs minimize MRSA rate. This is good news for the infection prevention committee and the management; if there is a need for screening and decolonization is sought most HCWs are ready to do that. Screening of HCWs for MRSA and decolonization is recommended if there is an outbreak in the hospital and the HCWs are the source of that outbreak (Sigel, et al., 2006). On the other hand, related to MRSA screening nurses and HCWs have a concern, for example, nurses in Dutch health facilities and Norwegian nursing homes, they feel that the impact of MRSA screening has a huge impact on their life including stress and work banning (Thorstad et al., 2011; van Heuvel et al., 2020). Therefore, it is important also to consider the impact of MRSA screening on patients as well as staff while doing MRSA screening and decolonization.

The overall attitude of HCWs towards MRSA prevention and control at TASH is significantly different between male and female HCWs. Being female HCWs have good attitudes than male HCWs ( OR ,95 CI, 1.75, 1.25-2.46). This might be females are taking more safety measures and are more curious than male HCWs.

Similarly, the overall attitudes score is significantly associated with the professional category and level of education, and nurses and degree holder HCWs at TASH had more good attitude for MRSA prevention and control. Of course, nurses and degree holders of all categories are the dominant groups in the current study and such differences could occur due to the background of the respondents. There was also a previous report, that nurses are taking more hand hygiene adherence than doctors and other health cadres (Erasmus et al., 2010). In the Korean study, dentists had a higher knowledge score compared to dental hygienists and dental technologists while dental hygienists had higher practice related scores compared to dentists and dental technologists indicating even in the same field the knowledge and practice could be different (Yoo et al., 2018)

There is also a significant difference between overall attitude score with cell phone types, cell phone cover, frequency of changing gown, use of the mobile phone during actual clinical practice, source of information about MRSA, availability of sufficient hand hygiene materials, guidelines, and leaflets about MRSA ( p-value below 0.05). It has been also described that availability of personal protective equipment, training, and education, lack of teamwork are important factors for preventing MRSA (da Silva et al., 2010).

Finally, the practice of HCWs towards MRSA prevention and control at TASH indicated that only 17.8 % of HCWs (104/583) wash their hands before and after patient contact. Likewise, 53.3 % (310/ 582) HCWs very often and or often wear aprons and gowns during contact with patients. Surprisingly,41.1% (234/ 581) of HCWs in TASH do not clean their cell phone and or cell phone cover. This is in line with other studies that reported that MRSA mobile phone contamination is higher among HCWs who did not clean their mobile phones (Heyba et al., 2015). Those doctors who failed to clean their mobile phone had a 2.4 times risk of contamination than those who disinfect it. A low level of practice about over infection prevention (36.3 %) was reported among HCWs in West Arsi zone, southeast Ethiopia. The presence of training and Infection prevention guidelines are associated with self-reporting safe infection prevention and being a member of infection prevention committee are associated with safe practice (Geberemariyam, et al., 2018). However, our participants are working in tertiary university hospital compared to this study that may bring slightly higher MRSA prevention practice.

A very low practice score of 24.6 % was reported about infection control among the HCWs of Alnnasar Hospital Saudi Arabia and the overall practice for all staff was found to be least (24.6%). The overall compliance with standard precautions was found to be 26.9% (Hamid et al., 2019).

On the other hand, we used only a questionnaire to collect the KAP data and the perceived practice mentioned by HCWs above may not be the actual one. HCWs are expected to apply hand hygiene using the well-known five moments during their clinical practice ( Sax[b] et al., 2007). A study done in Brazil affirms that the perceived and observed adherence to hand hygiene was found to be 87.9 % and 19 .0 % respectively (de Oliveira et al., 2017) which underscore the use of observational study to assess practice-related activities.

About 16.1 % of HCWs never or seldom wash their hands before and after patient contact, 22.9 % of HCWs never or seldom wear aprons and gowns during contacts with patients, 39.4 % of HCWs never and or seldom wear a mask during caring or examining patients, 47 % never and or seldom trained about hand hygiene and other personal protective devices for preventing MRSA ,11 % of HCWs at TASH never or seldom clean their gowns and other PPDs and 55.8 % of HCWs at TASH does not clean cell phone or cell phone cover very often, often or some times. All these practice gaps are a concern and have to be addressed well.

About 25.6 % and 16.7 % of HCWs at TASH never or seldom encourage their colleagues to clean their gowns and cell phones, 37.9 % of them never consult appropriate personnel about MRSA, and 14.1 % of them do it seldom.43. 6 % of HCWs at TASH do not request regular medical checkup and 14.1 % of them make it seldom and 35.8 % of HCWs at TASH never teach families and other friends about MRSA transmission and 15.3 % of them make it seldom. From MRSA quality improvement study in the US , HCWs comfort increased from baseline as they remind other staff about proper hand hygiene from 61% to 70 % and contact precautions ( Burkitt et al., 2010).

The overall practice score has been summarized by considering a 50 % score and above from the total 10 practice-related questions. Therefore, 68.4 % (398/ 582) had good practice while 31.6 % (184/ 582) of HCWs had poor practice about MRSA prevention and control measures in general

In the Saudi Arabia study, there was a variation of practice and knowledge score among sex, occupation, working in high-risk medical wastes, and lack of enough training for medical staff which is vital for good practice for infection prevention (Hamid et al., 2019). While in our case slightly significant number of females have higher overall practice score than male partners which might be linked with their high number. in TASH, similarly, those who are young also have higher practice scores, and nurses and medical doctors have overall higher practice scores than other cadres as these two groups have more contacts with patients related to MRSA and related infection.

Moreover, there is no significant difference between the overall practice of HCWs and MRSA training status, type of mobile phone, presence of mobile phone cover or not, availability of guideline, /leaflets, history of hospital admission, and surgical intervention. An effective educational program in Egypt has shown a change in overall knowledge, attitude, and practice score among Nurses, physicians, and housekeeping staff (Abdel-Rasoul et al., 2016) underscoring the need for continuous training of HCWs about MRSA and other infection prevention in general.

## 4.6. Strength and Limitation of the Study

### 4.6.1. Strength

Various sources were used to isolate *S. aureus* and or MRSA including HCWs, administrative staff, stored isolates from nasal swabs of mother-child pair and diabetic patients, and clinical isolates. This could help us to understand the dynamic of MRSA / *S. aureus* in TASH and help us to design appropriate control and prevention methods.

Second, our study has also tried to include information about the carriage rate of MRSA among HCWs nasal source in relation to contamination of HCWs gowns and Mobile phones by this bugs.

The study included the KAP of HCW to identify gaps to help in future interventions that could help in the planning of infection control and preventive measures in the study setting.

Last the study included both phenotypic and molecular tests to generate results about the burden of MRSA in the context of one of the biggest tertiary Hospitals in Ethiopia , that could serve as a baseline for studies in other hospitals with a similar setting in Ethiopia and elsewhere.

### 4.6.2. Limitation of the study

Due to resource limitations and time constraint we are unable to do MLST and WGS for more isolates to understand the real dynamics of MRSA in our study settings.our finding may be affcated by recall bias of HCWs for KAP study and we took only a single swab for MRSA colonization study which we can not estimate MRSA carriage persistence.

## 4.7. Conclusion and Recommendations

### 4.7.1. Conclusion

We have attempted to determine the burden of MRSA in TASH from HCWs, administrative staff, patient colonization, and the burden from clinical isolates of *S. aureus*. Accordingly, the burden of MRSA nasal colonization was higher among HCWs than administrative staff . No significant difference was seen between MRSA nasal carriage and sex of HCWs but significantly HCWs with the age group of 20-26 years and being a nurse were more affected ( p-value < 0.05)

Remarkably, 2.72 % (16/588) mobile phones of HCWs were contaminated by MRSA compared to 1.3 % of administrative staff's mobile phones. More than half of mobile phone contamination was seen among females HCWs.

The existence of MRSA in the gown of HCWs is a cause for concern as 2.89 % of the gowns of HCWs were contaminated. On the other hand, the MRSA rate from stored isolates from mother child pair was found to be very low. Only two individuals were having MRSA in their nasal cavity which is much lower than the previous study done in Ethiopia. While MRSA rate is relatively high among *S. aureus* analysed from stored isolates of clinical sources based on the Mec A gene.

Antimicrobial susceptibility tests showed that penicillin is the least effective drug for *S. aureus* and only 13.02 % of isolates were susceptible to this drug. Importantly, resistance to Erythromycin and Clindamycin was 15.1 % and 8.8 % respectively. Overall, there is a significant difference in the resistance patterns of drugs between MRSA and MSSA strains. Still, vancomycin and rifampicin are the most effective antibiotics as we did not get resistance isolates in this study unlike some previous study which report vancomycin-resistance even for carriage isolates.

The spa typing observed in this study is very diverse as the source of the isolates were different, Hence we have seen 131 spa types from 189 *S, aureus* isolates from colonization and contamination of HCWs gowns and mobile phones. Spa type t355 are the most frequently seen spa-type followed by t223, t085, t131, and t003 which occurred 20 times, 19 times, 13 times, and 12 times respectively. Spa type *t701, t1828, t080, t2235, t2302 and t14805,t314, t380, t3841, t11375, t5338, t14350,* t062,

t318 ,t693 and t937 were also observed with less frequencies. Much more spa types are also generated with a frequency of one. We feel that we have analysed many *S. aureus* as a result new spa types is reported from Ethiopian perspectives. Spa CC showed CC15 was the most abundant one followed by spa CC 22.

MLST ST 152, ST 4666, ST 5, ST744, ST 88, ST 80, are the most important MLST ST generated from the representative *S. aureus* isolates. Interestingly some of these STs were MRSA and CC5, CC8, and CC15 are the very common clones both for MRSA and MSSA groups. The 16 SrRNA sequences phylogenetic tree showed our isolates are diverse and mostly poorly separated. Based on the average nucleotide identity and many of our *S.aureus* isolates showed high similarity with *S. aureus* strains identified from various clinical and nonclinical isolates.

Finally, the overall knowledge of HCWs about MRSA prevention and control in TASH was 85.5 %. The majority of HCWs in TASH have a positive attitude towards the importance of system-wide approach to prevent MRSA in general in the hospital (95.2 %, 542/ 582). While 54.68 % of HCWs have a positive attitude and 68.4 % of them have good practice about MRSA prevention and control measure at TASH and 54.68 % of HCWs have a positive attitude and 68.4 % of them have good practice about MRSA prevention and control measure at TASH.

### 4.7.2. Recommendations

Based on our findings the following recommendations are forwarded:

- Updating the real burden of MRSA is essential using phenotypic and genotypic methods to take preventive measures and track changes for intervention in TASH
- TASH as a tertiary level specialized teaching hospital, should use emerging technologies including advanced molecular methods should be availed for identification and AST of Microbes including *S.aureus* / MRSA and other multi-drug resistant organisms
- TASH should avail adequate personal protective devices such as gowns, hand rub, and uniforms for all HCWs practicing in the hospital with an appropriate guideline to prevent acquisition and transmission of MDRO within and outside the hospital settings
- Awareness and Practice measures of HCWs and the hospital communities are required to understand the transmission of MDRO including MRSA.
- Policy related to the use of Universal gown and hand hygiene use is required at TASH.
- Large-scale studies are required to generate more robust and representative data at the regional and national level.
- Even though, we generated huge evidence on the burden of MRSA at TASH , it is difficult to recommend decolonization of patients and HCWs at this stage. Additional evidence from studies on the microbiological, managerial, and policy aspects in TASH and other hospitals in Ethiopia is needed.

- The existence of MRSA and other pathogens in the nasal cavity of HCWs, their mobile phones, and gowns in TASH require consorted efforts primarily by the HCWs themselves, the management at all levels of TASH/ CHS/AAU, and the policymakers.

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# 6.0. Appendices

## Appendix I: Information Sheet for HCWs

**Title of the project:**

Burden of Methicillin *Resistant Staphylococcus aureus (*MRSA) and associated factors at TASH: Evidence from colonization of patients, health care workers, Administrative staffs and selected inanimate objects.

**Dear study participants,**

You are invited to take part in a research study entitled

Burden of Methicillin *Resistant Staphylococcus aureus (*MRSA) and associated factors at TASH: Evidence from colonization of patients, health care workers, Administrative staffs and selected inanimate objects.

. The researcher is inviting health care workers in TASH to participate in this study.. This study is being conducted by **Kassu Desta Tullu** , a PhD candidate from the Department of Medical Microbiology Parasitology and Immunology, School of Medicine, College of Health Sciences, Addis Ababa University.

**Purpose of the study:**

The aim of this study is to determine colonization rate of Methicillin Resistant *Staphylococcus aureus* in health care workers , administrative staff, and inanimate objects such as your cell phones, and gown . We would also like to know the knowledge. Attitudes and practice of health care workers on sources, transmission and control of MRSA at TASH ,Addis Ababa, Ethiopia.

**Procedures:**

If you agree to be in this study, you will be asked to:

• be involved in providing sociodemograpic data

- To fill out self-administered questionnaire related to KAP ,which will take about 20 minutes.

• Provide swab samples from the nasal cavity and from your cell phone and gown that may take only three minutes.

**Voluntary Nature of the research:**

This research work is completely voluntary. Everyone will respect your decision of whether or not to participate in the study. No one at the hospital or the college or university will treat you differently if you decide not to be involved in the study. If you decide to join the study now, you can still change your mind later. You may stop at any time.

**Risks and Benefits of Being in the Study:**

Being involved in this study, no major risk is anticipated, except a minor discomfort during sample collection from the anterior nares of the nasal cavity. . Being in this study would not pose risk to your safety or well being. There will not be direct benefit to you , but your participation in the study could help in the design of appropriate preventive measures to prevent transmission of MRSA in the Hospital. The outcomes of the study will also identify gaps for planning further training on control and prevention of Hospital acquired infections. .

**Privacy:**

Any information you provide will be kept confidential. The researcher will not use your personal information for any purposes outside of this research project. Moreover, we will not include your name or anything other personal identifiers in the study reports. Data will be kept secured by storing paper questionnaires in locked filing cabinets and in electronic form on password protected computers. Data will be kept for a period of at least 3 years as required according to the rules and regulation of the country

**Contacts and Questions:**

You may ask any questions you have now for further clarification related to the study. Or if you have questions later, you may Contact the researcher via telephone at 0911 10 70 99 or email at kassudesta2020@gmail.com. If you want to talk privately about your rights as a participant, you can call: IRB of the college of health science, Addis Ababa University , telephone no. 0118961396 ;

**Appendix II: Consent form for study participants ( HCWs)**

I have read the information sheet and I feel I understand the study well enough to make a decision about my involvement in the research. I have understood that by agreeing to be part of the study, swabs from my nasal cavity will be taken and also swabs samples from my cell phone and gown. I will be asked information related to socio demographic data and will also fill the questionnaire to KAP related information..

Name : ______________________________ Date ___________________

Signature ____________________________

Name of person conducting the informed consent process:_______________________________

Date : _____________________ Signature _________________

## Appendix III : Information Sheet for Study participants ( admintrative staff )

**Title of the project:**

Burden of Methicillin *Resistant Staphylococcus aureus (*MRSA) and associated factors at TASH: Evidence from colonization of patients, health care workers, Administrative staffs and selected inanimate objects.

**Dear study participants,**

You are invited to take part in a research study entitled

Burden of Methicillin *Resistant Staphylococcus aureus (*MRSA) and associated factors at TASH: Evidence from colonization of patients, health care workers, Administrative staffs and selected inanimate objects**.**

. The researcher is inviting administrative staffs CHS to participate in this study.. This study is being conducted by Kassu Desta Tullu , a PhD candidate from the Department of Medical Microbiology Parasitology and Immunology, School of Medicine, College of Health Sciences, Addis Ababa University.

**Purpose of the study:**

The aim of this study is to determine colonization rate of Methicillin Resistant *Staphylococcus aureus* in health care workers, administrative staff, and inanimate objects such as your cell phones.

**Procedures:**

If you agree to be in this study, you will be asked to:

• be involved in providing sociodemograpic data

- To fill out self-administered questionnaire related to sociodemographic factors work experience and few questions on history of hospital admission and surgical intervention you might encountered , which will take about 10 minutes.

• Provide swab samples from the nasal cavity and from your cell phone that may take only three minutes.

**Voluntary Nature of the research:**

This research work is completely voluntary. Everyone will respect your decision of whether or not to participate in the study. No one at the hospital or the college or university will treat you differently if you decide not to be involved in the study. If you decide to join the study now, you can still change your mind later. You may stop at any time.

**Risks and Benefits of Being in the Study:**

Being involved in this study, no major risk is anticipated , except a minor discomfort during sample collection from the anterior nares of the nasal cavity. . Being in this study would not pose risk to your safety or well being. There will not be direct benefit to you , but your participation in the study could help in the design of appropriate preventive measures to prevent transmission of MRSA in the Hospital. The outcomes of the study will also identify gaps for planning further training on control and prevention of Hospital acquired infections.

**Privacy:**

Any information you provide will be kept confidential. The researcher will not use your personal information for any purposes outside of this research project. Moreover, we will not include your name or anything other personal identifiers in the study reports. Data will be kept secured by storing paper questionnaires in locked filing cabinets and in electronic form on password protected computers. Data will be kept for a period of at least 3 years as required according to the rules and regulation of the country.

**Contacts and Questions:**

You may ask any questions you have now for further clarification related to the study. Or if you have questions later, you may Contact the researcher via telephone at 0911 10 70 99 or email at kassudesta2020@gmail.com. If you want to talk privately about your rights as a participant, you can call: IRB of the college of health science, Addis Ababa University , telephone no. 0118961396 ;

**Appendix IV: Consent form for study participants (administrative staff)**

I have read the information sheet and I feel I understand the study well enough to make a decision about my involvement in the research. I have understood that by agreeing to be part of the study swabs from my nasal cavity will be taken and also swabs samples from my cell phone. I will be asked information related to socio demographic data and some questions.

