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Bacterial contamination and antimicrobial susceptibility pattern of þÿ i s o l a t e s f r o m h e a t h c a r e w o r k fomites at Felege Hiwot Referral Hospital, North West Ethiopia.

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Bacterial contamination and antimicrobial susceptibility pattern of isolates from heath care worker's fomites at Felege Hiwot Referral Hospital, North West Ethiopia.

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A thesis submitted to the Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Bahir Dar University in partial fulfillment for the requirements of Masters of Science (MSc) in Medical Microbiology

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APPROVED BY THE BOARD OF EXAMINERS

This thesis by Workneh Ayalew is accepted in its present form by the board of examiners as satisfying thesis requirement for the Degree of Masters in Medical Microbiology.

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ABBREVIATIONS

AMR Antimicrobial resistance

BSI Blood Stream Infection

CDC Center for Disease Control and Prevention

CLSI Clinical Laboratory and Standard Institute

CONS Coagulase Negative *Staphylococcus*

ESBL Extended Spectrum *Beta Lactamase*

HAIs Health care Associated Infections

HCW Health Care Worker

ICU Intensive Care Unit

KPC K. *pneumoniae* Carbapenemases

MDR Multi Drug Resistant

MPDs Mobile Phone Devices Surfaces

MRCoNS Methicillin Resistant *Coagulase Negative Staphylococcus*

MRSA Methicillin Resistant *Staphylococcus aureus*

PPE Personal Protective Equipments

SSI Surgical Site Infections

UTI Urinary Tract Infection

WHO World Health Organization

ABSTRACT

Background: Health care associated infections also known as hospital acquired infections (HAIs), are infections that are not present (may be incubating) at the time of admission but acquired during the delivery of health care. Though, HAIs are major public health problem in hospitals worldwide, the prevalence is two to three folds higher in developing countries compared to developed countries. Health care worker's fomites are highly prone to bacterial contamination from the health care setting and are considered as potential sources for HAIs. However, there is scarcity of data that shows the magnitude of bacterial contamination and antimicrobial susceptibility pattern of isolates from health care workers (HCWs) fomites in Ethiopia.

Objective: To determine the bacterial contamination and antimicrobial susceptibility pattern of isolates from HCWs fomites at Felege Hiwot Referral Hospital (FHRH), Ethiopia.

Methods: A cross-sectional study was conducted from February 2017 to April 2017 from 422 HCWs fomites surface samples using convenient sampling techniques by simple-rinse method. Nutrient broth moisten sterile cotton swab used to rub the fomite. The swab was aseptically placed in 1 ml tryptic soy broth and diluted in 9ml of normal saline, then inoculated to plate count agar and incubated. Colonies were counted and calculated in terms of CFU/ml. Bacterial isolation and antimicrobial susceptibility testing were done following standard bacteriological techniques. Demographic and other explanatory variables were collected by face-to-face interview using structured questionnaire. The data from the finding was coded, entered and analyzed using Statistical Package for Social Science (SPSS) version 23. Descriptive statistics were used to get summary values. Binary logistic regression analysis was computed to see association between variables. A P-value < 0.05 was considered as statistical significant.

Result: Overall, 243 (57.6%) of fomites were contaminated with aerobic bacteria colony count >5 CFU/ml. The highest contamination was found in mobile phones 165 (59.2%) with the isolation rate of 103 (62.4%) ($P = 0.006$). Differences, in field of specialization ($P=0.05$), working wards ($P<0.001$) and laundry washing of white coats ($P=0.015$) of HCWs were significantly associated with bacterial contamination. Coagulase negative *Staphylococcus spp.* (44%) was the leading isolates followed by *S.aureus* (32%) and

K.pneumoniae (10.2%). Moreover, *K.pneumoniae* and *E.coli* showed 100% and 87.5% resistance to ampicillin and cotrimoxazole, respectively. Multidrug resistant was seen in 88.9, 92.6 and 100% of *S.aureurs, K.pneumoniae* and *E.coli* isolates, respectively.

Conclusions: Bacterial contamination of HCWs fomites is a major problem in the study area. Multiple drug resistance of isolates is alarmingly high in both Gram positive and Gram negative bacteria. Therefore, health care workers of the hospital need to implement proper handling of fomites to reduce contamination.

Keywords: Health care worker fomites, Bacterial contamination, Antimicrobial susceptibility, Felege Hiwot Referral Hospital, Bahir Dar, Ethiopia.

1. INTRODUCTION

1.1 Back ground

Health care associated infections also known as hospital acquired infections (HAIs), are infections that are not present (may be incubating) at the time of admission but acquired during the delivery of health care **(Allegranzi *et al*., 2007).** Though HAIs are major public problem in hospitals worldwide, the prevalence is two to three folds higher in developing countries compared to developed countries. About 5% to 10% of patients admitted to modern hospitals in the developed countries acquire one or more HAIs **(Magill *et al*., 2014**). In sub-Saharan Africa, prevalence of HAIs ranges from 2.5% to 14.8% **(Rosenthal *et al., 2011),* at 17.8% (Melaku *et al*., 2012) and 14.9% (Yallew *et al*., 2016**).

