

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*.

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).

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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 PVL*

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, ATCATTAGGTAATAATGTCTGCACATGATCCA 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

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)	Work experience (n = 587)	
Professional Category (n= 588)		1-2 Years	197(33.6)
Medical doctors/ specialist/residents	167(28.4)	3-4 Years	158(26.9)
Nurses	289(49.1)	5-7 Years	111(18.9)
Medical Laboratory Personnel	36 (6.1)	8-10 Years	45(7.7)
Pharmacy personnel	29 (4.9)	More than 10 Years	76(12.9)
Others	67 (11.4)		

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 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.

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, (67/585) had history of surgical intervention, and only respectively. From the participating HCWs, 16.9 % 11.9 % (70 /585) had MRSA related training. (99/587) had a history of hospital admission, 11.5 %

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)	
Single gown	88 (15.0)	Trained	70(12.0)
Two gowns	231(39.3)	Untrained	515(88.0)
Three gowns	176(29.9)	Guidelines/leaflet on MRSA (n = 585)	
Four gowns	58(9.9)	Present	81(13.8)
Five gowns	23(3.9)	Absent	433(74.0)
Six or more gowns	12(2.0)	Do not know	71(12.1)
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**).

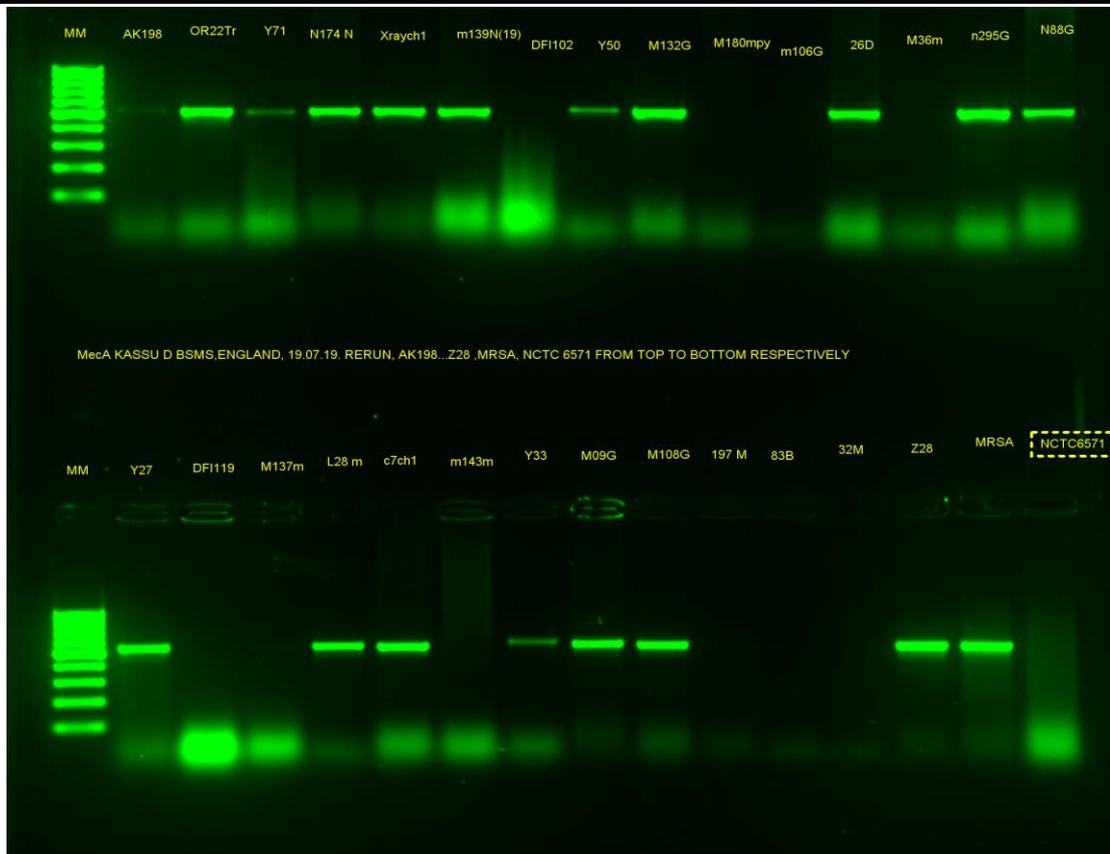


Figure 1. PCR products of *MecA* genes from *S. aureus* isolate. MM is for molecular markers of 100 base pairs (bp), PCR products of *S. aureus* isolates, MRSA and National Collection of Type Culture (NCTC) are positive and negative controls and others are *S. aureus* isolates

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 %)	>0.05
27-33 years	2 (2.4 %)	83 (97.6 %)	
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%)	>0.05
Married	9 (3.8%)	228 (6.2%)	
Divorced	0 (0%)	5 (100 %)	
HCWs (n= 588)			
Nurse	4 (2.4 %)	163 (97.6%)	>0.05
Medical doctors	10 (3.5 %)	279 (6.5%)	
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 %)	>0.05
Degree	12 (3.3 %)	357 (96.7 %)	
MD degree	2 (3.6 %)	54 (96.4 %)	
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 %)	
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%)	

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* isolates 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.

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 to previous 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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