Name : ____________________________ Date ____________________

Signature _____________________________

Name of person nel conducting the informed consent process:________________

Date : _____________________ Signature _________________

**Appendix V. ለጤና ባለሙያዎች ለሆኑ የሚሰጥ መረጃ (Information sheet for HCWs)**

**የፕሮጀክቱ ርዕስ** ፡- መድሀኒት የተላመደ አስታፊሎኮከስ አውረስ (*Staphylococcus aureus* ) መጠን እና ተያያዥ ችግሮች በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል ከታካሚዎች: ከጤና ባለሙያዎች: ከአስተዳደር ሰራተኞች እና ከሚጠቀሙባቸው አንዳንደ ዕቃዎች ላይ ምን እንደሚመስል ያጠናል ።

የተከበራችሁ የጥናቱ ተሳታፊዎች ከላይ በርዕሱ በተጠቀሰው ጥናት እንዲሳተፉ **እየጋበዝ**ን ጥናቱን የሚያደርገው ግለሰብ አቶ ካሱ ደስታ ቱሉ ሲሆኑ እሳቸውም በጤና ሣይንስ ኮሌጅ በህክምና ትምህርት ቤት የሜዲካል ማይክሮ ባዮሎጂ ፓራሳይቶሎጂ እና **የ**ኢሚኖሎጂ ትምህርት ክፍል የ3ተኛ ዲግሪ ተማሪ ነው።

**የጥናቱ አላማ**

የዚህ ጥናት ዋና አላማ መድሀኒት የተላመዱ የእስታፊሎኮከስ አውረስ ባክቴሪያ መጠን በጤና ባለሙያዎች ዘንድ በአስተዳደር ሰራተኞች ላይ እና በአንዳንድ መጠቀሚያ እቃዎች ላይ ማለትም የሞባይል ቀፎዎች ላይ ጋዋን ላይ ምን ያህል እንደሆነ ለማወቅ ነው። በተጨማሪም የጤና ባለሙያው ለመድሀኒት ስለተላመደ እስታፊሎኮከስ አውረስ መነሻ የሚተላለፍበት ሁኔታ እና ስለመቆጣጠሪያው ያላቸው የእውቀት ፤ የአመለካከት ፡የተግባራዊ ልምድን ለመገምገምና ለማወቅ ጭምር ነው።

**የጥናቱ አካሄድ**

በጥናቱ ከተስማሙ የሚከተሉትን መረጃዎች እና ከአፍንጫ ጫፍ ላይ ከጋውኖች ላይ እና ከተንቀሳቃሽ ስልኮ ላይ ናሙና መስጠት ይጠበቅቦታል ። ስለራስዎ የተወሰነ መረጃ እንዲሰጡ በተዘጋጀው የመጠይቅ ፎርም ላይ ስለመረጃው እንዲሞሉልን ይህም ወደ 20ደቂቃ ብቻ የሚወስድ ይሆናል ። ከአፍንጫ ጫፍ በጥጥ ተጠርጎ ለሚወሰዱ ናሙናዎች እንዲሁም ከሚጠቀሙበት ተንቀሳቃሽ የስል ቀፎ እና ከጋዋኖት ላይ በጥጥ ተጠርጎ ለሚወሰድ ናሙና ፈቃደኛ ተሳታፊ እንዲሆኑ ይጠበቃል ።

**በዚህ ጥናት ላይ መሳተፍ በራስ ፈቃድ ላይ የተመሰረተ ስለመሆኑ**

በዚህ ጥናት ላይ መሳተፍ የርሶ ነፃ ፍቃድ ላይ የተመሰረተ ነው፤፤ ማንም ሰው እርሶ በዚህ ጥናት ላይ ለመሳተፍ ወይም ላለመሳተፍ ውሳኔዎን ያከብራል።በዚህ ጥናት ለመሳተፍ በሆስፒታሉ ወይም በኮሌጁ ውስጥ ወይም በዩኒቨርሲቲ ውስጥ በተለየ መልኩ ሊታዩ ወይም

ሊገለሉ አይችሉም። ምንም እንኳን አሁን በጥናቱ ለመሳትፍ ቢፈልጉም ውሳኔዎን በሌላ ጊዜ መቀየር ይችላሉ። ተሳትፎዎን በማናቸውም ጊዜ ሊያቋርጡ ይችላሉ።

**በጥናቱ በመሳተፍ ሊኖር የሚችል ስጋት እና የሚኖረው ጥቅም**

በዚህ ጥናት በመሳተፍ ከአፍንጫ ላይ በጥጥ የሚወሰዱ ናሙናዎች ከሚያመጣው እጅግ አንስተኛ ያለመመቸት ስሜት በስተቀር ሌላ ጉዳት በፍፁም አያስከትልም።

በዚህ ጥናት በመሳተፍዎ ለርሶ በቀጥታ የሚይስገኘው ጥቅም የለም። ነገር ግን እርሶ በዚህ ጥናት በመሳተፍዎ በሆስፒታላችን ውስጥ መድሀኒት የተላመደን የስታፊሎኮከስ አውረስ ባክቴሪያን ለመቆጣጠር እና እቅድ ለማውጣት ይጠቅማል። ጥናቱ በሆስፒታላችን ውስጥ ለሚከሰቱ ኢንፌክሽንን ለመቆጣጠርና ለመከላከል ለሚደረጉ ሰልጠናዎችን ለማቀድ እና ለማዘጋጀት ትልቅ ግብአት ይሆናል።

**ሚስጥር ስለመጠበቅ**

በዚህ ጥናት ውስጥ በመሳተፍ የሚሰጡትን መረጃ ሁሉ በሚስጥር ይይዛል። ተመራማሪዎቹ የርሶን መረጃ በዚህ ጥናት ላይ ከተጠቀሰው አላማ ውጪ አያውሉም። እርሶን የሚገልፁ መረጃዎችን ስሞዎንም ቢሆን በሪፖርቱ ውስጥ አይካተትም ። ከእርሶ የምናገኘውን መረጃ በሚቆለፍ ሳጥን ውስጥ እንዲቀመጥ ይደረጋል። በኮምፒተር ውስጥም በሚስጥር ቁልፍ የሚያዝ ይሆናል። ጠቅላላ መረጃው ለሶስት ተከታተይ አመታት ህግ እና ደንብ በሚፈቅደው መሰረት ይቀመጣል።

**ስለ ጥናቱ ጥያቄ ካለዎት**

ስለጥናቱ ማንኛውንም ጥያቄ መጠየቅ ይቻላል ። ጥያቄ ከለዎት አቶ ከሱ ደስታ ቱሉን በስልክ ቁጥር 0911107099 ወይም በኢሜል Kassudesta2020@gmail.com መጠየቅ ይቻላል። በግልዎ ስለራስዎ መብት መጠየቅና መረዳት ካስፈለግዎ በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የምርምርና ስነምግባር ቢሮ በስልክ ቁጥር 118961396 በመደወል መጠየቅ ይችላሉ።

## Appendix VI. የስምምነት ቅጽ (Consent form for HCWs)

ከላይ የተገለፀውን መረጃ በአግባቡ የተረዳሁ እና በዚህ ጥናት ላይ ለመሳተፍ እና ውሳኔ ላይ እንድደርስ ጠቅሞኛል። በዚህም መሰረት በዚህ ጥናት ስሳተፍ ከአፈንጫ ጫፍ ላይ ከተንቀሳቃሽ ስልኬ ላይ እና ከጋውኔ ላይ በጥጥ ተጠርጎ የሚወሰድ ናሙና ለመስጠት እንዲሁም ስለራሴ የሚገልፅ መረጃ ለመስጠት ተስማምቻለሁ። በተጨማሪም ከእውቀት ፤ አመለካከት እና ተግባር ጋር የተያያዘ መጠይቅ ለመሙላት እና ለመስጠት ተስማምቻለሁ።

**የተሳታፊ ስም** ------------------------------------------------------------ **ቀን**---------------

**ፊርማ**--------------------------------

**የስምምነት ቅፁን ያስሞላው ሰው ስም** ----------------------------------**ቀን** -------

**ፊርማ**-------------------------------

## Appendix VII. አስተዳደር ሰራተኞች ለሆኑ ተሳታፊዎች የሚሰጥ መረጃ (Information sheet for Administrative staff )

**የፕሮጀክቱ ርዕስ** ፡- መድሀኒት የተላመደ አስታፊሎኮከስ አውረስ (*Staphylococcus aureus* ) መጠን እና ተያያዥ ችግሮች በጥቁር አንበሳ እስፔሻላይዝድ ሆስፒታል ከታካሚዎች: ከጤና ባለሙያዎች: ከአስተዳደር ሰራተኞች እና ከሚጠቀሙባቸው አንዳንደ ዕቃዎች ላይ ምን እንደሚመስል ያጠናል ።

የተከበራችሁ የጥናቱ ተሳታፊዎች ከላይ በርዕሱ በተጠቀሰው ጥናት እንዲሳተፉ **እየጋበዝን** ጥናቱን የሚያደርገው ግለሰብ፣ አቶ ካሱ ደስታ ቱሉ ሲሆኑ እሳቸውም በጤና ሣይንስ ኮሌጅ በህክምና ትምህርት ቤት የሜዲካል ማይክሮ ባዮሎጂ ፓራሳይቶሎጂ እና **የ**ኢሚኖሎጂ ትምህርት ክፍል የ3ተኛ ዲግሪ ተማሪ ነው።

**የጥናቱ አላማ**

የዚህ ጥናት ዋና አላማ መድሀኒት የተላመዱ የእስታፊሎኮከስ አውረስ ባክቴሪያ መጠን በጤና ባለሙያዎች ዘንድ በአስተዳደር ሰራተኞች ላይ እና በአንዳንድ መጠቀሚያ እቃዎች ላይ ማለትም የሞባይል ቀፎዎች ላይ ጋዋን ላይ ምን ያህል እንደሆነ ለማወቅ ነው። በተጨማሪም የጤና ባለሙያው ለመድሀኒት ስለተላመደ እስታፊሎኮከስ አውረስ መነሻ የሚተላለፍበት ሁኔታ እና ስለመቆጣጠሪያው ያላቸው የእውቀት ፤ የአመለካከት :የተግባራዊ ልምድን ለመገምገምና ለማወቅ ጭምር ነው።

**የጥናቱ አካሄድ**

በጥናቱ ከተስማሙ የሚከተሉትን መረጃዎች እና ከአፍንጫ ጫፍ ላይ እና ከተንቀሳቃሽ ስልኮ ላይ ናሙና መስጠት ይጠበቅቦታል ። ስለራስዎ የተወሰነ መረጃ እንዲሰጡን በመጠይቅ መረጃውን ለመስጠት ወደ 10 ደቂቃዎች ብቻ የሚወስድ ይሆናል ። ከአፍንጫ ጫፍ በጥጥ ተጠርጎ ለሚወሰዱ ናሙናዎች እንዲሁም ከሚጠቀሙበት ተንቀሳቃሽ የስል ቀፎ ላይ በጥጥ ተጠርጎ ለሚወሰድ ናሙና ፈቃደኛ ተሳታፊ እንዲሆኑ ይጠበቃል ።

**በዚህ ጥናት ላይ መሳተፍ በራስ ፈቃድ ላይ የተመሰረተ ስለመሆኑ**

በዚህ ጥናት ላይ መሳተፍ የርሶ ነፃ ፍቃድ ላይ የተመሰረተ ነው፤፤ ማንም ሰው እርሶ በዚህ ጥናት ላይ ለመሳተፍ ወይም ላለመሳተፍ ውሳኔዎን ያከብራል።በዚህ ጥናት ለመሳተፍ በሆስፒታሉ ወይም በኮሌጁ ውስጥ ወይም በዩኒቨርሲቲ ውስጥ በተለየ መልኩ ሊታዩ ወይም

ሊገለሉ አይችሉም። ምንም እንኳን አሁን በጥናቱ ለመሳትፍ ቢፈልጉም ውሳኔዎን በሌላ ጊዜ መቀየር ይችላሉ። ተሳትፎዎን በማናቸውም ጊዜ ሊያቋርጡ ይችላሉ።

**በጥናቱ በመሳተፍ ሊኖር የሚችል ስጋት እና የሚኖረው ጥቅም**

በዚህ ጥናት በመሳተፍ ከአፍንጫ ላይ በጥጥ የሚወሰዱ ናሙናዎች ከሚያመጣው እጅግ አንስተኛ ያለመመቸት ስሜት በስተቀር ሌላ ጉዳት በፍፁም አያስከትልም። በዚህ ጥናት በመሳተፍዎ ለርሶ በቀጥታ የሚይስገኘው ጥቅም የለም። ነገር ግን እርሶ በዚህ ጥናት በመሳተፍዎ በሆስፒታላችን ውስጥ መድሀኒት የተላመደን የስታፊሎኮከስ አውረስ ባክቴሪያን ለመቆጣጠር እና እቅድ ለማውጣት ይጠቅማል። ጥናቱ በሆስፒታላችን ውስጥ ለሚከሰቱ ኢንፌክሽንን ለመቆጣጠርና ለመከላከል ለሚደረጉ ሰልጠናዎችን ለማቀድ እና ለማዘጋጀት ትልቅ ግብአት ይሆናል።

**ሚስጥር ስለመጠበቅ**

በዚህ ጥናት ውስጥ በመሳተፍ የሚሰጡትን መረጃ ሁሉ በሚስጥር ይይዛል። ተመራማሪዎቹ የርሶን መረጃ በዚህ ጥናት ላይ ከተጠቀሰው አላማ ውጪ አያውሉም። እርሶን የሚገልፁ መረጃዎችን ስሞዎንም ቢሆን በሪፖርቱ ውስጥ አይካተትም ። ከእርሶ የምናገኘውን መረጃ በሚቆለፍ ሳጥን ውስጥ እንዲቀመጥ ይደረጋል። በኮምፒተር ውስጥም በሚስጥር ቁልፍ የሚያዝ ይሆናል። ጠቅላላ መረጃው ለሰስት ተከታተይ አመታት ህግ እና ደንብ በሚፈቅደው መሰረት ይቀመጣል።

**ስለ ጥናቱ ጥያቄ ካለዎት**

ስለጥናቱ ማንኛውንም ጥያቄ መጠየቅ ይቻላል ። ጥያቄ ከለዎት አቶ ከሱ ደስታ ቱሉን በስልክ ቁጥር 0911107099 ወይም በኢሜል Kassudesta2020@gmail.com መጠየቅ ይቻላል። በግልዎ ስለራስዎ መብት መጠየቅና መረዳት ካስፈለግዎ በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የምርምርና ስነምግባር ቢሮ በስልከ ቁጥር 118961396 በመደወል መጠየቅ ይችላሉ።

**Appendix VIII. የስምምነት ቅጽ አስተዳደር ሰራተኞች ለሆኑ ተሳታፊዎች (Consent form for Administrative staffs)**

ከላይ የተገለፀውን መረጃ በአግባቡ የተረዳሁ እና በዚህ ጥናት ላይ ለመሳተፍ እና ውሳኔ ላይ እንድደርስ ጠቅሞኛል። በዚህም መሰረት በዚህ ጥናት ስሳተፍ ከአፈንጫ ጫፍ ላይ እና ከተንቀሳቃሽ ስልኬ ላይ በጥጥ ተጠርጎ የሚወሰድ ናሙና ለመስጠት እንዲሁም ስለራሴ የሚገልፅ መረጃ ለመስጠት ተስማምቻለሁ።

**የተሳታፊ ስም--** ------------------------------------------------------------ **ቀን---------------**

**ፊርማ--------------------------------**

**የስምምነት ቅፁን ያስሞላው ሰው ስም** ----------------------------------- **ቀን** ----------------
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**ፊርማ-------------------------------**

**Appendix IX. Questionnaires for survey of knowledge attitude, perceived practice and information source for MRSA among health care personnel in Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia. You can keep a right mark ( √ ) on the bracket and write your response in the blank space.**

Code No. ___________________________________________

**IX. a. General Information**

1. Sex [ ] M [ ] F
2. Age in years [ ]
3. Marital status: [ ] Single [ ] Married [ ] Divorced
4. Current professional category

   [ ] Medical Doctor [ ] Nurse

   [ ] Medical Laboratory personnel [ ] Pharmacy personnel

   [ ] Others, Specify ___________________________________

5. Educational status

   [ ] Diploma [ ] Degree

   [ ] General Practioner [ ] MSc [ ] Specialist

   [ ] Others , Specify ___________________________________

6. Year of service as a health care worker

   [ ] 1-2 yrs [ ] 3-4 yrs

   [ ] 5-7 yrs [ ] 8-10 yrs [ ] >10 yrs

7. How many gowns do you have? 1 [ ] 2 [ ] 3 [ ] 4 [ ] 5 [ ] 6 or more [ ]

8. Types of gowns Short sleeves 1.[ ] 2. Long sleeves [ ]

9. Frequency of changing gown/s 1.[ ] Daily 2. [ ] every other day 3. [ ] weekly 4. [ ]

   every two weeks 5. [ ] others specify ____________________________

10. Type of cell phone 1. [ ] Ordinary cell phone 2. [ ] Smart phone [ ]

11. Does the cell phone has cover 1.[ ] Yes 2. [ ] No

12. Do you use your cell phone in the operating rooms or during procedures or while you assist, examine or care patients ? 1. [ ] Yes 2. [ ] No 3. [ ] Some times

13. Source of information regarding MRSA (one or more responses)

    1.[ ] Radio and television

    2. [ ] The hospital itself

    3. [ ] Colleagues

    4. [ ] Health personnel

    5. [ ] Training

6. [ ] Personal experience
7. [ ] Others specify ___________________________

14. Current work in wards / departments

1.[ ] OPD 2. [ ] Laboratory 3. [ ] Pharmacy 4. [ ] internal medicine 5. [ ] . Obstetrics and gynecology [ ] 6.Pediatrics/ Neonatology [ ] 7. Surgery [ ] 8. Emergency [ ] 9. Orthopedics [ ] 10. ICUs [ ] 11. others ( specify)____________________________

15. Have you ever taken any short term training about MRSA and other Multidrug resistance bacteria ?

1.[ ] Yes 2. [ ] No

16. If Yes, when

1.[ ] Last year 2.[ ] this year 3. [ ] before 3 years back

4. [ ] Before 5 yrs back [ ] 5. Other (mention the date)_____________

17. Are there any guidelines and leaf lets about MRSA related matter in your work place ?

1.[ ] Yes 2. [ ] No 3. [ ] do not know

18. Do you feel that there are sufficient hand hygiene materials like soap, water, antiseptics and functional sinks in your working place?