Health care associated infections are associated with high rate of mortality, morbidity, length of stay in the Intensive Care Unit (ICU), and hospital costs **(Endalafer *et al*., 2011; Alp *et al*., 2015; Eldegla *et al*., 2016).** Health care workers (HCWs) move from patient to patient, inadequate sanitation protocols regarding uniforms, equipment sterilization, washing and other preventive measures that may either be unnoticed by hospital personnel and the emergence of the resistant strains of microorganisms are reasons for the spreading of nosocomial infections **(Samuel *et al*., 2010; Mbim *et al*., 2016).**

Contaminated hands of HCWs play a major role in spreading infections in health care settings **(WHO, 2009).** Objects with frequent hand contact can serve as reservoirs from which infections can spread to the hands of HCWs and then to patients. Such inanimate objects which become contaminated with pathogenic bacteria and then spread the infection to others are often referred to as fomites (**Maryam *et al*., 2014; Segujja *el al*., 2016)**. Of these, HCWs fomites, stethoscopes, mobile phones and white coats are highly contaminated with hospital pathogens.

The most common type of hospital care infections that are correlated with hospital care worker fomites are surgical site infections (SSI), urinary tract infections (UTI), respiratory tract infection (RTI), and blood stream infection (BSI) **(Haun *et al*., 2016; Segujja *el al*., 2016)**.

Health care worker's' fomites in the hospital environments can become contaminated by different reasons like transfer of bacteria contaminating health worker's hands or direct patient shedding of microorganisms in the immediate environment of patients setting, settlement of airborne bacteria, and contact with other solid objects **(Chikere *et al*., 2008; Maryam *et al.*, 2014).**

According to previous studies conducted in Africa including Ethiopia, *Staphylococcus aureus,* coagulase negative *Staphylococcus* (CoNS), *Escheichia coli*, *Klebsiella. pneumoniae, Proteus* species and *Pseudomonas aeruginosa were* isolated from health care worker's stethoscopes, mobile phones and white coats **(Chikere *et al.*, 2008; Misgana *et al.*, 2015; Haun *et al.*, 2016; Segujja *el al*., 2016)**.

Mobile phones are the most commonly used non-medical portable electronic devices in hospital setting by HCWs. They are not only used for communication but also for network consultant and use of applications for patient care e.g. calculation of infusions doses, electrolyte corrective formula (**Selim *et al.*, 2015).** They are often used in close proximity to patients and inside patients' zones which are loaded with microorganisms. Unlike fixed phones, mobile phones serve as a perfect habitat for the microbes to breed–providing higher temperature and humid conditions. This could enhance pathogen transmission and intensify the difficulty of containing disease spread **(Sepehri *et al*., 2009; Gashaw *et al*., 2014).**

The use of stethoscope often occurs in hospital wards and outpatient departments. This crucial medical equipment can be used as vehicles for the transmission of pathogenic bacteria after touching contaminated hospital environment and the health care worker's did not follow the standard protocol set to prevent infections **(Shiferaw *et al*., 2013).**

Health care worker's wear their white coats on the way to their hospital environments and even in the non-clinical and non-practical rooms, library, cafeteria, and in the resting areas around their working environments. However, it has been shown to harbor potential contaminants and may have a role in the nosocomial transmission of pathogenic microorganisms **(Banu *et al*., 2012).**

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents (**WHO, 2014**; **Alp *et al.*, 2015)**. The worldwide estimates of global antibiotic resistance, published by the World Health

Organization listed *E.coli*, *K.pneumoniae*, and *S. aureus* as the three most common agents of greatest concern, associated with both hospital and community acquired infections **(WHO, 2014)**. The increased frequency of bacterial pathogen in a hospital environment is associated with a background rise in various types of HAIs **(Endalafer *et al*. 2011).**

Though, HCWs fomites have a potential role of contamination and act a major reservoir and source of nosocomial infections by resistance species. The contributions of such surfaces to the spreading of resistant strains were not studied before in the study area. Therefore; this study was conducted to determine the bacterial contamination and antimicrobial susceptibility pattern of isolates from HCWs fomites at FHRH.

1.2. Statement of the problem

Despite continuing efforts of hospital infection control measures, hospital associated infections are still a major public health problem globally and are on the increase in developing countries especially in Sub -Saharan Africa. It contributes significantly to morbidity and mortality of all age groups **(Alp *et al.*, 2015; Eldegla *et al.*, 2016).** Besides harming patients, HAIs can affect health care workers and anyone who has contact with the hospital **(Maryam *et al*., 2014)**. The Center for Disease Control (**CDC, 2013**) estimates that about 10% of all hospital patients acquire some type of HAIs as a result of contact with some contaminated hospital equipment. Approximately 40 million people are admitted to hospitals annually, 2 to 4 million people may develop an infection they did not have upon entering the hospital.

Transmission of pathogens within the hospital environments remains a hazard for hospitalized patients. They can be transmitted via environmental surfaces and inanimate objects. Health care worker's fomites when contaminated with pathogenic bacteria can transfer them to a new host thereby serving as vehicles in transmission. Identification of common fomites and associated pathogens in any hospital settings is an important opportunity to interrupt the spread of infection **(Maryam *et al*., 2014; Lopez *et al*., 2013)**.