1.[ ] Yes 2. [ ] No 3. [ ] do not know

19. Have you ever been admitted in a hospital ? , if yes when _______( date , year)

1.[ ] Yes 2. [ ] No 3. [ ] do not know

20. Have you had any surgical intervention during the last three years ?

1.[ ] Yes 2. [ ] No 3. [ ] do not know

21. Have you had any surgical intervention for the last one year ?

1. [ ] Yes 2. [ ] No 3.[ ] do not know

**IX. b. Knowledge of health care personnel towards MRSA and related questions**

1. The most important and effective measure to prevent transmission of MRSA infection is
   1. [ ] Use of prophylactic drugs
   2. [ ] Environmental hygiene
   3. [ ] Proper hand hygiene
2. What is MRSA ( Methicilin resistant Staphylocoous aureus )
   1. [ ] *Staphylocoous auerus* which is resistant to Methicilin drug alone

2. [ ] *Staphylocoous auerus* which is resistant at least to penicillin and cephalosporions groups

3. [ ] *Staphylocoous auerus* which is sensitive to all drugs

3. MRSA could be transmitted from health care personnel to patients

1. [ ] Correct

2. [ ] Incorrect

4. Clean hands protect MRSA related illness in health care institutions

1. [ ] Correct

2. [ ] Incorrect

5. Hand washing practice does not help to interrupt transmission of MRSA in hospital set up

1. [ ] Correct

2. [ ] Incorrect

6. It is not fair to decolonize MRSA carrying patients before surgical procedures

1. [ ] Correct

2. [ ] Incorrect

7. Contaminated hands , cell phone and gowns can carry MRSA and or other germs and be transmitted to patients

1. [ ] Correct

2. [ ] Incorrect

8. If TASH plan decolonization of patients with MRSA ( Nasal) which drugs is more appropriate

1. [ ] Hot water bath 2. [ ] Mupirocin 3. [ ] TTC ointment

**IX. c. Attitudes of health care personnel towards MRSA related issues at Tikur Anbesa Specialized Hospital**

1. System wide approach about MRSA prevention is important in TASH

1. [ ] Strongly agree 2. [ ] Agree 3. [ ] Undecided 4.[ ] Disagree

5. [ ] strongly disagree

2. Health care workers can carry MRSA and they could transmit it to patients during handling in the hospitals ?

1. [ ] Strongly agree 2. [ ] Agree 3. [ ] Undecided 4. [ ] Disagree

5. [ ] strongly disagree

3. Adherence of health care workers on personal protective devices PPD is key factors for control of MRSA related illness / infection ?

1. [ ] Strongly agree 2. [ ] Agree 3. [ ] Undecided 4. [ ] Disagree

5. [ ] strongly disagree

4. Use of frequent hand hygiene could causes irritation on hands and does not halt MRSA transmission

1. [ ] Strongly agree 2.[ ] Agree 3. [ ] Undecided 4.[ ] Disagree
5. [ ] strongly disagree

5. All health care workers should be screened for MRSA colonization during outbreak condition

1. [ ] Strongly agree 2. [ ] Agree 3. [ ] Undecided 4. [ ] Disagree
5. [ ] strongly disagree

6. Cell phone and gown could carry MRSA and it could be a source of infection for Patients

1.[ ] Strongly agree 2.[ ] Agree 3. [ ] Undecided
4.[ ] Disagree 5. [ ] strongly disagree

7. Health care personnel who is colonized with MRSA should be decolonized before patient contact

1.[ ] Strongly agree 2.[ ] Agree 3. [ ] Undecided
4. [ ] Disagree 5.[ ] strongly disagree

8. If health care workers had MRSA in the nares the person can involve in minor Surgical intervention.

1. [ ] Strongly agree 2.[ ] Agree 3. [ ] Undecided
4. [ ] Disagree 5. [ ] strongly disagree

9. When " you feel ill" , you should not be isolated from work place.

1. [ ] Strongly agree 2.[ ] Agree 3. [ ] Undecided
4. [ ] Disagree 5. [ ] strongly disagree

10. Safety measures taken by health care personnel does not minimize MRSA rate in a hospital

1. [ ] Strongly agree 2.[ ] Agree 3. [ ] Undecided
4. [ ] Disagree 5. [ ] strongly disagree

**IX. d. Self reported practice of health care personnel towards MRSA colonization and intervention measures in TASH.**

1. Wash hands before and after patient contact

1.[ ] Never 2.[ ] Seldom 3.[ ] Sometimes 4. [ ] Often 5.[ ] Very often

2. Wear aprons and gowns during contacts with patients.

1[ ] Never 2.[ ] Seldom 3.[ ] Sometimes 4.[ ] Often 5.[ ] Very often

3. Wear mask during caring / examining patients

   1.[ ] Never 2.[ ] Seldom 3.[ ] Sometimes 4.[ ] Often 5.[ ] Very often

4. Trained about hand hygiene and other personal protective devices (PPDs) methods to prevent MRSA transmission

   1.[ ] Never 2.[ ] Seldom 3.[ ] Sometimes 4.[ ] Often 5.[ ] Very often

5. Clean gowns and other personal protective devices

   1.[ ] Never 2.[ ] Seldom 3.[ ] Sometimes4. [ ] Often 5.[ ] Very often

6. Does not clean cell phone and or cell phone cover

   1.[ ] Never 2.[ ] Seldom 3.[ ] Sometimes 4.[ ] Often 5.[ ] Very often

7. Encourage other staffs to adhere on cleaning of gowns and cell phone/cover.

   1.[ ] Never 2.[ ] Seldom 3.[ ] Sometimes4. [ ] Often 5.[ ] Very often

8. Consult appropriate personnel about MRSA transmission while you had infection.

   1.[ ] Never 2.[ ] Seldom 3.[ ] Sometimes 4.[ ] Often 5.[ ] Very often

9. Perform / request regular medical check up

   1.[ ] Never 2.[ ] Seldom 3.[ ] Sometimes 4. [ ] Often 5.[ ] Very often

10. Teach families and other close friends that you might transmit MRSA and other related infection to them

    1.[ ] Never 2.[ ] Seldom 3.[ ] Sometimes4. [ ] Often 5.[ ] Very often

**Appendix X. Questionnaires for survey of MRSA colonization study for administrative staff of CHS, TASH ,AAU, Addis Ababa, Ethiopia. You can keep a right mark (√ ) on the bracket and write your response in the blank space.**

Code No. ___________________________________________

**I. General Information (You can keep a right mark ( √ ) on the bracket and write your response in the blank space).**

1. Sex [ ] M [ ] F

2. Age in years [ ]

3. Marital status: [ ] Single [ ] Married [ ] Divorced

4. Current professional category ( Please Specify) ______________________________

5. Educational status

   [ ] Diploma [ ] Degree [ ] MSc [ ] Certificate

   [ ] Others , Specify _______

6. Year of service as a administrative staffs

   [ ] 1-2 yrs [ ] 3-4 yrs [ ] 5-7 yrs [ ] 8-10 yrs [ ] >10 yrs

7. Type of cell phone 1. [ ] Ordinary cell phone 2. [ ] Smart phone [ ]

8. Is the cell phone has cover 1.[ ] Yes 2. [ ] No

9. Current working departments / units __________________________________

10. Have you ever admitted in hospital? , if yes when ______________( date , year)

   1.[ ] Yes 2. [ ] No 3. [ ] do not know

11. Have you had any surgical intervention for the last three years?

   1.[ ] Yes 2. [ ] No 3. [ ] do not know

12. Have you had any surgical intervention for the last one year ?

   1. [ ] Yes 2. [ ] No 3.[ ] do not know

## Appendix XI. Additional tables

**Table 3.27. Overall Knowledge score of HCWs with the variability of gowns and mobile phone use in TASH, 2019, Addis Ababa, Ethiopia, 2019**

| Variables | Over all Knowledge status of HCWs | | |
|---|---|---|---|
| | **Good no. ( %)** | **Poor no. ( %)** | **$X^2$ p value** |
| **No. Gowns (n= 587)** | | | |
| One | 71 ( 12.09 %) | 17 ( 2.89 %) | |
| Two | 202 ( 34.41 %) | 29 ( 4.94 %) | |
| Three | 150 ( 25.55 %) | 25 ( 4.25 %) | 2.92; 0.712 |
| Four | 50 ( 8.51 %) | 8 ( 1.36 %) | |
| Five | 19 ( 3.23 %) | 4 ( 0.68 %) | |
| Six and above | 11 ( 1.87 %) | 1( 0.7 %) | |
| **Type of gown ( n= 587)** | | | |
| Short | 176 ( 29.98 %) | 33 ( 5.62 %) | |
| Long | 295 ( 50.25 %) | 42 ( 7.5 %) | 3.26; 0.196 |
| Both | 32 ( 5.45 %) | 9 ( 1.53 %) | |
| **Frequency of changing Gown (n=585)** | | | |
| Daily | 7 ( 1.19 %) | 4 ( 0.68 %) | |
| Every other day | 112 ( 19.14 %) | 31 ( 5.29 %) | 15.08; 0.005 |
| Weekly | 247 ( 42.22 %) | 34 ( 5.81 %) | |
| Every other week | 89 ( 15.21 %) | 10 ( 1.70 %) | |
| As required | 47 ( 8.03 %) | 4 ( 0.68 %) | |
| **Type of cell phone (n= 586)** | | | |
| Touch pad / ordinary | 74 ( 12.62 %) | 23 ( 3.92 %) | |
| Smart | 421 ( 71.84 %) | 54 ( 9.21 %) | 24.85; 0.000 |
| Both | 7 ( 1.19 %) | 7 ( 1.19 %) | |
| **Cell phone cover ( 582)** | | | |
| Present | 235 ( 40.37 %) | 42 ( 7.21 %) | 0.684; 0.408 |
| Absent | 266 ( 45.70 %) | 39 ( 6.70 %) | |
| **Use of Mobile phone during clinical practice (n= 582)** | | | |
| Yes | 347 ( 59.62 %) | 40 ( 6.87 %) | |
| No | 91 ( 15.63 %) | 20 ( 3.43 %) | 14.31; 0.001 |
| Sometimes | 63 ( 10.82 %) | 21 ( 3.60 %) | |

**Table 3.28. Over all Knowledge score of HCWs with source of information and hospital exposure at TASH, Addis Ababa, Ethiopia, 2019**

| Variables | Over all Knowledge status of HCWs | | |
|---|---|---|---|
| | **Good no. ( %)** | **Poor no. ( %)** | **$X^2$ p value** |
| **Source of information for MRSA (n=585)** | | | |
| Radio and TV | 31 ( 5.29 %) | 13 ( 2.22 %) | |
| Hospital itself | 104 ( 17.77 %) | 19 ( 3.24 %) | |
| Colleagues | 45 ( 7.69 %) | 6 ( 1.02 %) | 20.817; 0.013 |
| Health personnel | 80 ( 13.67 %) | 11( 1.88 %) | |
| Training | 52 ( 8.88 %) | 9 ( 1.53 %) | |
| Personal experience | 60 ( 10.25 %) | 14 ( 2.39 %) | |
| Other books, journals | 27 ( 4.61 %) | 3 ( 0.51 %) | |
| 2 responses of above | 59 ( 10.08 %) | 1 ( 0.17 %) | |
| 3 responses | 31 ( 5.29 %) | 3 (0.51 %) | |
| 4 or more responses | 13 ( 2,22 %) | 4 ( 0.68 %) | |
| **Training on MRSA (n= 584)** | | | |
| Yes | 48 ( 8.21 %) | 22 ( 3.76 %) | 19.92; 0.000 |
| No | 454 ( 77.73 %) | 60 ( 10.27 %) | |
| **Presence of guidelines and or leaf lets ( n= 584)** | | | |
| Yes | 57 ( 9.76 %) | 24( 4.10 %) | 19.063; 0.000 |
| No | 379 ( 64.89 %) | 53 ( 9.07 %) | |
| Do not know | 65 ( 11.13 %) | 6 ( 1.02 %) | |
| **Availability of sufficient hand hygiene materials ( n=585)** | | | |
| Present | 158 ( 27.00 %) | 31( 5.29 %) | 19.425; 0.000 |
| Absent | 338 ( 57.77 %) | 45 ( 7.69 %) | |
| Do not know | 6 ( 1.02 %) | 7 ( 1.19 %) | |
| **Hospital admission ( n=586)** | | | |
| Yes | 79 ( 13.48 %) | 20 ( 3.41 %) | 3.80; 0.149 |
| No | 420 ( 71.67 %) | 63 ( 10.75 %) | |
| Do not know | 3 ( 0.51 %) | 1( 0.17 %) | |
| **Surgical intervention (n= 584)** | | | |
| Yes | 56 ( 9.58 %) | 11 ( 1.88 %) | |
| No | 441 ( 75.51 %) | 70 ( 11.98 %) | 6.6; 0.037 |
| Do not know | 3( 0.51 %) | 3 ( 0.51 %) | |

**Table 3.31. Overall attitude score of HCWS with use of gown, cell phone and at TASH, 2019, Addis Ababa, Ethiopia, 2019**

| Variables | Over all Knowledge status of HCWs | | |
|---|---|---|---|
| | Good attitude no. ( %) | Poor attitude no. ( %) | $X^2$ p value |
| **No. Gowns (n= 584)** | | | |
| One | 46 ( 7.87 %) | 41 ( 7.02 %) | |
| Two | 131 ( 22.43 %) | 99 ( 16.95 %) | |
| Three | 98 ( 16.78 %) | 76 ( 13.01 %) | 4.75; 0.446 |
| Four | 25 ( 4.28 %) | 33 ( 5.65 %) | |
| Five | 13 ( 2.22 %) | 10 ( 1.71 %) | |
| Six and above | 5 ( 0.85 %) | 7 ( 1.19 %) | |
| **Type of gown (n= 584)** | | | |
| Short | 104 ( 17.80 %) | 104 ( 17.80 %) | |
| Long | 194 ( 33.21 (%) | 142 ( 24.31 %) | 3.44; 0.179 |
| Both | 20 ( 3.42 %) | 20 ( 3.42 %) | |
| **Frequency of changing Gown (n=582)** | | | |
| Daily | 2 ( 0.34 % ) | 9 ( 1.54 %) | |
| Every other day | 72 ( 12.37 %) | 71 ( 12.19 %) | 13.66 ; 0.008 |
| Weekly | 151 ( 25.94 %) | 128 ( 21.99 %) | |
| Every other week | 55 ( 9.45 %) | 43 ( 7.38 %) | |
| As required | 37 ( 6.35 %) | 14 ( 2.40 % ) | |
| **Type of cell phone (n= 583)** | | | |
| Touch pad / ordinary | 36 ( 6.17 %0 | 61 ( 10.46 %) | |
| Smart | 277 ( 47.51 %) | 195 ( 33.44 ) | 17.15; 0.000 |
| Both | 5 ( 0.85 %) | 9 ( 1.54 %) | |
| **Cell phone cover (n= 579)** | | | |
| Present | 138 ( 23.83 %) | 138 ( 23.83 %) | 4.45 ; 0.035, OR/CI **1.2** (1.01-1.44) |
| Absent | 178 ( 30.74 %) | 125 ( 21.58 %) | |
| **Use of Mobile phone during clinical practice (n = 579)** | | | |
| Yes | 222 ( 38.34 %) | 162 ( 27.97 %) | |
| No | 49 ( 8.46 %0 | 62 (10.70 %) | 6.52 ; 0.038 |
| Sometimes | 45 ( 7.77 %) | 39( 6.73 %) | |

**Table 3.32. Over all attitude score of HCWs with source of information and hospital exposure at TASH, Addis Ababa, Ethiopia, 2019**

| Variables | Over all attitude of HCWs | | |
|---|---|---|---|
| | **Good no. ( %)** | **Poor no. ( %)** | **$X^2$ p value** |
| **Source of information for MRSA(n=583)** | | | |
| Radio and TV | 20 ( 3.43 %) | 24 ( 4.11 %) | |
| Hospital itself | 59 ( 10.12 %) | 64 ( 10.97 %) | |
| Colleagues | 28 ( 4.80 %) | 23 ( 3.94 %) | 30.17; 0.00 |
| Health personnel | 40 ( 6.86 %) | 50 ( 8.57 %) | |
| Training | 42 ( 7.20 %) | 19 ( 3.25 %) | |
| Personal experience | 32 ( 5.48 %) | 42 ( 7.20 %) | |
| Other books, journals | 20 ( 3.43 %) | 10 ( 1.88 %) | |
| 2responses of the above | 39 ( 6.68 %) | 21 ( 3.60 %) | |
| 3 responses of the above | 26 ( 4.45 %) | 7 ( 1.20 %) | |
| 4 or more responses of above | 12 ( 2.05 %) | 5 ( 0.85 %) | |
| **Training on MRSA (n= 582)** | | | |
| Yes | 31( 5.32 %) | 39 ( 6.7 %) | 3.32 ; 0.068 |
| No | 286 ( 49.14 %) | 226( 38.8 %) | **OR/CI:1.26** (1.002-1.589) |
| **Guidelines and or leaf lets ( n= 582)** | | | |
| Yes | 27 ( 4.63 %) | 54( 9.27 %) | 16.96; 0.000 |
| No | 250 ( 42.95 %) | 181( 31.09 %) | |
| Do not know | 40 ( 6.87 %) | 30 ( 5.15 %) | |
| **Availability of sufficient hand hygiene materials ( n= 583)** | | | |
| Present | 84 ( 14.40 %) | 105 (18.01 %) | 24.52; 0.000 |
| Absent | 233 ( 39.96 %) | 149 ( 25.55 %) | |
| Do not know | 1( 0.17 %) | 11( 1.88 %) | |
| **Hospital admission ( n= 584)** | | | |
| Yes | 58 ( 9.93 %) | 41 (7.02 %) | 4.32; 0.115 |
| No | 256 ( 43.83 %) | 225 ( 38.52 %) | |
| Do not know | 4 (0.68 %) | 0 ( 0%) | |
| **Surgical intervention ( n= 582)** | | | |
| Yes | 35( 6.01 %) | 32 ( 5.49 %) | |
| No | 280 ( 48.10 %) | 229 (39.34 %) | 1.25 ; 0.529 |
| Do not know | 2 ( 0.34 %) | 4 (0.68 %) | |

**Table 3.35. Over all practice score with gown use by HCWs of TASH, Addis Ababa, Ethiopia, 2019**

| Variables | Over all Practice status of HCWs | | |
|---|---|---|---|
| | Good practice no. ( %) | Poor practice no. ( %) | $X^2$ p value |
| **No. of Gowon (n= 582)** | | | |
| One | 58 (9.96 %) | 28 ( 4.81 %) | |
| Two | 157 ( 26.97 %) | 71 (12.19 %) | |
| Three | 116 ( 19.93 %) | 59 (10.13 %) | 4.65; 0.460 |
| Four | 44 (7.56 %) | 14 ( 2.40 %) | |
| Five | 13 (2.23 %) | 10 ( 1.71 %) | |
| Six and above | 10 ( 1.71 %) | 2 ( 0.34%) | |
| **Type of Gown (n= 582)** | | | |
| Short sleeves | 149 ( 25.60 %) | 58 (9.96 %) | 3.45; 0.178 |
| Long sleeves | 219 (37.62 %) | 116 ( 19.93 %) | |
| Both type | 30 ( 5.15 %) | 10 ( 1.71 %) | |
| **Frequency of changing gown (n=580)** | | | |
| Every day | 8 (1.37 %) | 2 (0.34 %) | |
| Every other day | 105 (18.10 %) | 37 (6.37 %) | |
| Weekly | 187 (32.24 %) | 92 ( 15.86 %) | 5.39; 0.249 |
| Every other week | 67 (11.55 %) | 32 (5.51 %) | |
| As required | 29 (5.0%) | 21 (3.62 %) | |
| **Current working department (n=580)** | | | |
| OPD | 51 (8.79 %) | 14 (2.41 %) | |
| Laboratory | 37 (6.37 %) | 11 (1.89 %) | |
| Pharmacy | 14 (2.41 %) | 3 (0.51 %) | |
| Medical wards | 32 (5.51 %) | 14 (2.41 %) | 21.19; 0.031 |
| Obstetrics & gynaecology | 51 (8.79 %) | 38 (6.55 %) | |
| Paediatrics & neonatology | 42 (7.24 %) | 22 (3.79 %) | |
| Surgery | 41 (7.06 %) | 27 (4.65 %) | |
| Emergency | 22 (3.79 %) | 9 (1.55 %) | |
| Orthopedics | 15 (2.58 %) | 3 (0.51 %) | |
| ICU | 42 (7.24 %) | 16 (2.75 %) | |
| Radiology/ imaging | 37 (6.37 %) | 14 (2.41 %) | |
| Others | 12 ( 2.06 %) | 13 (2.24 %) | |

## Appendix XII. Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by Department of Medical Microbiology, Immunology and Parasitology, CHS, Addis Ababa University, Ethiopia

The Department of Medical Microbiology, Immunology and Parasitology, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia,will facilitate the transfer of research materials (bacterial isolates and derivatives (ACDP Hazard Group 2) (the Research Material) related to the stated protocol under the thesis title "Burden of Methicillin *Resistant Staphylococcus aureus (*MRSA) and associated factors at TASH: Evidence from colonization of patients, health care workers, administrative staffs and selected inanimate objects"

**<u>Provider</u>:**

Department of Medical Microbiology, Immunology and Parasitology,
College of Health Sciences,
Addis Ababa University,
P.O. Box 9086, Addis Ababa, Ethiopia.

**<u>Recipient:</u>**

The University of Sussex, whose principal administrative offices are at Sussex House, Falmer, Brighton, BN1 9RH UK
Tel: +44 1273 877889, Fax: +44 1273 877884

The Provider agrees to transfer to the Recipient the Research Material which will be characterized using different molecular techniques.

1. The Research Material will only be used for research purposes as described in the protocol by the Recipient's investigator in a designated laboratory for the research project described below, under suitable containment conditions. This Research Material will not be used for commercial purposes such as screening, production or sale for which a commercialization license may be required. The Recipient agrees to comply with all National and International guidelines rules and regulations applicable to the Research Project and the handling of the Research Material.

a) Are the Research materials of human origin?

Yes ☑ No ☐

Description: Bacterial isolates (ACDP Hazard Group 2) from human samples.

b) If yes, are they collected according to the details in the protocol and in adherence to National Health Research Ethics Review Committee (NERC), IRB of CHS, AAU recommendations and their approval?

Yes ☑ No ☐

**2.** The Research Material and its derivatives will be used by Recipient's investigator solely in connection with the following research project:

**"**Burden of Methicillin *Resistant Staphylococcus aureus (*MRSA) and associated factors at TASH: Evidence from colonization of patients, health care workers, administrative staffs and selected inanimate objects".

3. In all presentations or written publications concerning the research project, the Recipient will seek agreement from the Provider and acknowledge the Provider's contribution of the Research Material unless requested otherwise.

4. The Research Material represents a significant contribution on the part of the Provider and is considered proprietary to the Provider. The Recipient therefore agrees to retain control over this research material and further agrees not to transfer the research material to other people not under her/his direct supervision without advance written approval of Provider. The Research Material will be disposed of as agreed upon per protocol at the end of completion of the project.
5. The Provider is responsible for shipment of the Research Material and shall use a courier with suitable skill and experience to safely transport the Materials in accordance with all applicable laws.
6. The Provider does not take any responsibility for loss, damage, wastage or spoilage of the Research Material during or after shipment to the address provided by the Recipient under conditions agreed to in the protocol on shipment of the isolates. This Research Material is provided as a service to the research community. The material is supplied to the recipient with no warranties, express or implied, including any warranty of mechantability or fitness for a particular purpose. The Provider makes no representations that the use of the Research Material will not infringe any patent or proprietary right of third parties.

7. The Recipient shall notify the Provider in witting of any intention, improvement, modification discovery or development to the material or the information made by the recipient or parties, collaborating with the recipient, here in after referred to as "invention". Nothing in this agreement shall, however, be construed as conveying to the Provider any rights under any patents or other intellectual property to such invention, other than as explicitly provided herein. At their discretion the Provider shall be entitled to receive sample of any materials derived from the materials for its own research and evaluation purposes only.

8. The under-signed Provider and Recipient expressly certify and affirm that the contents of any statements made herein are truthful and accurate.

9. The Provider acknowledges the Recipient does not warrant the validity of any of the results generated by the Recipient through the use of the Research Material and the Provider shall rely upon those results at their own risk.

10. The Provider maintains ownership rights of the Research Material and its derivatives unless stated otherwise.

11. The Provider will retain a copy (aliquot) of bacterial isolates sent abroad as much as possible, as stipulated by Ethiopian National Ethics Review Committee Guidelines for Local Research needs.

**Material Transfer Agreement Signature page**

The under-signed Provider and Recipient expressly certify and affirm that the contents of statements made herein are truthful and accurate.

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Signature: ______________ Date: 3rd November 2017

**Appendix XIII. Publications from the PhD Extrcat** .

**ORIGINAL RESEARCH 1**

**High Levels of Methicillin-Resistant Staphylococcus aureus Carriage Among Healthcare Workers at a Teaching Hospital in Addis Ababa Ethiopia: First Evidence Using mecA Detection**

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## Abstract

**Background**: Staphylococcus aureus is a major human pathogen and causes healthcare and community-acquired infection. Data on the extent of MRSA colonization among health-care workers (HCWs) in sub-Saharan Africa are limited. Hence, we determined the burden of MRSA colonisation among HCWs and administrative staff in Tikur Anbessa Specialised Hospital (TASH), College of Health Sciences (CHS), Addis Ababa University, Ethiopia.

**Methods:** Using a cross-sectional study design, participants were screened for MRSA colonisation between June 2018 and August 2019 using nasal swabs. The swabs were analysed using standard laboratory methods including antibiotic resistance gene, mecA. Anonymised sociodemographic data were collected by pretested questionnaires to evaluate HCWs factors associated with MRSA carriage.

**Results:** A total of 588 HCWs and 468 administrative staff were screened for MRSA. Women were over-represented. Overall, 49.1% (289/588) of HCWs were nurses and 25% (117/468) of the administrative staff were cleaners or laundry workers. Overall, 138 S. aureus isolates were retrieved from the nasal swabs of both groups (16.3%, 96/588 from HCWs). The burden of MRSA colonisation was 4.8% (28/580, 95% CI: 3.1–6.5%) among HCWs compared to 0.2% (1/468, 95% CI: 0.18–0.6%) of administrative staff (p value).

**Conclusion:** This is the first report in Ethiopia on MRSA colonization using mecA and revealed that; (i) overall carriage rates of MRSA in HCWs are comparable with observations reported in some other countries and (ii) HCWs exhibit a higher burden of MRSA carriage than administrative staff. Our data support strategic screening of MRSA and antimicrobial stewardship for better intervention measures. (**Infection and Drug Resistance** 2022:15 3135–3147).

**Keywords**: MRSA, HCWs, administrative staff, mecA, TASH, Ethiopia

## Introduction

Globally, Staphylococcus aureus is a major human pathogen that can cause a wide range of infections, both health care associated and community-acquired. It also exists as a commensal and approximately 30% of the human population is colonized with S. aureus at one point in time[1] and 5% are colonised with methicillin resistant S. aureus (MRSA) strains. Carriage precedes infection.[2]

Methicillin resistance is primarily mediated by penicillin-binding protein 2a which has a low affinity for β-lactam antibiotics. The mecA gene is part of a 21 to 60 kb Staphylococcal chromosome cassette mec (SCCmec), a mobile genetic element that may also contain genetic structures such as Tn554, pUB110, and pT181 which encode additional resistance to non-β-lactam antibiotics.3 MRSA can also acquire vancomycin resistance through a plasmid-mediated mechanism (vanA, vanB) transferred from enterococcal species.[4] MRSA screening and suppression in hospitalised patients, patients undergoing surgery or haemodialysis procedures, cancer patients, neonates (especially those who are underweight) and patients in ICU, have been practiced routinely in many developed countries to minimize the prevalence and spread of MRSA infection.[5,6] Health-care workers (HCWs) who are colonised with MRSA can transmit it to patients and the community. The burden of MRSA carriage among HCWs has been described in many countries around the globe.[7–11] However, in low and middle-income countries including Ethiopia, there has been very little research describing carriage of MRSA among HCWs.12,13 Importantly, the few published studies use phenotypic methods to characterise MRSA, which can result in over- or underestimated prevalence rates. We aimed to determine the burden of MRSA carriage among HCWs and administrative staff at a large group of teaching hospitals in Addis Ababa, Ethiopia, using both phenotypic and genotypic methods. Moreover, we compare nasal carriage rate of HCWs and administrative staff. Knowing the real burden of MRSA will enable health-care providers and policymakers to design appropriate strategies for the control and prevention of MRSA at the study site and other similar settings.

## Materials and Methods

**Study Site, Design and Duration**: A prospective cross-sectional study design was conducted at Tikur Anbessa Specialised Hospital (TASH), a teaching and referral hospital in Addis Ababa, Ethiopia between June and September 2018 for phenotypic analysis, while the molecular tests were performed from May to August 2019. During the data collection period, there were 1245 HCWs (400 medical doctors, 600 nurses, 70 laboratory personnel, 60 pharmacy personnel, 115 other HCWs) and 1200 administrative staff in the Hospital. All HCWs have direct patient contacts while administrative staff do not have direct roles in patient management or care. During the time of data collection CHS had two functional hospital buildings in the same compound, one administration building adjacent to the hospital and another administrative and academic campus located four kilometers away from the hospital.

TASH provides medical, surgical, obstetrics and gynaecology, radiology and imaging, laboratory and pharmacy services.

**Study Participants and Outcome Variables**: All HCW and administrative staff working at TASH for at least 6 months prior to the data collection period were approached to join the study. Participants who had taken antimicrobial drugs in the 15 days prior to data collection, staff on leave of absence and those who have been admitted to the hospital in the month prior to data collection were excluded. The burden of MRSA from nasal swabs of study participants was the outcome variable, while sociodemographic and other work-related data were collected as independent variables.

**Sample Size and Sampling Technique:** The single population proportion method was used to calculate the sample size. We used a 12.7% MRSA rate from a previous study in Dessie, Ethiopia[12] with a 95% confidence interval, 5% margin of error, and 10% contingency level, resulting in a sample size of 520 participants. However, we have included more HCWs than the calculated sample size to include different cadres of HCWs. The number of HCWs per cadre was allocated based on their proportion and convenience of selection. We selected administrative staff using convenience sampling until the required sample size was fulfilled.

**Data Collection** : Socio-demographic data were collected from HCWs using pretested self-administered questionnaires, while administrative staff were interviewed by data collectors. Sociodemographic data including age, gender-identity, information on availability of guidelines and leaflets about MRSA prevention and control, availability of adequate hand hygiene materials. Past medical and surgical history were collected both for HCWs and administrative staff.

**Sample Collection and Isolate Characterisation** : A nasal sample was collected from both anterior nares using a single cotton-tipped sterile moistened swab (Amie's, Oxoid, England), placed in Amie's transport media and transported to the laboratory in the Department of Microbiology, Immunology, and Parasitology (DMIP) at CHS, AAU for further analysis. If there was a delay, samples were kept in the refrigerator for no more than 12 hours.

All nasal swabs were cultured on mannitol salt agar or CHROMagar MRSA (Oxoid, England) and incubated overnight at 35–36°C for primary isolation of *S. aureus*. As per routine testing methods, colonies resembling Staphylococci species were further tested; those exhibiting catalase and coagulase, mannitol fermentation, or DNAse were identified as *S. aureus.* Antimicrobial susceptibility testing was determined using clinical laboratory standard institute (CLSI, 2018) methodology including, rifampin (5µg), clindamycin (2µg), trimethoprim-sulfamethoxasole (1.25/ 23.75µg), erythromycin (15µg), tetracycline (30µg), and penicillin (10 Units). Methicillin resistance was detected using the cefoxitin (30 µg) disc diffusion test and mecA detection. The MHA plates were incubated at 36°C for 16–18 hours, and the zone of inhibition around the disc was measured in millimetre using graduated callipers and the isolates were classified as sensitive, intermediate, or resistant according CLSI guidelines. All isolates were stored in skimmed milk glycerol medium at −30 to −70°C.[13,14,15]

**Resistance Mechanism Genotyping:** Genotyping was undertaken at the Department of Global Health and Infection, Brighton and Sussex Medical School, University of Sussex, UK. DNA preparation was performed using Instagene matrix solution kits (IMS, BIO-RAD, Munchen, Germany) following the manufacturer's instructions. Briefly, 1–2 loopful of overnight growth colonies of S. aureus were suspended in a sterile Eppendorf tube and washed with sterile double distilled water (DDH2O). The pellets were suspended in 200 ul of 6% IMS and the mixture was heated in a heat block at 56°C for 20 min, then vortexed and heated for 8 min at 100°C and centrifuged at 8000x g for 2–3 minutes. The DNA quality and concentration was assessed using NanoDrop™ one Spectrophotometers (Thermo Scientific™).