Hospital fomites like stethoscopes are commonly used to assess the health of patients and have been reported to be potential sources for HAIs in various parts of the world. Previous studies in Ethiopia reported bacterial contamination rate at of 85.8% in

stethoscopes **(Shiferaw *et al.*, 2013**), 71.2% in HCWs mobile phones (**Misgana *et al.*, 2015)**,73.3% in white coats (**Qaday *et al*., 2015)**.

According to previous reports, HCWs including medical students did not follow the standard protocol set and aseptic procedure to prevent infections in using crucial medical equipment like stethoscopes (**Whittington *et al*., 2009)**. The degree of a strict adherence of the guideline also varies. Studies showed that degree of bacterial contamination from different HCWs fomites like mobile was an increasing concern, 32% (**Sepehri *et al*., 2009)**, 61.7% **(Misgana *et al*., 2015**) from Iran and Ethiopia, respectively.

Gram positive and gram negative bacteria are able to survive up to months on dry inanimate surfaces with longer persistence under humid and lower temperature conditions with a concentration sufficient to cause transmission on most cases. Moreover, multidrug resistance (MDR) bacteria have been reported as contaminating microorganisms of stethoscopes, mobile phones and white coats **(Haun *et al*., 2016).**

Previous studies also explain the presence of high level of resistance potential pathogens in health care fomites like mobile phone surfaces used by various groups. The results suggest that mobile phone devices surfaces (MPDs) may act as a vehicle for the transmission of antibiotic resistant bacteria to the community or/and family. This is shown that the need for maintaining good hygienic practices by the members of the above-mentioned groups to prevent the spread of antibiotic-resistant bacteria in hospital settings and community **(Asmari *et al*., 2015).**

Bacteria isolated from fomites are highly resistance to antimicrobial agents and capable of causing serious infections in hospital patients as well as in the community. Most of them are the most common causative agents for the most type of HAIs like UTI, RTI, SSI and BSI **(Daka *et al*., 2015)**

A healthcare provider uses their white coats and stethoscope outside but near to the hospital environment such as in cafeterias and in other places **(Haun *et al*., 2016).** Mobile phones are the most commonly used non-medical portable electronic devices used for communication, applications for patient care in the hospital setting. However, there was no specific policy that bans using mobile phones, strict practice of hand washing and habit of disinfection. Hence, a fomite was harbor pathogenic bacteria

(Sepehri *et al*., 2009). The level of potential bacterial contamination from HCWs is varies in frequency of hand washing, type and concentration of antiseptic type, ward to ward, profession to profession **(Daka *et al*., 2015).**

Frequency of changing and of laundering HCWs white coats may vary for the contribution of potential bacterial pathogens while providing care to patients in different clinical and epidemiological conditions **(Banu *et al*., 2012).** Health care workers are wearing their white coats during procedures and patient-care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions however; they move from the working area to non-clinical setting (**Qaday *et al*., 2015)**. Provision of scientific information, the conclusive impact of improved hand hygiene on HAIs and active participation in hand hygiene promotion was not implemented by HCWs (**Gashaw *et al*., 2014)** as well as without following standard infection prevention practices **(Shiferaw *et al*., 2013)** in the hospital setting.

Though, numerous medical devices and attires are carried by HCWs; the hand washing practices, regularly use antiseptic, regularly use disinfectant, infection prevention practices vary in terms of inter clinical setting and inter-health professional which results in varied load of contamination of fomites from hospital to hospital and among health care workers. Heath care worker's fomites are major means of spreading, selection and subsequent development of drug resistant species. However, there is limited of data on the distribution of pathogenic bacterial isolates and possible associated factors of contamination of stethoscopes, white coats and mobile phones in FHRH.

Therefore, the aim of this study was used to determine the bacterial contamination of HCWs fomites and its antimicrobial susceptibility patterns of the isolates. The study showed that a significant proportion of bacterial contamination, increased resistance of bacteria to antimicrobials. Hence, heath care worker's fomites were act as a major source of infections.

2. LITERATURE REVIEW

The hospital environment is a potential reservoir of bacterial pathogens since it houses a large number of patients with diverse pathogenic microorganisms and at high risk of contracting infections. The increased frequency of bacterial pathogen in hospital environment is associated with a background rise in various types of HAIs like surgical site infection, urinary tract infection, pneumonia and blood stream infection **(Rhomberg *et al.*, 2006).**

An estimated of 20 to 40% of HAIs have been attributed to cross infection via the hands of HCWs, who have become contaminated from direct contact with the patient or indirectly by touching contaminated hospital environmental surfaces **(Maryam *et al.*, 2014).** Health care worker's fomites that can be the predisposing factors for the transmission of pathogenic bacteria includes frequent contact with contaminated hands, no regular hand washing, large amount of exposure to equipment, no intensive follow up of infection prevention, the absence of delegated supervisor or infection prevention committee **(Loveday *et al.*, 2014).**