**PCR for mecA, vanA, and vanB Detection** :The presence of mecA was amplified by polymerase chain reaction (PCR) using mecA forward and reverse primers AAAATCGATGGTAAAGGTTGGC and AGTTCTGGAGTACCGGATTTGC, respectively, following previously described methods with slight modification.[14] Briefly, 22.5µl of a master mix composed of 50 units/mL of Taq DNA polymerase, 400µM of each dATP, dGTP, dCTP and dTTP, 3mM MgCl2 was mixed with 10 mmol of 0.5µl of each primer and 1.5µl of DNA product was mixed and PCR was done in a thermocycler (MRB Research, UK) with an initial 35 cycles of amplification (denaturation at 94°C for 60s, primer annealing at 60°C for 90s and primer extension at 70°C for 60s), and final extension at 72°C for 5 minutes. Then, 5µl of PCR products and 1 µL of loading dye was mixed and the band was resolved in 1.2% agarose gel prepared in 1× Tris borate EDTA (TBE) buffer containing 0.5 µg/mL of SYBER green solution. A hundred (100) bp DNA ladder was used as a molecular marker, the amplification products were electrophoresed for 1h at 100V and visualized under a gel image instrument LICOR Odyssey Fc Imager and the images saved in the computer.

vanA and vanB were amplified using PCR protocols as described elsewhere16 with slight modification. Briefly, we used the GTGACAAACCGGAGGTAATA forward and TCA CCC CTT TAA CGC TAA TA reverse primers, for vanA, and forward primer of CAG TGCATGTGCCATGGATA and reverse primer of CCG CCA TCC TCC TGC AAA AAA for vanB. A master mix (composed of 50 units/mL of Taq DNA polymerase, 400µM of each dATP, dGTP, dCTP and dTTP, 3mM MgCl2) of 22.5µl was mixed with 10 mmol of 0.5µl of each forward and reverse primers of vanA and vanB, and 1.5µl of DNA product of each gene was mixed. PCR reaction was resumed in a thermocycler (MRB Research, UK) with an initial 35 cycles of amplification (denaturation at 94°C for 60s, primer annealing at 60°C for 90s and primer extension at 70°C for 60s), and final extension at 72°C for 5 minutes. 5µl of PCR products and 1µl of loading dye were mixed and the band was resolved in 1.2% agarose gel prepared in 1× TBE buffer containing 0.5µg/mL of SYBER green solution. A hundred (100) bp DNA ladder was used as molecular marker, and the amplified products were electrophoresed for 1h at 100V. The gel was visualized under a gel image instrument, LICOR Odyssey Fc Imager and the image saved into the computer. In this study, we considered MRSA if the isolate was resistant to cefoxitin and positive for mecA.

**Quality Assurance Measures**: All pre-analytical, analytical, and post-analytical quality control (QC) measures were taken following standard operating procedures (SOPs) for isolation, identification, antimicrobial susceptibility testing (AST) and molecular testing. S. aureus (ATCC 25923), MRSA 252 Newman strains and E. faecium 1024 were used as QC strains. About 5% of AST was duplicated to check for any difference in performances. Known gram-positive and negative organisms were used to assess the QC of gram staining reagent, coagulase, DNAse, and catalase tests. Positive and negative controls were implemented for all PCR reactions and gel electrophoresis reading. Isolates were stored at negative 80°C with glycerol and skimmed milk. Data Analyses Data analyses and cleaning were done using SPSS version 20.0 software. Comparison was made for different variables among MRSA positive and negative participants and statistically tested using chi-square or Fisher's exact test. A comparison was made between the MRSA colonization rate of HCWs and administrative staff. A p-value of <0.05 was considered as statistically significant.

**Ethical Considerations :**This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board of CHS, AAU (Ref. no. AAUMF 03-008) and from the national research and ethics review committee (Ref. no. MoST 310/160/18). Written informed consent was obtained from each participant. The confidentiality of all information gathered from the participants was maintained. Participants were informed on the overall findings including MRSA carriage in the hospital. The principal investigator showed photographs of MRSA cultures for HCWs, to create some awareness on MRSA and other drug-resistant bacteria.

**Operational Definition and Abbreviations** :Health-care workers: These are qualified health-care professionals including nurses, doctors, laboratory personnel, pharmacy personnel, radiographers and radiologists, physiotherapists, anesthetist, interns and residents who provide different types of patient care in TASH. Methicillin resistant S. aureus: These are S. aureus isolates resistant to cefoxitin and positive for mecA. Administrative staff: these are other than HCWs like secretary, drivers, garage workers, cleaners and laundry workers, finance and personnel officers, library staff who have no direct contacts with patients and working in TASH and CHS. AST, Antimicrobial Susceptibility Testing; ATCC, American Type Culture Collection; CHS: College of Health Sciences, CLSI, Clinical and Laboratory Standard Institute; MRSA: methicillin resistant S. aureus; Multiple Drug Resistance; QC, Quality Control; TASH: Tikur Anbessa Specialised Hospital.

## Results

**Socio-Demographic Characteristics of HCWs and Administrative:** Staff Overall, 1056 HCWs and administrative staff were recruited for the study; 588 were HCWs and 468 administration staff. Of the HCWs, 58.4% identified as female and the mean age was 29 years (SD ± 6.7 years, range of 20–57 years). Overall, 75% of HCWs in TASH were within the age group of 20–26 years, 40.3% (237/586) of HCWs were married; most were nurses (49.1%, 289/588) followed by medical doctors (28.4%, 167/588). In terms of educational level, 63% (369/586) of HCWs had Bachelor of

Science degrees, and 60.5% (355/587) had 1–4 years of work experience in TASH **(Table 1).**

**Table1. Socio-demographic characteristics and departments of participants in TASH, 2019**

| Variables | Frequency (%) | Variables | Frequency (%) |
|---|---|---|---|
| **Healthcare workers** | | **Administrative staff** | |
| **Gender identity (n= 580)** | | **Gender identity ( n = 468)** | |
| Male | 241 (41.6) | Male | 168 (35.9) |
| Female | 339 (58.4) | Female | 300 (64.1) |
| **Age group (n = 574)** | | **Age group (n = 468)** | |
| 20-26 Years | 441 (76.8) | 17-25 Years | 127 (27.13) |
| 27- 33 Years | 85 (14.8) | 26-35 Years | 140 (29.91) |
| 34- 40 Years | 21 (3.7) | 36-45 Years | 120 (25.64) |
| >= 41 Years | 27 (4.7) | 46-55 years | 59 (12.6) |
| | | >= 56 years | 22 (4.7) |
| **Marital status (n = 586)** | | **Marital status (n = 468)** | |
| Single | 344 (58.7) | Single | 177 (37.82) |
| Married | 237 (40.4) | Married | 288 (61.54) |
| Divorced | 5 (0.9) | Divorced | 3 (0.64) |
| **Professional Category (n = 588)** | | **Current working area (n = 468)** | |
| Medical doctors | 167 (28.4) | CHS offices | 271(57.90 %) |
| Nurses | 289 (49.1) | Sefere-Selam campus | 65 (13.88 %) |
| Medical Laboratory Personnel | 36 (6.1) | TASH offices | 19 (4.05 %) |
| Pharmacy personnel | 29 (4.9) | TASH wards | 113 ( 24.15 %) |
| Others | 67 (11.4) | **Educational level (n = 468)** | |

| | | | |
|---|---|---|---|
| **Educational level (n = 586)** | | Diploma | 64 (13.67 ) |
| Diploma | 6 (1.0) | Degree | 52 (11.11 ) |
| Degree | 369 (63.0) | MSc | 7 ( 1.49 ) |
| Medical Doctor | 56 (9.6) | Certificate | 36 (7.69 ) |
| MSc | 39 (6.7) | Secondary level education | 142 (30.34 ) |
| Specialty certificate | 112 (19.1) | Primary level education | 155 ( 33.11) |
| Others | 4 (0.7) | Read and write | 12 (2.56 ) |
| **Work experience (n = 587)** | | **Work Experience** | |
| 1-2 Years | 197 (33.6) | 1-2 years | 179 (38.24%) |
| 3-4 Years | 158 (26.9) | 3-4 years | 63 (13.46 %) |
| 5-7 Years | 111 (18.9) | 5-7 years | 49 (10.47 %) |
| 8-10 Years | 45 (7.7) | 8-10 years | 25 (5.34 %) |
| More than 10 Years | 76 (12.9) | More than 10 years | 152 (32.47 %) |

NB: n is different for variables since there were missing values from the participants' responses

Of the administrative staff, 64.1% identified as female and only 4.7% (22/468) were 56 years old or above. More than 65% of participants had secondary-level education or below. Nearly 25% and 5% of them had a history of hospital admission or surgical intervention, respectively. More than 37% of the administrative staff had served for at least 8 years, and more than 57% of these were in CHS administrative offices. Based on our findings, 16.4% (96/585) and 15.38% (90/585) of HCWs were working in the out-patient or emergency department and Gynaecology and Obstetrics department of TASH, respectively **(Figure 1)**.

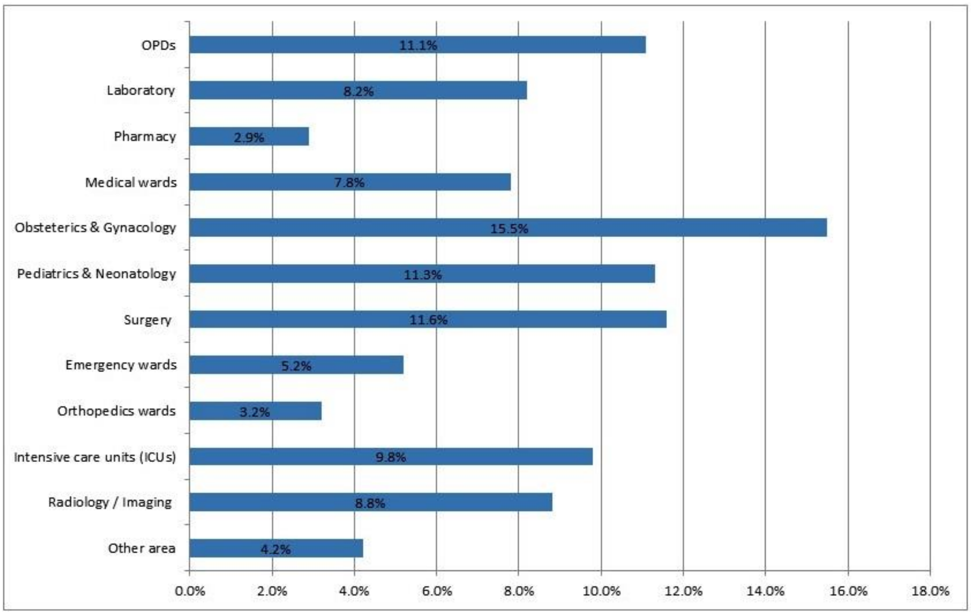


**Figure1.** Distribution of HCWs in the various Departments of TASH, 2019, the bar indicate the proportion of HCWs in each department as showed in the y-axis and x –axis is the proportion (%).

Importantly, 86.1% (504/586) and 84.6% (496/190/586) of HCWs at TASH said that there was no MRSA-related guideline or leaflets, and insufficient hand hygiene materials in TASH, respectively, while 88% (515/585) had not had training on MRSA prevention and control. Among the HCWs in TASH, 82.5% (484/587) had no history of hospital admission in the 3 years prior to data collection, and only 11.5% (67/585) had surgical intervention in the hospital in the same period. Of the 468 administrative staff, 25% (117/468) were cleaners and laundry workers **(Figure 2)**. Only 24.6% (115/468) and 5.1% (24/468) had a hospital admission or surgical intervention in the 3 years prior to data collection.

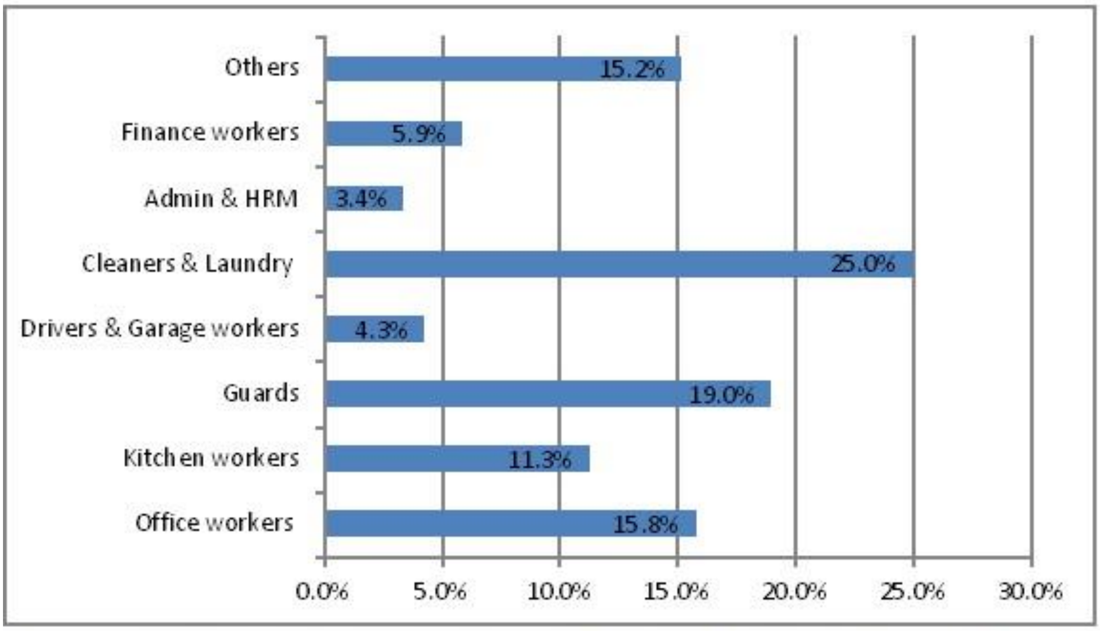


**Figure 2.** Distribution of administrative staff of TASH/CHS, AAU, by working department. The bar indicates the proportion of each group of administrative staff in each department as showed in the y-axis and x axis shows proportion (%). (HRM: Human Resource Management).

### The Isolation Rate of S. aureus, and Antimicrobial Susceptibility Testing Patterns

The proportion of S. aureus isolates from nasal swabs of HCWs and administrative staff was 16.3% (96/588) and 9.1% (42/468), respectively. All 138 S. aureus isolates underwent AST **(Table 2)**. Among S. aureus isolates, 21.7% were susceptible to penicillin and all isolates were susceptible to rifampicin. Twenty-nine isolates were MRSA as characterised by resistance to cefoxitin (oxacillin) and contained mecA. In this study, both MSSA and all MRSA isolates were tested for vancomycin and all of them were sensitive using the molecular method. MRSA strains had higher resistance rate to most drugs tested than MSSA isolates (p-value <0.05).

Table 2. Antimicrobial susceptibility patterns of *S.aureus* isolates from nasal swabs of HCWs and administrative staff of TASH, CHS, AAU, Ethiopia.

| **Antibiotics tested** | **AST status** | **HCWs n (%)** | **Admin staff n (%)** | **Total n (%)** | **P value** |
|---|---|---|---|---|---|
| Penicillin 10 U | S | 17 (17.7) | 13 (31.0) | 30 (21.7) | 0.067 |
| | R | 79 (82.3) | 29 (69.0) | 108 (78.3) | |
| Cefoxitin 30ug | S | 67 (69.7) | 41 (97.6) | 108 (78.3) | 0.001 |
| | R | 28 (30.3) | 1 (2.4) | 29 (21.0) | |
| | I | 1 (1.0) | 0 (0) | 1 (0.7) | |
| Erythromycin (15µg) | S | 79 (82.3) | 34 (80.9) | 113(81.9) | 0.513 |
| | R | 17 (17.7) | 8 (19.1) | 25(18.1) | |
| Clindamycin (2µg) | S | 80 ( 83.3) | 41(97.6) | 121 (87.7) | 0.013 |
| | R | 16 (16.7) | 1 (2.4) | 17 (12.3) | |
| Tetracycline (30µg) | S | 50(52.0) | 38 (90.5) | 88 (63.8) | < 0.001 |
| | R | 46 (48.0) | 4 (9.5) | 50(36.2) | |
| Trimethoprim-Sulfamethoxazole (1.25/23.75µg) | S | 62 (64.6) | 32 (76.2) | 94(68.1) | 0.125 |
| | R | 34 (35.4) | 10 (23.8) | 44 (31.9) | |
| Rifampin (5µg) | S | 96 (100) | 42 (100) | 138(100) | NA |
| | R | 0 (0) | 0 (0) | 0 (0) | |

NA ; not applicable

Multidrug-Resistant Pattern of S. aureus Isolates One hundred and twenty-one (87.6%) of S. aureus isolates were resistant to at least one antibiotic, 41 isolates were resistant to two different classes of antibiotics, and one isolate was resistant to six antibiotics **(Figure 3)**.

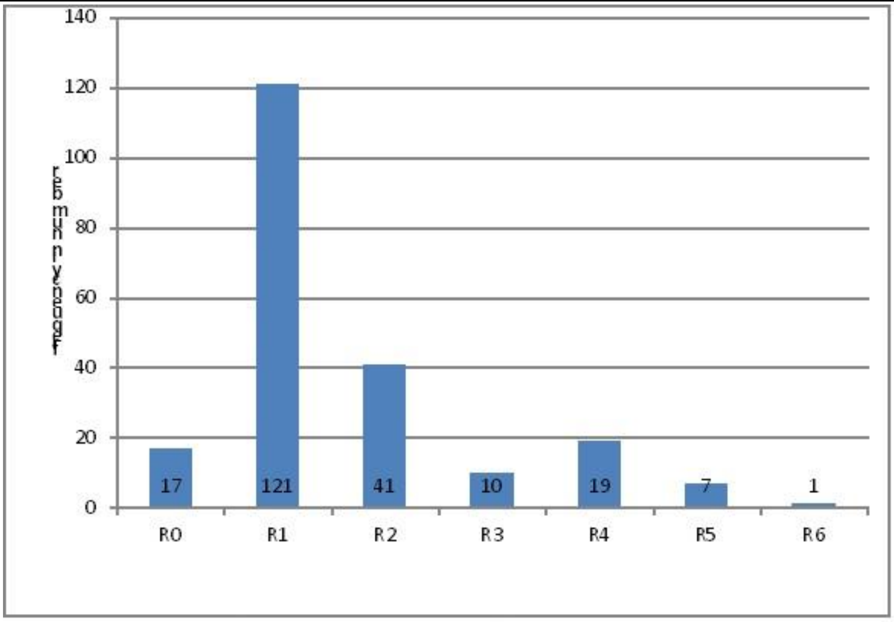


**Figure 3.** Multiple antibiotic resistance pattern of *S aureus* isolates at TASH, CHS, AAU, 2019

Key: R0: No resistant, R1: Resistant to 1 drug, R2: Resistant for 2 drugs, R3: Resistant for 3 drugs, R4: Resistant to 4 drugs, R5: resistant to 5 drugs, R6: resistant to 6 drugs.