The source and spread of organisms in the hospital are important issues; human related organisms or the body normal flora also found in clothing, medical equipment and other HCWs fomites **(Samuel *et al.* 2010).**

Fomites are inanimate objects that can serve as vehicles for pathogens transfer at health care setting. Items including white coats, stethoscopes, and mobile electronic devices, with varied pathogens including *S. aureus*, CoNS, *Bacillus* spp., *E .coli* , *Pseudomonas* spp. and *Klebsiella* spp **(Maryam *et al.*, 2014; Haun *et al.*,2016).** Health care worker's fomites like stethoscopes, mobile, and white coats are commonly contaminated with bacterial pathogens including *S. aureus* (including MRSA) and gram negative rods, though there was high inter-facility and inter-study variability (**Haun *et al.*,2016).**

Bacterial contamination of fomites

A study conducted at Wisconsin (America) reported bacterial contamination (MRSA & gram negative rods) from stethoscope (0-42%, 0-31%), Mobile phones (0-20%, 0-75%), and white coats (0-16%, 0-42%), respectively (**Haun *et al.*, 2016).** The degree of bacterial contamination of white coats that studied in Iran was 94 % **(Askari *et al.*,**

2015). The study by **(Banu *et al.*, 2012)** showed those white coats bacterial contamination was differing in gender 62.8% female and 74.3% male HCWs, respectively.

Very little from African countries has been published on the issue of HCWs clothing and the potential for contamination. However, in Tanzania and Jimma, Ethiopia 73.33% and 85.8% bacterial contamination of white coats and stethoscopes have been documented **(Qaday *et al.*, 2015;** (**Shiferaw *et al*.,2013))**.

Bacterial isolates from health care worker's fomites

Different researchers showed that *S. aureus, CoNS, E.coli, P. aeruginosa, K. pneumoniae, Proteus spp. and Bacillus spp.* were the potential pathogenic bacteria commonly isolated from HCWs fomites **(Shiferaw *et al*., 2013; Chikre *et al*., 2008; Segujja *el a.*, 2016).**

The most common bacterial species that isolated from HCWs fomites (mobile, stethoscope and white coat) were gram-positive *S. aureus* and CoNS; *E. coli, K. pneumonia, Proteus* spp. and *P. aeruginosa* from gram negatives **(Loveday *et al*.,2014; Haun *et al*., 2016).** A study conducted in Iran **(Sepehri *et al*., 2009)** reported that bacterial contamination of mobile phones at 32.0% and hands at 39.3%. Among these, *S. epidermidis* was found to be the most commonly cultured organisms from all sites.

The most dominant pathogen that isolated in Tanzania **(Qaday *et al.*, 2015)** medical doctors and students white coats were *S. aureus* (91.67%), *P. aeruginosa* (6.82%) and *E. coli* (2.27%) respectively. In Nigeria**,** samples were obtained from doctors, nurses fomites showed that *S. epidermidis* (39.2%) and *S. aureus* (28.5%); and *E. coli* (7.1%), *K. pneumoniae* (5.3%), *Proteus spp.*(3.5%) and were predominant pathogenic bacteria respectively **(Chikere *et al*., 2008).** Prevalence studies conducted in Uganda **(Segujja *el al*., 2016)** from the surfaces of wards were reported at 57.59%. In Ethiopia, at 52% of HCWs stethoscopes were harbored potential bacterial pathogens **(Shiferaw *et al.*, 2013).**

The rate of isolates from both Uganda and Ethiopia were that *S. aureus* (25.75% vs.30.9%), *K. pneumoniae* (20.96% vs. 4.7%), *E. coli* (18.55% vs.0.8%), *P. aeruginosa*

(11.98% vs1.2%), *P. mirabilis* (5.39% vs. 3.5%), and *Bacillus* spp. (2.40%vs 5.1%) respectively. From a study conducted at Gondar, the most frequently pathogenic bacteria and their rate were CoNS (47.5%), *S. aureus (*27.1%), and *E.coli* (6.8%) recovered from the HCWs mobiles **(Gashaw *et al*., 2014).**

Antimicrobial Susceptibility Patterns

A study conducted in Iran **(Parhizgari *et al.*, 2014)** HCWs mobile phones reported 86% and 87% sensitivity of gram positive isolates to gentamicin and ciprofloxacin, respectively. Moreover, a study in Ethiopia **(Gashaw *et al*., 2014)** also reported 71.7% and 89.1% sensitivity of gram positive isolates to ceftriaxone and ciprofloxacin, respectively.

The antimicrobial drug resistance profile of bacterial isolates from mobile phones and stethoscopes of HCWs of Jimma University specialized hospital showed that, 26.6%-38.4%% of the *S. aureus* and 29.6%-30.1% of *CoNS* isolates were methicillin resistant. All methicillin resistant strains were susceptible to vancomycin from stethoscopes, but 38.5% of them were resistant strain isolated from mobile phones. About 75.9% of *S.aureus* and 87.4% of *CoNS* isolated from stethoscope were resistant to penicillin. However; 10.4 % of *S. aureus* and 9.7% of *CoNS* from stethoscopes were resistant against clindamycin **(Misgana *et al., 2015;* Shiferaw *et al*., 2013).**

A study conducted on white coats bacterial contamination at Nigeria **(Maryam *et al*., 2014)** showed that gram negative and gram positive bacteria were highly sensitive to gentamicin and erythromycin, respectively.

Factors with bacterial contamination from health care worker's fomites

Contamination may be occurred either transfer of microorganisms contaminating health workers' hands or direct patient shedding of microorganisms in the immediate environment of a patient's bed **(Mbim *et al*., 2016)**.

Health care workers and admitted patients in various hospitals use mobile phones for communication. The stethoscope is a popular instrument used by HCWs to evaluate the lung, heart, and abdominal sounds of their patients. Clinical setting like inpatients, emergency wards and out patients are providing health service by HCWs with wearing a white coats (**Haun *et al*., 2016)**.

Poor environmental hygiene, frequent contact with hands, no antiseptic use of practice, improper personal hygiene, and increased patient length of stay (hospitalization), educational status of HCWs and drug resistant were major factors for the transmission of HAIs. Studies for Hand Hygiene in Healthcare Settings (London) with provision of specific recommendation among HCWs with regarding of hand washing and hand antisepsis practice showed that improved hand-hygiene practices reduce transmission of pathogenic microorganisms to patients and personnel **(Loveday *et al.*,2014; WHO, 2014)**.

Health care workers are associated particularly with hospital acquired infections (HAIs) that remain a major cause of patient morbidity and mortality. An estimated high percentage of HAIs have been attributed to cross infection via HCWs fomites , which have become contaminated from direct contact with the patient or indirectly by touching contaminated hospital environmental surfaces **(Maryam *et al*., 2014)**.

According to **Daka *et al*., 2015**, the rate of routine cleaning of HCWs mobile phones was at 5.3%, which means 94.7% of the participants never cleaned their mobile phones either daily or weekly. A study among 176 stethoscopes by Shiferaw and his colleagues showed that only 2.8% of the respondents (owners) reported that they disinfect their stethoscope before and after examining each patient. In addition, the report showed that 98.1% of the medical students do not disinfect regularly. A study conducted in India **(Banu *et al*. 2012)** showed that 9% of the HCWs did not believe that white coats can be a potential transmitting agent for pathogens.

Prevention and control

Improved chemical disinfection, environmental monitoring, epidemiological surveillance, self-cleaning surfaces, automated disinfection systems, avail infection prevention guideline, periodic supervision, reduce multi-bed rooms and crowded patients in a single room have contributed to the prevention and control of HAIs measures **(Chikere *et al.*, 2008; Samuel *et al*., 2010)**.

Contamination of bacteria from HCWs fomites like stethoscopes, mobile phones, and white coats could be minimized by hand washing, disinfection practice after exposing contaminated area, proper waste disposal system, using antiseptic practice, proper personal hygiene**,** decrease patient length of stay (hospitalization), and education of

health care worker's **(Smith *et al.*, 2012).** Five Moments for Hand Hygiene (before touching a patient, before the clean / aseptic procedure, after body fluid exposure risks, after touching patients, and after touching patient's surroundings) is the simplest but the most cost effective measures for HCWs associated infections **(WHO, 2009)**

3. SIGNIFICANCE OF THE STUDY

This study was carried out to assess the bacteria contamination and antimicrobial susceptibility pattern of pathogenic bacteria spp. from HCWs fomites that could be of potential health risk in a hospital setting. Therefore, determining the magnitude of bacterial contamination of HCWs fomites and identifying the most common contaminants is believed to preserve local knowledge, generate base line and multi-centered national data that would play a major role for the infection control practices and promotions of new interventions.

Moreover, determining the antimicrobial susceptibility pattern of isolates would also to have document of current antimicrobial resistance patterns used to implement active measures in containing the spread of drug resistance strains in the hospital that would help to minimize the burden of HAIs and for appropriate selection of antibiotics.

4. OBJECTIVES OF THE STUDY

4.1. General objective

To determine the bacterial contamination and antimicrobial susceptibility pattern of isolates from HCW's fomites in FHRH, North West Ethiopia

4.2. Specific objectives

- To determine the load of bacterial contamination of HCW's fomites.
- To identify bacterial pathogens responsible for HCW's fomites contamination.
- To determine the antimicrobial susceptibility pattern of bacterial isolates to the commonly prescribed antimicrobial drugs.
- To identify factors associated with bacterial contamination of HCW's fomites.

5. MATERIALS AND METHODS

5.1. Study design, Period and Setting

A cross-sectional study was conducted from February 2017 to April 2017 at FHRH which is located in Bahir Dar town, 565 kilometers away from the capital City of Ethiopia, Addis Ababa.

Felege Hiwot Referral Hospital is one of the highest patient loaded governmental hospitals in Ethiopia and has more than 430 beds. At its outpatient department, it provides health care services for 690 patients per day. The hospital consists of an Operating room, Intensive care units, Medical, Surgical, Orthopedics, Pediatric, Gynecology/Obstetrics, Maternity, and Emergency wards, Outpatient departments (OPDs), Laboratory and Pharmacy units. According to the 2016 human resources and development department reports of FHRH, it has 107 Medical Doctors (University and Hospital), 174 Nurses, 30 Midwives, 37 Pharmacists, 43 medical laboratory professionals and 120 medical intern students **(FHRH, 2016).**

5.2. Population

5.3. Source population

All HCWs working in FHRH.