## MRSA Burden Among HCWs and Administrative Staff

The burden of MRSA nasal colonisation among HCWs was 4.8% (95% CI: 3.1–6.5%, 28/580) and was comparable among those identifying as male and female (4.9% and 4.7%, respectively, p-value 0.517). **Figure 4** shows the amplified mecA.

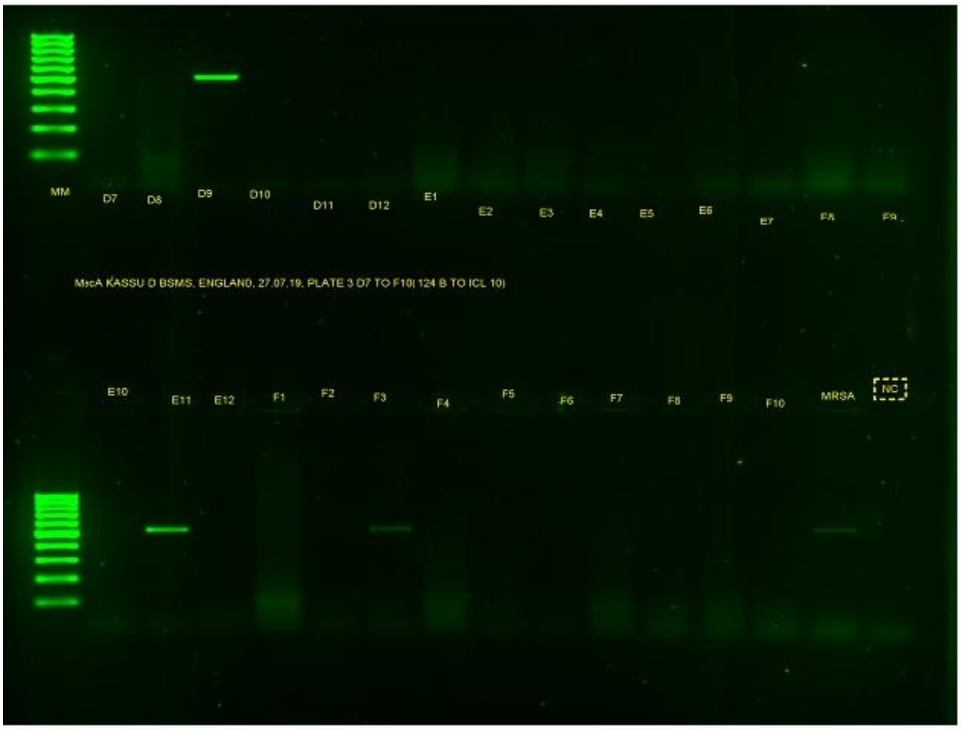


**Figure 4**.Gel band result of *mecA*. MM is for molecular markers of 100 bp; the letters from D7 to F10 are PCR products of *S. aureus* isolates; MRSA is a positive control and NC is a negative control.

The burden of MRSA was higher in HCWs within the age group of 20–26 years **(Table 3)**. Nurses had the highest MRSA rate in TASH (22/28 from the total positive). Most of the HCWs had 1–2 years of work experience and nasal MRSA colonisation was found in 6.59% (13/197). MRSA colonisation of HCWs did not differ according to - whether HCWs had had MRSA-related training or not; availability of guidelines; source of information; history of hospital admission; surgical intervention; availability of sufficient hand hygiene materials; or department in which they were working (P-value >0.05; data not shown). On the other hand, among 468 administrative staff, only one individual had MRSA carriage in the nasal cavity, with a magnitude of 0.2% (95% CI: 0.18–0.6%, 1/468). The MRSA-positive administrative staff member was a 26-year-old female cleaner with work experience of 4 years, but no history of hospital admission or surgical intervention for the last three years during data collection time

**Table 3 Nasal Colonisation of HCWs with MRSA working at TASH, CHS, AAU, 2019.**

| Variables | MRSA + ve No. (%) | MRSA -ve No. ( %) | Significance |
|---|---|---|---|
| **Gender-identity ( n= 580)** | | | |
| Male | 12 (4.97 %) | 229 (95.03 %) | 0.05 |
| Female | 16 (4.72 %) | 323 (95.30 %) | |
| **Age Group** | | | |
| 20-26 years | 26 (5.89 %) | 415 (94.11 %) | |
| 27-33 years | 0 (0 %) | 85 (100 %) | <0.03 |
| 34-40 years | 1 (4.76 %) | 20 (95. 24 %) | |
| >/= 41 years | 1 (3.70 %) | 26 (96.30 %) | |
| **Marital status** | | | |
| Single | 19 (5.52%) | 325 (94.48%) | >0.05 |
| Married | 9 (3.79 %) | 228 (96.21%) | |
| Divorced | 0 (0%) | 5 (100 %) | |
| **Professional category** | | | |
| Medical doctor | 4 (2.39 %) | 163 (97.61 %) | |
| Nurses | 22 (7.61 %) | 267(92.39 %) | <0.023 |
| Medical Laboratory personnel | 0 ( 0 %) | 36 (100 %) | |
| Pharmacy personnel | 1 (3.44%) | 28 (96.55%) | |
| Others | 1 (1.49%) | 66 (98.51%) | |
| **Educational level** | | | |
| Diploma | 0 (0 %) | 6 (100 %) | |
| Degree | 23 (6.23 %) | 346 (93.77 %) | |
| Medical doctor | 1 (1.78 %) | 55 (98.22 %) | >0.05 |
| MSc | 1 (2.56 %) | 38 (97.44 %) | |
| Speciality certificate | 3 (2.67 %) | 109 (97.33%) | |
| Others (PhD,PGD) | 0 (0 %) | 4 (100 %) | |
| **Work experience (years)** | | | |
| 1-2 | 13 (6.59 %) | 184 (93.41 %) | |
| 3-4 | 6 (3.79 %) | 152 (96.21 %) | >0.05 |
| 5-7 | 6 (5.40 %) | 105 (94.60 %) | |

| | | | |
|---|---|---|---|
| 8-10 | 2 (4.44 %) | 43 (95.56%) | |
| More than 10 years | 1 (1.31 %) | 75 (98. 69 %) | |

MRSA –ve means those who had growth of MSSA or had no growth of MSSA or MRSA at all

## Discussion

MRSA-colonised patients and HCWs could increase the risk of transmission and contamination of the hospital environment. Screening of patients and HCWs has been implemented in some hospital settings.[17] HCWs are on the boundary between the community and hospital, and hence they may be at increased risk of MRSA colonisation or infection.[18] In the current study, higher rate of S. aureus carriage was seen among HCWs than administrative staff (16.3% and 9.0% respectively) which is slightly lower than two previous studies done in Mekele and Adigrat/Wukro hospitals of Ethiopia (28.8% and 20.3% respectively).[13,19] Higher rate of S. aureus carriage was also reported from Uganda (48%) and Gaza (25.5%).[20,21] The discrepancies seen could be explained as there is difference in sample size, laboratory methods and difference in study sites. The 78.2% penicillin resistance in this study was comparable with the 71% resistance reported in the Netherlands[22] but lower than two former studies done in Dessie and Adigrat, Ethiopia (100% resistant)[19,23] and 90.6% to 100% in Southern Brazil and Gaza.[21,24] The rate of multidrug-resistance was similar to reports from Dessie and Jimma, Ethiopia.[23,25] The rate of resistance to cefoxitin (oxacillin) in this work (21%) was higher than two previous studies in Ethiopia namely Adigrat/Wukro hospital (5.8%) and Dessie Hospitals (12.7%).[19,23] This might be due to the higher number and diversity of the sources of our isolates and the use of oxacillin as a surrogate marker in the aforementioned studies. Erythromycin resistance in this study (18.1%) is lower than 29–100% resistance rates reported from India, Nigeria and Libya.[26–28] These differences in availability and antibiotics use in the study sites could contribute the inconsistency in the resistance rate. Resistance rate of 12.3% (17 isolates) to clindamycin in this study was lower than an earlier study in North Ethiopia (17.2%).[19] Clindamycin resistance was higher for strains isolated from HCWs than administrative staff isolates, indicating that exposure to resistant strains is more likely in the hospital setting than in the community. Higher resistance was also reported from studies from Libya and India (15–69%).26,28 Resistance to tetracycline (36.2%) and trimethoprim-sulfamethoxazole (68.9%) in our study was comparable with the findings from North Ethiopia (55.2% and 51.7% respectively).[19] However, a higher resistance rate to tetracycline (86.1%) was reported from Mekele, North Ethiopia.[13] Probably our isolates mainly from administrative staff could comprise susceptible strains and contribute to low levels of resistance to this drug. One of the encouraging results in our study is that, all isolates were sensitive to rifampicin and vancomycin, which is in line with studies in Nepal[29] and Southern Brazil,[24] suggesting these two drugs are still useful and effective for managing S. aureus and MRSA-related infections. Although the source population was different, a recent study in North Ethiopia also indicated the absence of resistance to these drugs.[30]

Resistance to rifampin was reported among 50% of MRSA and 6% of MSSA in one of the Indian studies.[26] Since rifampicin is not routinely used for the treatment of S.

aureus infection in Ethiopia, this might favour the absence or selection of resistant strains. However, 5.6% rate of resistance to vancomycin was reported in a former study in Mekele, Ethiopia,[13] rate of 1.3% and 6% in Nigerian studies,[27,31] and 12% in Libya[28] were also seen. We have used PCR for the detection of vanA and vanB unlike the other studies in Ethiopia, Nigeria and Libya that used only the disc diffusion test and may contribute the existence of vancomycin resistance. Generally, we found higher rate of drug resistance for MRSA than MSSA isolates and among HCWs than administrative staff (p-value <0.05).

The 4.8% (28/580) MRSA rate in our HCWs is in line with the figure of 4.6% from a meta-analysis of 127 studies around the world,[18] rate of 1.8–4.4% from a systematic review in Europe and the USA,34 and of 4.6% from a metaanalysis in nine German acute care hospitals[35] in a non-outbreak setting. However, our MRSA burden is lower than previous studies done in Ethiopia with a rate of 5.8% to 14.1%,[12,13,20,25,36] and elsewhere with MRSA rates of 11.7% to 52.59%.[21–28,37] The higher rate might be, other studies used resistant testing based on disc diffusion alone and so overestimate the MRSA rate, whereas we used cefoxitin disc diffusion and mecA methods together. Moreover, our sample size was reasonably large and includes diverse cadres of HCWs. Lower MRSA burdens than ours have been reported elsewhere, with MRSA rates of 0% to 2.3% in Kenya, Germany, Australia, and Nigeria.[27,38–40] Better infection prevention practices in Europe and the involvement of HCWs who had very low or limited contact with patients in Kenyan and Nigerian studies might have favored for the lower MRSA colonization rates. Almost all Ethiopian studies reported higher rates of MRSA carriage than ours, as they used coagulase tests alone for identification purposes, rather than coagulase and DNAse tests. These findings need further investigation as we have seen discrepancies in S. aureus identification that could potentially result in inflated rates of MRSA.[41] This is expected in low income settings where the microbiology laboratory infrastructure and personnel are not well supported. We feel that there might be over-reporting of MRSA in most of the previous studies done in Ethiopia and other similar settings around the globe. Earlier work from Ethiopia and elsewhere[9,12,13,21,22,26,27,33,37] indicated that MRSA rates might be higher among nursing staff, in agreement with this study. This may be the consequence of more frequent contact with patients and hence exposed them for MRSA. In the current study, the majority of MRSA carriage (92.8%; 26/28) was seen among nurses and HCWs in the age group of 20–26 years, which is similar to other studies.[29,31] This age group is active and may have more interaction with patients and colleagues, potentially exposing them to more MRSA colonization. As HCWs become more senior, they may have less contact with patients. Other variables like educational, marital status, work experience, history of hospital admission and surgical procedure were not statistically associated with MRSA burden in line with previous studies in Ethiopia.[12,13] All in all, this study generates robust evidence on the burden of MRSA using phenotypic and genotypic methods which are very rarely done in Ethiopia and other sub-Saharan Africa. Our work also corroborates that, HCWs had higher rates of MRSA. As a limitation, we did not analyse a second swab after an interval of time to understand the persistence of MRSA carriage. Second, SCCmec typing was not done which could predict the source of MRSA. Finally, we did not perform co-colonisation of MRSA and MSSA among our study participants which might be addressed in future works. Conclusion MRSA carriage was much higher among HCWs of TASH than administrative staff. Importantly, vancomycin and rifampicin are still the most effective antibiotics. Updating the real burden of MRSA carriage using phenotypic and genotypic methods is essential to inform preventive measures and track changes for intervention in TASH and other similar settings. Moreover, TASH being as a tertiary level specialised teaching hospital should have emerging technologies including molecular identification methods, drug resistance testing and strategic screening of HCWs for MRSA. Although we have generated evidence on the burden of MRSA carriage at TASH, it is difficult to recommend decolonisation of HCWs without acquiring additional evidences. Data Sharing Statement We have presented the most important data with this report. However, upon reasonable request, additional data could be available from the corresponding author.

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**Author Contributions** : All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article was submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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# Original Article 2.

## Methicillin Resistant *Staphylococcus aureus* contamination of Health care worker gowns and Uniforms: A cross-sectional Study from the biggest teaching hospital in Ethiopia

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**Abstract**

**Introduction:** Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a global public health problem. Personal protective equipment (PPEs), including gowns and uniforms prevents transmission of pathogens including MRSA. Data are limited on the contamination of gowns and uniforms by MRSA in Ethiopia and many other developing nations using molecular markers.

**Objective:** To determine the rate of MRSA contamination of gowns and uniforms of health care workers (HCWs) at Tikur Anbessa Specialized Hospital (TASH), in Ethiopia.

**Methods:** A cross-sectional study design was used, and pooled swab samples from 588 HCW's reusable gowns/uniforms were tested for the presence of *S.aureus* MRSA and drug-resistant testing using conventional methods and polymerase chain reaction (PCR) based *mecA* and Panton-Valentine leukocidin (PVL) detection. Socio-demographic data and information on the use of gowns and uniforms were collected using a questionnaire and analysed by SPSS version 20 software. A p value less than 0.05 was considered statistically significant.

**Results:** Female HCWs are slightly higher in number than males (58.4 % and 41.6 %, respectively). The mean age and standard deviation of HCWs were 29.13 ± 6.6 years. In TASH, 15 % (88/588) and 57.5 % (338/588) of HCWs had single and long sleeve gowns and uniforms, respectively. Forty-seven *S.aureus* were isolated making MRSA contamination rate of 2.9 % (17/588) (*mecA* positive and cefoxitin resistant) and a significant difference was seen among HCWs with history of surgical intervention. Ten of 17 MRSA contaminations were seen among HCWs who changed their gown once in a week and 2/3 of *S.aureus* carried PVL.

**Conclusion:** Gowns and uniforms of HCWs in TASH harbored MRSA as confirmed by *mecA* and PVL, which has implications for infection control and prevention. TASH should provide an adequate number of gowns and urgently develop a policy covering gown use to curb MRSA transmission. **[*Ethiop. J. Health Dev.* 2023; 37(1) 000-000]**

**Keywords:** Health care workers' gowns/uniforms; Tikur Anbessa Specialized Hospital, Methicillin - Resistant *Staphylococcus aureus* . **Corresponding author**, kassudesta2020@gmail.com/kassu.desta@aau.edu.et, Tel:+251911107099

## Introduction

Personal protective equipment (PPE) is an items including single-use gowns or aprons, face protection (masks or respirators) and eye protection that are used by HCWs to use PPEs to create a barrier between the body part and the microbes which prevents the acquisition of pathogens by the wearer and onward transmission to susceptible individuals. Moreover, the inadequacy of gowns and uniforms used by HCWs could affect the overall infection prevention practice in the hospital (1).

Transmission of pathogenic microorganisms in healthcare settings occurs through direct and indirect means during the interaction of HCWs and patients. Other means of transmission include contact with hospital environments and HCW's clothing (uniform) or other PPEs (2-5).

HCW’s hands and their uniforms could carry multi-drug resistant bacteria such as MRSA, Vancomycin-resistant Enterococcus (VRE), and extended spectrum βlactamase (ESBL) producing Gram negative bacteria within and outside healthcare settings (6-7).

HCW’s gowns and uniforms potentially acts as fomites in that they may harbor pathogenic microorganisms and efforts are required to reduce the burden of infections associated with HCW’s gowns and uniforms in hospitals and long-term settings (8-10).

Evidence from Maryland and Michigan nursing homes shows that MRSA contaminated HCWs gowns (14 %) and gloves (24%) that have been linked to the transmission of the pathogens to patients (11). Similarly, other evidence from a medical intensive care unit at the University of Maryland Medical Center showed, 17 % (24/137) of HCWs caring for patients with MRSA and/or VRE acquired MRSA and VRE from their gloves, gowns, or both (12). It is also noted that *S. aureus* produces the versatile virulence factor, Panton-Valentine leukocidin (PVL), a cytotoxin that forms pores in the membrane and has been associated with several skin infections and is linked with disease severity (13-14).