5.4. Study population

All Medical Doctors, Nurses, Midwives, Anesthetists, Medical Laboratory and Pharmacy professionals working in different wards, operating rooms, departments, and units of FHRH.

5.5. Sample size determination and sampling technique

The sample size was determined using a single population proportion formula [$N = Z^2 p (1-p)/ d^2$] considering 0.5 proportion (p) (hence, there was no similar study conducted so far in Ethiopia), 95 % confidence level (z), and 5 % margin of error (d). Accordingly, the minimum sample size calculated was 384. Considering a 10 % (38) non-response rate, the final sample size was 422 (the number of HCWs fomites). The calculated sample size was proportionally allocated to different health care professionals according to their population size. Study participants included conveniently until the required sample size fulfilled. Swabs either from stethoscope,

white coat or mobile phones were collected from medical doctors or intern student while Swab either from the white coat or mobile phones were collected from nurses, midwives, pharmacy and medical laboratory professionals.

5.6. Inclusion and Exclusion Criteria

5.6.1. Inclusion criteria

Stethoscope, mobile phones or coats of medical doctors, anesthetists, medical intern students; and mobile phone or white coat of nurses, midwives and medical laboratory & pharmacy professionals working at FHRH were included.

5.6.2. Exclusion Criteria

Fomites from physiotherapist, radiologist, dermatologist, psychiatrist, dentist, environmental officer, ophthalmologist, department head and matron were excluded.

5.6.3. Variables of the study

Bacterial contamination of fomites and antimicrobial susceptibility pattern of bacterial isolates were the dependent variable whereas demographic characteristics such as age, sex, heath care worker's qualification, field of specialization, service year of participants, hand washing practice, infection prevention practice & working wards were the independent variables.

5.7. Data collection

5.7.1. Socio demographic data

Demographic and other data related to HCW's fomites bacterial contamination were collected by the investigator via face-to-face interview using structured questionnaire. Moreover, hand hygiene practices of HCWs were collected by observation using checklists.

5.7.2. Sample Collection and Processing

Sample Collection and transportation:

A total of 422 HCW's fomite samples were swabbed aseptically from stethoscope, mobile phones and coats with moisten sterile cotton swab by simple-rinse method.

Specifically, swabs from the cuff and pocket mouth of the dominant hand and the abdominal region of white coat was collected using sterile 0.85% normal saline dipped cotton swabs by gently passing them up and down twice over the site. The entire

surface of the diaphragm and ear pieces of each stethoscope was swabbed with a sterile swab moistened in sterile 0.85% normal saline. Moreover, sterile moistened swab was fully stretched on the screen and back side of mobile phones where the most frequent areas of contact with the fingers and was inoculated according to the standard protocol.

The collected moistened swab sample was inserted to 1ml of tryptic soy broth (TSB) **(Parhizgari *et al.* 2014**) and transported to FHRH Microbiology Laboratory within 15 minutes and diluted with 9ml ml of sterile normal saline.

Mesophilic Colony Counting: A quantity (1 ml) of the diluted sample was aseptically pipetted and inoculated onto 5% sheep Blood Agar Plates (BAP) using pour plate method. All inoculated media were incubated at 35◦ c -37◦c for 18 to 24 hrs.
After overnight incubation, aerobic mesophilic bacterial count was determined by taking discrete bacterial colonies using a colony counter. Load of bacteria was determined by dividing total colony forming unit to that of the total area sampled. Colony count greater than 5 CFU per ml and less than 5 CFU per ml were considered as contaminated and non-contaminated, respectively (**Harley-Prescott, 2002; Misgana *et al.*, 2015**).

5.7.3. Bacteria identification

Following colony count, identification of culture isolates was done according to the standard bacteriological methods. Colony morphology, hemolytic pattern, Gram reaction and microscopic features were used as primarily identification criteria. Catalase test was done to differentiate staphylococcal from streptococcal isolates. Mannitol fermentation and a slide coagulase tests were done to differentiate *S. aureus* from coagulase-negative staphylococci (CoNS). Identification of Gram negative isolates were done by using different biochemical tests such as urease test, carbohydrate fermentation tests (glucose and lactose fermentation), Citrate utilization test, motility test, Indole test and gas production **(Cheesbourgh** M, **2006)**.

5.7.4. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of isolates were performed by disk diffusion techniques according to Kirby-Bauer method on Muller-Hinton agar (**Oxoid, UK**). Pure colonies of the test organism were taken using a sterile wire loop and emulsify in 2 ml of sterile physiological saline. The bacterial suspensions turbidity was matched with 0.5

McFarland standards. Then, a sterile cotton swab was dip in to the suspension and squeeze free from excess fluid against the side of test tube. The test organisms were uniformly inoculated on the surface of Muller-Hinton agar plate. Then, twelve antibiotic disks were dispensed manually (by using clipper) on a 150-mm plate and placed no less than 24 mm apart. The medium was incubated at 35^{o}C for 18-24 hours. Each zone of complete inhibition clearly measured the more obvious margin to determine the zone diameter by using ruler including the diameter of the disk.

The results were expressed as sensitive, intermediate or resistant according to the criteria developed by Clinical and Laboratory Standards Institute **(CLSI, 2016)**. Isolates showing an intermediate level of susceptibility considered as sensitive. Multiple drug resistance (MDR) was defined as resistance of the isolate to two or more classes of antibiotics **(Jorgensen and Turnidge, 2015).** The antibiotic disks were selected according to local prescription pattern, availability and accessibility of these drugs in the study area, type of microorganisms isolated, cost and recommendation for first and alternative drug **(CLSI, 2016)**.

The drugs tested **(Oxoid, LTD, UK**) for both gram negative and gram positive bacteria were Chloramphenicol (C, 30µg), Norfloxacin (NOR, 10µg), Ciprofloxacin (CIP, 5µg), Tetracycline (TE, 30µg), Gentamicin (GEN, 10µg), Trimethoprim-sulfamethoxazole (TS, 25µg), and Doxycycline (DOX, 10µg). Ampicillin (AMP, 10µg), Ceftriaxone (CRO, 30µg), Amoxicillin-clavulanate (AUG, 30µg) and Nalidixic acid (NA, 30µg) were used for gram negative bacteria. However, Penicillin (P, 10units),) Cefoxitin (FOX, 30µg), Clindamycin (CD, 2µg), Erythromycin (E, 15µg) and Vancomycin (VA, 10µg) were tested for gram positive cocci.

5.8. Quality control issues

Data collection: The prepared questionnaire used for collection of information for the intended purpose was checked for its completeness and validity prior to the collection of data.

During culture media preparation: The manufacturers' instruction and bacteriological standard procedures were strictly followed during culture media preparation. Expiry date of the media and autoclave status were confirmed before preparation.

The quality of the prepared cultured media was checked by doing both sterility and performance testing. For sterility testing, 3-5% of the uninoculated media from each batch after preparation were incubated at 35 0^C for 2 days. The uninoculated media that showed growth were discarded together with the whole batch. Performance testing was assured by inoculating and observing the growth of standard reference strains of *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922 and *P. aeruginosa* (ATCC 27853). To standardize the inoculums density of bacterial suspension for the susceptibility test, 0.5 McFarland standards was used **(Cheesbourgh** M, **2006).**

Specimen collection and transportation: All materials (test tubes, TSB, normal saline, cotton tip applicators) were autoclaved and used aseptically throughout specimen collection and transported by biosafety box with personal protective equipment's. Strict bacteriological sample collection procedure was followed at the time of actual swabbing. The collected samples were given unique identification numbers, labeled with the fomite name, coded field of specialization and the name of working wards.

During sample processing: Performance of all prepared media of antimicrobial susceptibility tests were also checked by inoculating American Type Culture Collection (ATCC) standard reference strains **(***S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853).

All steps in data collection and recording were closely monitored by senior supervisors and performed through the principal investigator; and daily collected data was recorded and compiled for the next day of study. Keep proper records of results was done.

5.9. Data organization, presentation and analysis

The data from the finding was coded, entered and analyzed using Statistical Package for Social Science (SPSS) version 23 for Windows. Descriptive statistics were used to describe relevant variables. Bivariate logistic regression analysis was computed to see the association between dependent and independent variables. Those variables having p-values less than 0.05 at 95% confidence interval were considered a statistically significant.

5.10. Ethical considerations

Ethical clearance was obtained from ethical review committee of College of Medicine and Health Sciences, Bahir Dar University. Official permission was obtained from the Amhara National Regional State Health Bureau and the management committee of FHRH. All the study participants were informed about the purpose of the study and finally their written consent were obtained before observation and sample collection. Confidentiality of the results was maintained.

5.11. Operational definitions

Health care worker fomites: Are health care personnel attire such as white coats and devices like stethoscope, and mobile phones.

Health care workers: Are HCWs who work in hospital or health centers.

Multiple drug resistance: defined as resistance of the isolate to two or more classes of antibiotics.

5.12. Dissemination of the study

The findings of this study will be submitted to the Department of Medical Laboratory Sciences, Bahir Dar University. The finding will be disseminated to Amhara Regional State Health Bureau and FHRH. Finally, it will be also communicated to the concerned bodies and presented through seminars and workshops as well as further effort will be made to publish the findings on peer reviewed journal.

6. RESULTS

Socio-demographic characteristics

A total of 422 fomites from HCWs were included in the study. Of them, 63 (14.9%) were stethoscopes, 165 (39.1%) were mobile phones and 194 (46.0%) were white coats. Two hundred twelve (50.2%) of the fomites were from males. The median age of the participants was 28 years (range: 20 to 55). In terms of profession, 146 (34.6%) nurses , 87(20.6%) medical intern students, 86 (20.4%) medical doctors, 22 (6.9%) midwives, 16 (3.8%) anesthetists, 30 (7.1%) pharmacists and 35 (8.3%) were medical laboratory professionals. Two hundred nine (49.5%) of HCWs had bachelor's degree (BSc). In terms of working wards, 58 (13.7%), 54 (12.8%) and 52 (12.3%) of HCWs were from outpatient department, operation theater and surgical wards, respectively (**Table** 1).