In Ethiopia and many other developing nations, there is a lack of understanding or negligence that PPE could be contaminated with microbes that could be transmitted to HCWs and susceptible patients. Importantly, such data is lacking in low and middle-income countries and it is time to raise awareness of HCWs and hospital administrators in order to design appropriate intervention measures. To the best of our knowledge, we did not find literature that describes HCW’s gown and uniform contamination by MRSA in particular with molecular based evidence such as *mecA* and the presence of PVL genes containing *S. aureus* in Ethiopia.

To fill this gap, we determined the proportion of MRSA contamination of HCW's gowns and uniforms along with gown utilization in the biggest University Hospital, in Addis Ababa, Ethiopia using conventional and *mecA* PCR based testing. Moreover, we have screened the *S.aureus* isolates for their PVL status.

## Methods and Materials

### *Study hospital, design, and duration*

A prospective cross-sectional study design was conducted at TASH, the biggest teaching and referral hospital in Addis Ababa, Ethiopia from June 2018 to August 2019. TASH provides referral services in all disciplines of medicine. During data collection time the hospital and College of Health Sciences (CHS) had 1245 HCWs. Gowns and uniforms are routinely used in clothes in the hospital during clinical practice. However, uniforms are mainly used in operation rooms and intensive care units.

### Study participants and outcome variables

All HCWs working at TASH for at least 6 months before the data collection period were approached to join the study. The contamination rate of HCWs gowns and uniforms by MRSA was the outcome of interest and sociodemographic, other work-related data and gown utilization by HCWs were the independent variables.

### Sample size and selection of HCW's gowns for microbiological analysis

We have used a single population proportion formula to estimate the number of HCWs and their gowns required to address the objective. Since this study was part of the MRSA nasal colonization study of HCWs and administrative staff of TASH/ CHS, we used a 12.7% MRSA nasal colonization rate from a previous study in Dessie, Ethiopia (15) with a 95% confidence interval, 5% margin of error, and 10 % contingency level, resulting in a sample size of 520. However, to increase precision, we included 588 HCWs and their gowns to include different types of HCWs of TASH. The number of HCWs per cadre was apportioned based on their proportion and convenience of selection.

### Sociodemographic data

Data related to socio-demography, past medical history of HCWs, MRSA related training and guidelines in TASH and information on the number, type, and gown utilization and availability of adequate hand hygiene materials were collected using a pretested, self-administered questionnaire.

### Specimen collection and processing

Pooled swab samples from all pockets and both hand sleeves of gowns and uniforms were collected from each HCW during their actual clinical practice using a single cotton-tipped sterile moistened swab (Amie's, Oxoid, England), which was placed in Amie's transport media and transported to the laboratory for analysis.

Swab samples were cultured on mannitol salt agar and CHROMagar MRSA (Oxoid, England) and incubated overnight at 35-36°C for primary isolation of *S. aureus* followed by biochemical tests using catalase, coagulase, mannitol fermentation, or DNA testing to identify the isolate is *S. aureus*.

**Antimicrobial susceptibility testing and PCR for *mecA* and P*VL***

Antimicrobial susceptibility and *mecA* testing were done following standard procedures as described well in our previous work on MRSA nasal colonization of HCWs and administrative staff of TASH(16). We followed the disc diffusion method using Muller Hinton agar (MHA) (UK) based on clinical laboratory standard institute (CLSI) 2018 guidelines, and we used antibiotic discs of rifampicin (5 µg), clindamycin (2 µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), erythromycin (15 µg), tetracycline (30 µg), penicillin (10 Units). Methicillin resistance was detected phenotypically using a cefoxitin (30 µg) disc. The MHA plates were incubated at 36 °C for 16-18 hours, and the zone of inhibition around the disc was measured to the nearest millimeter by a graduated calliper. The isolates were classified as sensitive, intermediate, and resistant according to CLSI guidelines.

DNA extraction and PCR for *mecA* amplification and detection were made following a standard protocol as described well in our previous work (16). We used the forward and reverse *mecA* primers of AAAATCGATGGTAAAGGTTGGC and AGTTCTGGAGTACCGGATTTGC, respectively. While for PVL detection we used primers Luk PV-1, ATCATTAGGTAAAATGTCTGCACATG ATCCA and Luk PV-2, GCATCAACTGTATTGGATGCCAAAGC which amplify a 433 base pair fragment specific for *lukS/F –PV* genes, encoding the *PVL* S/F component proteins (Invitrogen, Thermo Fisher scientific, Great Britain) following previous work with slight modification (17). We prepared a master mix of 22.5 µl that is mixed,, with 0.5 µl of each forward and reverse PVL primer and 1.5 µl of DNA product that was mixed and amplification was done by initial denaturation at 94 °C for 4 min; 35 cycles of amplification (denaturation at 94 °C for 45 s, annealing at 57 °C for 45 s, and extension at 72 °C for 30 s); and a final extension at 72 °C for 2 min. To visualize the PCR product, 5 µl of the PCR amplicon was loaded with loading dye in 1.2 % agarose gel containing SYBERSAFE green, followed by electrophoresis at 100 V for 1 h and the gel was visualized under a gel image instrument, LICOR Odyssey Fc Imager, and the image saved into the computer.

**Quality control measures**

We applied pre-analytical, analytical, and post-analytical quality control (QC) procedures for isolation, identification, antimicrobial susceptibility testing (AST), and molecular testing. *S. aureus* (ATCC 25923), MRSA 252 Newman strains, and *E. faecium* 1024 were used as QC strains. We used known Gram-positive and negative bacteria to measure the QC of our staining, biochemical reagents, and molecular tests (*mecA* and *PVL* testing).

**Data analysis**

Data analysis and cleaning were done using SPSS version 20.0 software. The proportion was used to estimate MRSA contamination along with sociodemographic data. The difference in the proportion of gowns contaminated by the level of the independent variable was statistically tested by chi-square or Fisher's exact test. A p-value of <0.05 was considered statistically significant.

**Operational definition**

***Gowns and uniform***: For this research work it was defined as reusable clothing worn by HCWs for their daily clinical activities of any type in the outpatient units, wards, operation rooms, intensive care units, laboratories, pharmacies, and imaging rooms of the hospital.

***Health care workers*:** These are qualified health care professionals, including nurses, doctors, laboratory personnel, pharmacy personnel, radiographers and radiologists, physiotherapists, anesthetists, interns, and residents who provide several types of patient care in TASH. In this particular study, all HCWs had direct patient contact during investigation, care and specimen collection, or during other diagnostic and therapeutic work.

**Methicillin resistant *S. aureus (*MRSA*)*:** Defined in the context of this study, as *S .aureus* isolates that was resistant to cefoxitin and positive for *mecA by PCR*.

**Results**

***Characteristics of Health Care Workers (HCWs)***

A total of 588 HCWs were included, and 58.4 % of them were female. The mean age and SD of HCWs was 29.13 ± 6.6 years. Seventy-five percent of HCWs in TASH were within the age group of 20-26 years. Forty percent (237/586) of HCWs were married, and nurses were the dominant HCWs accounting for 49.1% (289/588). Concerning education, 63% (369/586) of HCWs had a Bachelor of Science degree, and 60.5% (355/ 587) had 1-4 years of work experience in TASH **(Table 1).** Of 586 HCWs, 58.7%, 40.4%, and 0.9 % were married, single, and divorced, respectively.

**Table 1. Socio-demographic characteristics and working departments of HCWs in TASH, 2019**

| Variables | Frequency (%) | Variables | Frequency (%) |
|---|---|---|---|
| **Gender ( n= 580)** | | **Educational level (n= 586)** | |
| Male | 241(41.6) | Diploma | 6 (1.0) |
| Female | 339(58.4) | Degree | 369(63.0) |
| **Age group ( n= 574)** | | | |
| | | Medical Doctor | 56(9.6) |
| 20-26 Years | 441(76.8) | MSc | 39(6.7) |
| 27- 33 Years | 85(14.8) | | |
| | | Specialty certificate | 112(19.1) |
| 34- 40 Years | 21(3.7) | Others | 4(0.7) |

| | | | |
|---|---|---|---|
| >= 41 Years | 27(4.7) | | |
| **Professional Category (n= 588)** | | **Work experience (n = 587)** | |
| Medical doctors/ specialist/residents | 167(28.4) | 1-2 Years | 197(33.6) |
| Nurses | 289(49.1) | 3-4 Years | 158(26.9) |
| MedicalLaboratory Personnel | 36 (6.1) | 5-7 Years | 111(18.9) |
| Pharmacy personnel | 29 (4.9) | 8-10 Years | 45(7.7) |
| Others | 67 (11.4) | More than 10 Years | 76(12.9) |

NB: n is different for some variables as there were missing values from the participants' responses
Others include: Anesthetist, physiotherapists, and radiographers

Among HCWs in TASH, 15 % (88/588) and 2% (12 / 588) of them had only single and six or more reusable gowns and uniforms, respectively. The majority of HCWs, 57.5 % (338/588) had long sleeve reusable gowns **(Table 2)**. Out of 586 HCWs, only 1.9 % of them changed their gowns or uniforms on a daily basis, while 24.4 %, 48.1 %, 16.9 %, and 8.7 % of the HCWs changed their gowns and uniforms every other day, weekly, every other week, and as required, respectively. From the participating HCWs, 16.9 % (99/587) had a history of hospital admission, 11.5 % (67/585) had history of surgical intervention, and only 11.9 % (70 /585) had MRSA related training.

**Table 2. Availability of gowns and MRSA-related training among HCWs in TASH, 2019.**

| Variables | Frequency (%) | Variables | Frequency (%) |
|---|---|---|---|
| **Number of reusable Gowns ( n= 588)** | | **MRSA training (n=585)** | |
| | | Trained | 70(12.0) |
| Single gown | 88(15.0) | | |
| | | Untrained | 515(88.0) |
| Two gowns | 231(39.3) | | |
| Three gowns | 176(29.9) | **Guidelines/leaflet on MRSA (n = 585)** | |
| | | Present | 81(13.8) |
| Four gowns | 58(9.9) | | |
| | | Absent | 433(74.0) |
| Five gowns | 23(3.9) | | |
| Six or | 12(2.0) | Do not know | 71(12.1) |

| | | | |
|---|---|---|---|
| more gowns | | | |
| **Type of Gowns ( n= 588)** | | **Hand hygiene materials in TASH ( n=586)** | |
| Short sleeves | 209(35.5) | Present | 190(32.4) |
| Long sleeves | 338(57.5) | Absent | 383 (65.4) |
| Both types | 41(7.0) | Do not know | 13 (2.2) |

NB: n is different as there are missing responses from HCWs. Clinical practice: activities in operation rooms, wards, examining patients, and other similar activities.

**MRSA contamination level of HCWs gowns and Uniforms**

Out of 588 HCWs gowns/uniforms tested, 47 *S. aureus* isolates were identified as contaminants of gowns and uniforms, and 2.9 % (17/588) , (95 % CI: 1.8-4.6%) were MRSA as identified as resistant to Cefoxitin and positive for the *mecA* gene **(Figure 1)**.

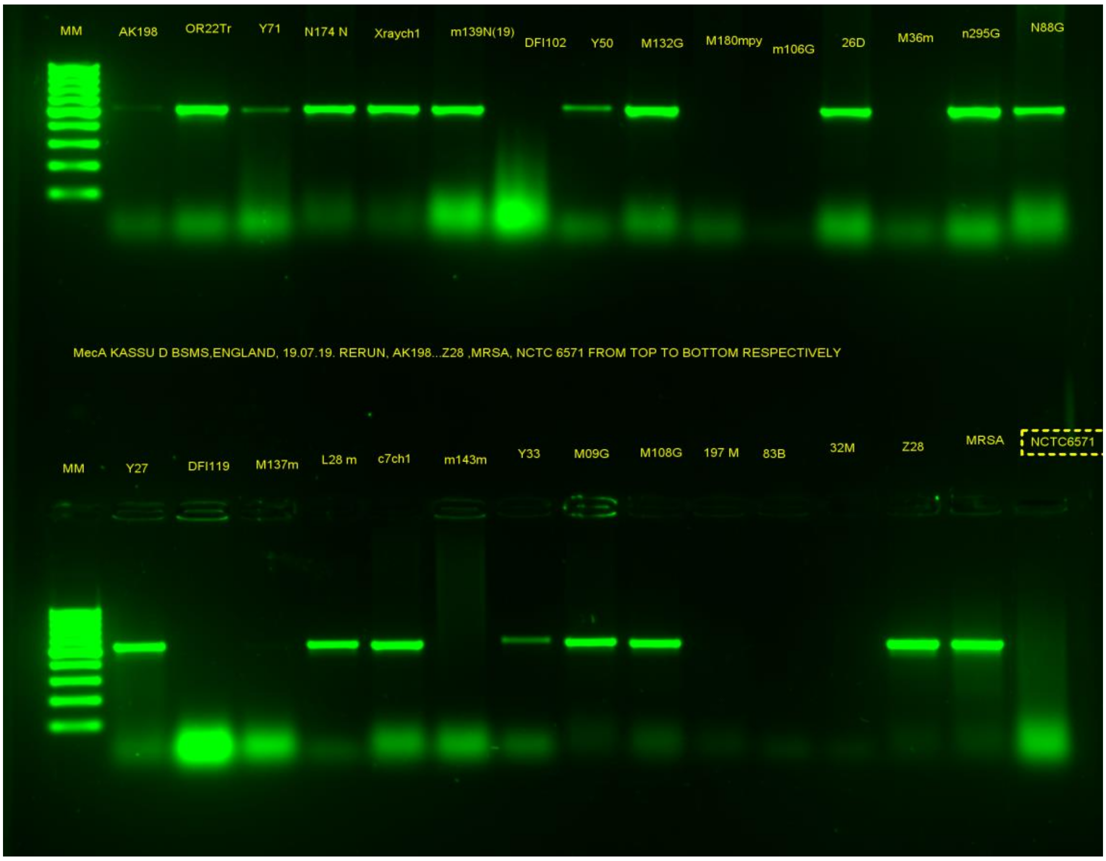


**Figure 1**.PCR products of *MecA* genes from *S aureus* isolate. MM is for molecular markers of 100 base pairs (bp),PCR products of *S.aureus* isolates, MRSA and National Collection of Type Culture (NCTC) are positive and negative controls and others are *S.aureus* isolates

All MRSA contamination of gowns and uniforms was found among HCWs in the age group of 20- 33 years, slightly higher in number among females than male HCWs (12 vs 5) though no significant difference was seen between gender, age, marital status, and level of education (P-value > 0.05) **(Table 3)**. There is a significant difference between MRSA contamination of gowns and uniforms with a history of surgical intervention (p-value = 0.007) but not with the number, type, and frequency of changing gowns and uniforms, the availability of MRSA related training and guidelines and hand hygiene materials (data not shown).

**Table 3. MRSA contamination level of HCWs gowns/uniforms practicing at TASH, 2019.**

| Variables | MRSA Present n. (%) | MRSA Absent, n (%) | P- value |
|---|---|---|---|
| **Gender ( n= 580)** | | | |
| Male | 5 (2.1 %) | 236 7.9 %) | >0.05 |
| Female | 12 (3.5 %) | 327 (96.5 %) | |
| **Age Group ( n= 574)** | | | |
| 20-26 years | 15 (3.4 %) | 426(96.6 %) | |
| 27-33 years | 2 (2.4 %) | 83(97.6 %) | >0.05 |
| 34- 40 years | 0 (0 %) | 21 (100 %) | |
| More than 40 years | 0 (0 %) | 27 (100 %) | |
| **Marital status (n= 586)** | | | |
| Single | 8 (2.32%) | 336 (97.7%) | |
| Married | 9 (3.8%) | 228 6.2%) | >0.05 |
| Divorced | 0 (0%) | 5 (100 %) | |
| **HCWs (n= 588)** | | | |
| Nurse | 4 (2.4 %) | 163 (97.6%) | |
| Medical doctors | 10 (3.5 %) | 279 (6.5%) | >0.05 |
| Lab. Personnel | 0 | 36 (100 %) | |
| Pharmacy personnel | 0 | 29 (100 %) | |
| Others | 3 (4.5 %) | 64 (93.5%) | |
| **Educational level (n= 586)** | | | |
| Diploma | 0 (0 %) | 6 (100 %) | |
| Degree | 12 (3.3 %) | 357 (96.7 %) | |
| MD degree | 2 (3.6 %) | 54 (96.4 %) | >0.05 |
| MSc | 0 (0 %) | 39 (100 %) | |
| Specialty certificate | 2(1.78 %) | 110 (98.2%) | |
| Others | 0 (0 %) | 4 (100 %) | |
| **HCWs (n= 588)** | | | |
| Nurse | 4 (2.4 %) | 163 (97.6%) | |
| Medical doctors | 10 (3.5 %) | 279 (96.5%) | >0.05 |
| Lab. Personnel | 0 | 36 (100 %) | |

| | | | |
|---|---|---|---|
| Pharmacy personnel | 0 | 29 (100 %) | |
| Others | 3 (4.5 %) | 64 (93.5%) | |
| **Educational level (n= 586)** | | | |
| Diploma | 0 (0 %) | 6 (100 %) | |
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| MD degree | 2 (3.6 %) | 54 (96.4 %) | >0.05 |
| MSc | 0 (0 %) | 39 (100 %) | |
| Specialty certificate | 2 (1.78 %) | 110 (98.2%) | |

NB: n is different as there are missing values in the responses from participants.

All *S.aureus* isolates were susceptible to rifampicin and 66 % of them were resistant to penicillin. Seventeen *S. aureus* isolates were resistant to oxacillin as measured by Cefoxitin disc **(Table 4).**

**Table 4. Antimicrobial Resistant patterns of *S. aureus i*solates from gowns and uniforms of HCWs in TASH, Ethiopia (n=47)**

| Antibiotic tested | Resistant n (%) | Intermediate n (%) | Susceptible n (%) |
|---|---|---|---|
| Penicillin 10 Units | 31 (66.0) | - | 16 (34.0) |
| Cefoxitin (30 µg) | 17 ( 35.4) | - | 31 (64.6) |
| Erythromycin ( 15 µg) | 4(8.3) | 2 (4.2) | 42 (87.5) |
| Clindamycin (2 µg) | 2 ( 4.2) | - | 46 (95.8) |
| Tetracycline ( 30 µg) | 16 (33.3) | - | 32 (66.7) |
| Trimethoprim - Sulfamethoxazole (1.25/23.75 µg) | 13( 27.1) | - | 35 (72.9) |
| Rifampicin (5µg) | 0 (0) | - | 47 (100) |

Out of 47 *S. aureus* isolates from gowns and uniforms, 40.4 % of them (19/47) were positive for the virulent PVL gene **(Figure 2)**. Among these 58.8 % (10/19) were from MRSA isolates.

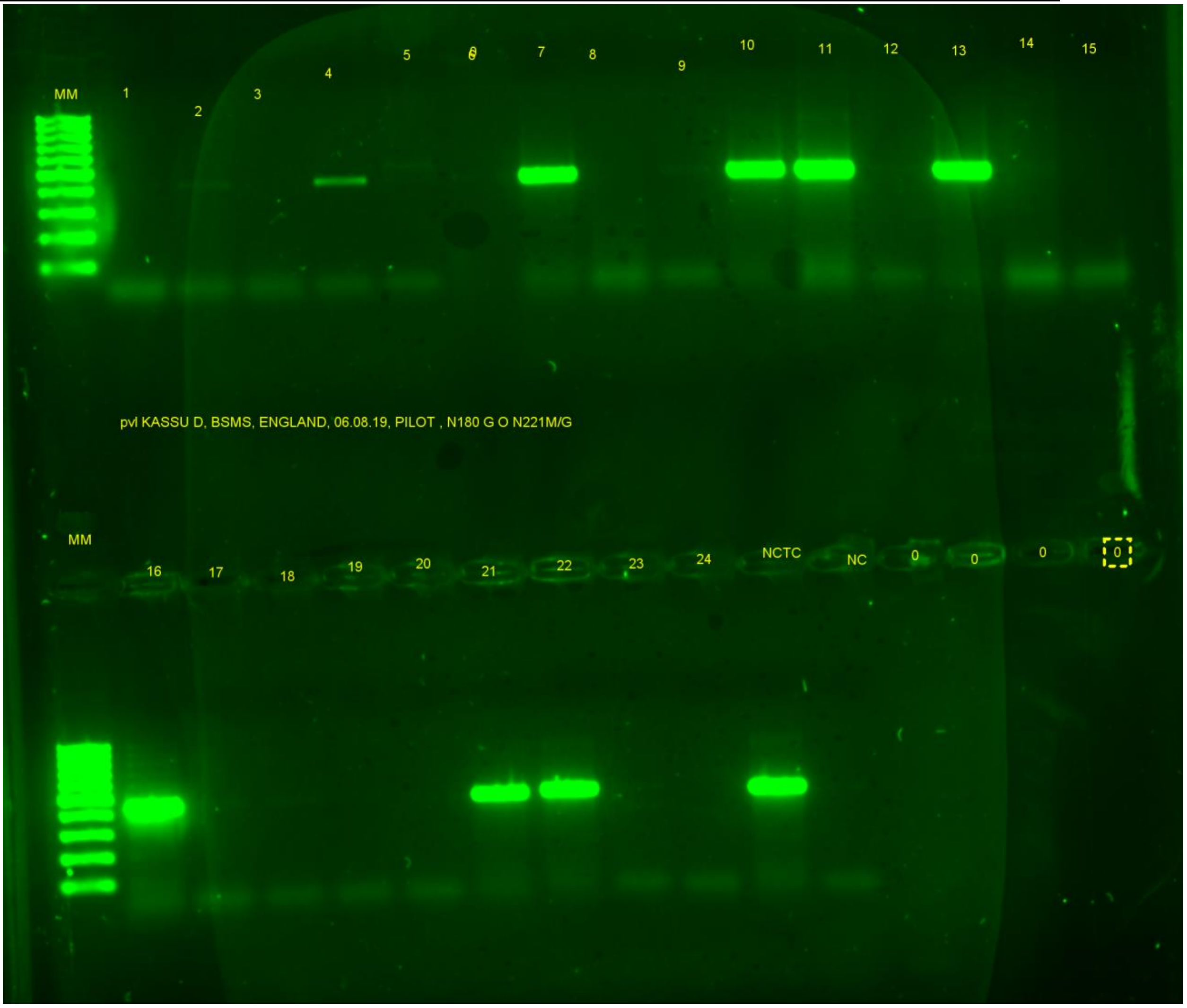


**Figure 2.** Gel band image for PCR result of PVL gene. Letter MM is for molecular markers, C1 to E4 is *S.aureus* isolates from mobile phones tested for PVL gene, MRSA (Newman 258) is a negative control and NCTC is a positive control for PVL.

**Discussion**

In the hospital environment, HCW's gowns and uniforms could serve as reservoirs of pathogenic microorganisms, including MRSA and other multidrug- resistant bacteria, and serve as a source of infection for hospitalized patients, HCWs themselves, and the community at large (2-5).

Keeping this in mind, we have isolated both methicillin- sensitive and resistant *S.aureus* with a rate of 5.1% (30/588) and 2.9 % (17/588), respectively from the gowns and uniforms of HCWs in the hospital. Our finding are in line with a recent meta-analysis report in Cyprus that included studies between 2000 to 2020 and the overall MRSA presence in HCWs attire ranged from 1.3 - 79 %. MRSA was found in 1.3- 14 % of gowns, 4-79 % of white coats, and 0-19.1 % of scrubs. Contamination was, 3.5-19.1 % among studies that considered long and short sleeve uniforms, and 2.5-32 % among studies that considered ties for HCWs (10). A higher level (10.7 %; n=15/140) of MRSA contamination was also reported in another study in Cyprus from hospital and long-term caregiver's uniforms (7). The contamination of HCWs gowns and uniforms in the current study is underestimated as we took a pooled swab sample of gowns and uniforms of

HCWs only from the pockets and on the sleeves. If we had included samples from other attire and clothes, the rate would have been higher.

All MRSA gown contamination was observed among HCWs whose age is between 20-33 years, implying this age group of HCWs actively interacts with patients and other colleagues with little infection prevention practice. Similar work from Brazil showed that MRSA was found in 53.3 % (out of 300) of gown samples taken from healthy university students (17). In fact, the MRSA contamination rate is higher than our findings as they used oxacillin disc as a marker of MRSA which may overstate their findings. Differences in isolation and identification methods of *S.aureus* in the two countries could also contribute to the change in the rate of MRSA contamination.

Nurses and doctors had more contact with patients than other HCWs and in our study,10 and 4 out of 17 cases of MRSA gown and uniform contamination was seen among nurses and medical doctors, respectively, which is in concordance with a report from a tertiary-care center in Maryland, the United States, that described, the transmission of MRSA through gloves and gowns of HCWs in 5.4 % of cases among staff who cared for patients very closely (18).

A similar observational study in nursing homes in Maryland and Michigan revealed that MRSA-contaminated HCW gowns (14%) and gloves (24%) were linked to the transmission of MRSA (11). Implying the existence of MRSA on HCWs' gowns and uniforms in our hospital could be a potential source of infection for the HCWs themselves, patients, colleagues, and the society at large and needs timely remedial action.

More importantly, 16 of the 17 cases of gown contamination were seen among HCWs who possessed only 1-3 gowns and changed their gowns on a weekly basis which underscores, having sufficient gowns / uniforms and frequent changing could minimize the level of contamination. This finding is essential for the hospital administrators and infection prevention committee to review the availability, adequacy and hygiene of gowns/uniforms and develop a guideline on the use of gowns and uniforms in TASH. It is known that the hands of HCWs have frequent contacts with patient and their gowns and uniforms, which spread the pathogens. This is more pronounced in situations where hand hygiene adherence is minimal (2-3,19). The majority of our HCWs stated that there were inadequate hand hygiene materials in TASH, which could contribute to poor hand hygiene adherence and increased contamination.

There are controversies over the use of short versus long sleeves gowns and the rate of microbial contamination, as long sleeve gowns may be easily contaminated during clinical practice. We did not see a difference in MRSA contamination of long sleeves (9 cases), and short ones (8 cases) which is in line with a study from Denver, Colorado Health Center (20).

The frequency of hand hygiene and its compliance is linked with the reduction of infection in the hospital (21-24) and HCWs washed their gowns and uniforms at home or hospital level in the case of Cyprus study (7). A significant number of HCWs in

TASH (n=88, 15%) had a single gown, this is a concern from an infection prevention and control point of view, and staff should have an adequate number of gowns /uniforms so that they can undertake hygiene measures as much as possible .Interestingly, during data collection time of this work,, we observed instances where HCWs washed their gowns and uniforms in the hospital or took them home to wash. This calls for hospital management to avail laundry services at the hospital to minimize transmission of MRSA / MSSA and other pathogens to family members and the public at large. If HCWs are taking their clothes home; they opt to import or export these pathogens to the hospital or family members.

Although we did not measure hand hygiene compliance in this study, previous studies in other hospitals in Ethiopia and developing countries showed, the compliance rate was minimal (25-28). In the current study, though not significantly, MRSA contamination was higher among HCWs who said there was inadequate hand hygiene material. However, HCWs gowns and uniforms being washed, ironed, and strictly used only in the patient-specific area are determinant factors for contamination and transmission of MRSA and other pathogens in the hospital (29-31).

In our most recent work, MRSA nasal colonization was higher among nurses (16) but not in this report (p-value > 0.05). Other behavioral factors may play roles in the occurrence of a high rate of MRSA in HCWs gown in relation to working departments. It also noted that the use of gowns and gloves with improved hand hygiene and minimal contact reduced MRSA acquisition in US intensive care units (32). Importantly, the dynamic of MRSA transmission between HCWs, the environment, and patients must be investigated periodically to generate more concrete evidence, like a similar study in the UK by Price and colleagues (33).

Except for rifampicin, our *S.aureus* isolates from gowns and uniforms were resistant to penicillin, oxacillin (cefoxitin), and other drugs. The trend is similar toprevious studies done in northern Ethiopia among different fomites (white coats, mobile phones and Stethoscopes) (34) though they used only phenotypic methods. Recent data from federal Police Hospital HCWs also showed the rate of MRSA contamination of working clothes was comparable to the current study (6/222) though we used only phenotypic methods (35).

The existence of the PVL gene in 60 % of *S.aureus* isolates in our study provides additional data from the perspectives developing countries perspectives that PVL is a virulence factor for *S.aureus* and potentially causes severe infection through contaminated gowns and uniforms (36). Though the source was not gowns or uniforms, in UK neonatal units, colonization of HCWs by PVL-producing methicillin-sensitive *S.aureus* was linked to the reported outbreak (37).

This study generates the first molecular based report on the existence of MRSA and *S.aureus* with PVL carriage from HCW's gowns and uniforms in Ethiopia. Nevertheless, it has some limitations. First, we took only a single pooled swab from the gowns and uniforms of HCWs at different shifts, and we cannot comment on the peak time of contamination. Second, we did not include all gowns and uniforms from each HCWs and other types of attire which could underestimate the contamination rate. Last but not least, we were not able to talk about the transmission of MRSA from patients to HCW gowns or vice versa.

**Conclusion**

The presence of MRSA in HCWs gowns and uniforms in our hospital is a cause for concern, as confirmed with molecular evidence for the presence of *mecA* and PVL hosting strains. Hence, hospital management should provide adequate PPEs including gowns, uniforms, and hand rubs or other hand hygiene materials for all HCWs with appropriate guidelines on the use of these PPEs to prevent the acquisition and transmission of MRSA and other pathogens. Importantly, policies related to the use of gowns, uniforms and hand hygiene materials are urgently required at TASH and other similar settings.

**Data Sharing Statement**

Important data used for this manuscript are included. However, it is possible to get some additional data upon fair request.

**Study Ethics and Consent to Participate**

This research was done following the Helsinki Declaration. Ethical approval was obtained from the Institutional Review Board of the College of Health Sciences, Addis Ababa University (Ref. no. AAUMF 03-008) and from the national research and ethics review committee (Ref.no. MoST 310/160/18). Written informed consent was obtained from study subjects. The confidentiality of all information gathered from the participants was maintained.

**Consent for Publication**

Not applicable as we did not take photographs and video records of our study subjects.

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**Author Contributions**

All authors contributed in one way or another to the concept of the study, data collection, analysis, drafting, or revising the article, have agreed on the journal to which the article was submitted, given final approval of the version to be published, and are be accountable for all aspects of the work.

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**Disclosure**

All authors declared that they do not have conflicts of interest in this particular work.

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**Appendix XIV. Additional publications**

**ORIGINAL RESEARCH 1, Abstrcat Only.**

**Extended-Spectrum Beta-Lactamase-Producing Gram-Negative Bacteria on Healthcare Workers' Mobile Phones: Evidence from Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**

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**Abstrcat**

**Background:** Mobile phones are widely used in hospital settings for different purposes. Mobile phones of healthcare workers (HCWs) could be colonized or harbor extendedspectrum beta-lactamase (ESBL) producing gram-negative bacteria and may act as source of infectious agents. The aim of this study was to determine the rate of extended-spectrum beta-lactamase-producing Gram-negative bacteria on mobile phones of healthcare workers, to assess their antimicrobial susceptibility patterns and associated factors. Methods: A laboratory-based cross-sectional study was conducted involving a total of 572 samples by rubbing swabs of the front screen, back, keypad, and metallic surfaces of mobile phones of healthcare workers using simple random sampling technique. All specimens were screened for ESBL using ESBL CHROME agar and confirmed using double-disk diffusion test (DDDT). Antibiotic susceptibility testing was done by the Kirby–Bauer disk diffusion technique on Mueller–Hinton agar. Data were analyzed using SPSS version 25, odds ratio and p-value was calculated to determine the association among variables.

**Results:** Overall, the number of mobile phones contaminated by gram-negative bacteria was 454 out of 572 (79.4%). Female sex (OR 0.651, p-value=0.039) and service year (OR 0.468, p-value=0.038) of healthcare workers were found to be the most significant factors associated with healthcare professionals' mobile phone and bacterial contamination. Nine percent of the isolates were ESBL-producers. K. pneumoniae

(27%) was the dominant ESBLproducing isolate followed by Acinetobacter spp. (14.5%) and E.coli (14.5%). ESBL-producers were highly resistant to ampicillin (95.8%), piperacillin (83.3%), cotrimoxazole (70.8%), and chloramphenicol (54.2%), but highly sensitive to meropenem (87.5%), amikacin (85.4%), and piperacillin-tazobactam (81.2%).

**Conclusion:** ESBL-producing Gram-negative bacteria were isolated from 8.3% of HCWs' mobile phones. As high as 79.4% of the isolates were multidrug resistant. Mobile phones can lead to bacterial cross-contamination and could be a source of nosocomial infections.

**Keywords**: mobile phones, extended-spectrum β-lactamase, Gram-negative bacteria, hospital-acquired infection, Addis Ababa, Ethiopia

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**Original Article 2. Abstrcat Only**

**Magnitude and drug resistance profile of Extended Spectrum β-Lactamase (ESBL) producing gram-negative bacteria from different inanimate objects at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia**

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**Abstract**

**Background**: Infections caused by gram-negative bacteria are causing morbidity and mortality worldwide. The production of Extended-Spectrum β-Lactamases (ESBLs) is an important mechanism that is responsible for resistance to the third-generation cephalosporin.

**Aim:** The purpose of this study was to determine the magnitude and drug resistance profile of ESBL producing gram-negative bacteria isolated from various inanimate objects at Tikur Anbessa Specialized Hospital (TASH).

**Methods**: Laboratory based study was conducted on stored isolates from January to March 2019. The samples were taken from different inanimate objects (Intensive care unit (ICU) tables, ICU sinks, ICU IV stands, ICU beds, Incubators, ICU pediatrics trolley, oxygen regulators, Operation room (OR) tables, OR beds, OR computers, OR doors, lift buttons, x-ray chairs, and some other items) in Tikur Anbessa Specialized Hospital(TASH) and 216 isolates were used for further analysis. Biochemical tests for identification and antimicrobial susceptibility test were done by disc diffusion method. Screening of ESBLs was done using ESBL CHROME agar and confirmed with a combined disk diffusion test. The data were analyzed using SPSS software version -20 and descriptive statistical tests including frequency and percentage were calculated.

**Results:** In this study out of 216 gram negative bacteria, 15.3% of them were found to be ESBL producers based on the confirmatory test (combined disk method) from the various inanimate objects of TASH. Klebsiella ozaenae, Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella rhinoscleromatis, Citrobacter spp, Escherichia coli, Serriatia spp and Acinetobacter spp were ESBL producing gram-negative bacteria and found to be 100% resistant to ceftazidime and ceftriaxone.

**Conclusion:** It is worrisome to detect ESBL producing gram-negative bacteria from the inanimate objects of TASH, calling for systematic screening of inanimate objects for ESBL and other multidrug-resistant bacteria in the hospital. Furthermore, strengthening the infection prevention practice is vital to halt the transmission of these microorganisms.
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**Keywords:** Extended spectrum β -lactamase, Inanimate objects, Gram negative, Ethiopia.

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## Appendix XV. Declaration form

**DECLARATION**

I, Kassu Desta Tullu , declare that this PhD thesis is my own original work and it has not been presented to any other university for a similar or any other degree award. Any material utilized as a references for this study is duly acknowledged.

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