Table 1: Demographic and professional related characteristics of study participants at FHRH, Bahir Dar, 2017.

Variables	Frequency (N=422)	Percent
Sex		
Male	212	50.2
Female	210	49.8
Age (in years)		
20-24	75	17.8
25-29	168	39.8
30-34	106	25.1
≥ 35	73	17.2
Field of specialization		
Nurses	146	34.6
Medical intern students	87	20.6

Medical doctors	86	20.4
Medical laboratory professionals	35	8.3
Pharmacy professionals	30	7.1
Midwives	22	5.2
Anesthetist nurse	16	3.8
Qualification		
Diploma	33	7.8
BSc	209	49.5
MD	61	14.4
MD+	32	7.5
Medical intern students	87	20.6
Working Wards		
OPD	58	13.7
Medical ward	54	12.8
OR	54	12.8
Surgical ward	52	12.3
Pediatrics	38	9.0
Laboratory	35	8.3
Gynecological and Obstetrics	30	7.1
Pharmacy	30	7.1
Maternity	29	6.9
Orthopedics	25	5.9

ICU	17	4.0
Health care workers fomites		
Stethoscopes	63	15.0
Mobile phones	165	39.0
White coats	194	46.0

6.1. Bacterial contamination

Out of the total fomites swabbed samples, 243 (57.6%) were contaminated. The proportion of bacterial contamination was not significantly higher in mobile phones (59.4%) compared to stethoscopes (58.7%) and white coats (55.6%) (P = 0.035) **(Table 2)**.

Table 2: The rate of bacterial contamination in HCW's fomites at FHRH, Bahir Dar, 2017.

	Contamination status		**P- value**
	Non-contaminated N (%)	**Contaminated N (%)**	
Fomites types			
Mobile phones (165)	67 (40.7)	98 (59.2)	0.035
Stethoscopes (63)	26 (41.3)	37 (58.7)	
White coats (194)	86 (44.3)	108 (55.6)	
Total (N=422)	**179 (42.4)**	**243 (57.6)**	

The proportion of fomites bacterial contamination was 40 (65.6%) in medical doctors and 53 (60.9%) in medical intern students. The highest proportion of fomites bacterial contamination was found in medical laboratory professionals (65.7%) and HCWs working at Intensive care unit (94.1%). Details of bacterial contamination with different categories of HCWs are depicted in **Table 3**.

Table 3: Rate of bacterial contamination of fomites in terms of different characteristics of HCWs at FHRH, Bahir Dar, 2017.

Characteristics	Contamination status		Total	P-value
	Contaminated	Non contaminated	N (%)	
	N (%)	N (%)		
Gender				
Female	120 (57.1)	90 (46.9)	210 (49.8)	
Male	123 (58)	89 (42)	212 (50.2)	0.86
Age (in years)				
20-24	46 (61.3)	29 (38.7)	75 (17.8)	
25-29	94 (56)	74 (44)	168 (39)	
30-34	57 (53.8)	47 (46.2)	106 (25.1)	
≥35	46 (63)	27 (37)	73 (17.3)	0.55
Year of services				
<5 years	116 (55.2)	94 (44.8)	210 (49.8)	
5-9 years	77 (56.6)	59 (43.4)	136 (32.2)	
≥10 years	50 (65.8)	26 (34.1)	76 (18)	0.27
Qualification of health care worker's				
Diploma	20 (60.6)	13 (39.4)	33 (7.8)	
BSc	113 (54.1)	96 (45.9)	209 (49.5)	
MD	40 (65.6)	21 (34.4)	61 (14.5)	
MD+	17 (53.1)	15 (46.9)	32 (7.4)	
Med. intern students	53 (60.9)	34 (39.1)	87 (20.6)	0.49
Field of specialization				
Lab. Professionals	23 (65.7)	12 (34.3)	35 (8.3)	0.05
Nurses	85 (58.2)	61 (41.8)	146 (34.6)	
Midwifery	14 (63.6)	8 (36.4)	22 (5.2)	
Pharm. professionals	12 (40)	18 (60)	30 (7.1)	
Doctor	52 (60.5)	34 (39.5)	86 (20.4)	
Intern students	53 (60.9)	34 (39.1)	87 (20.6)	
Anesthetists	4 (25)	12 (75)	16 (3.8)	
Health care worker's working in				

wards				
Surgical	29 (55.8)	23 (44)	52 (12.2)	
Medical	41 (75.9)	13 (24.1)	54 (12.8)	
Gynecology / Obstetrics	25 (83.3)	5 (16.7)	30 (7.1)	
Pediatrics	18 (47.4)	20 (52.6)	38 (9)	
Maternity	17 (58.6)	12 (41.4)	29 (6.9)	
Operation Theater	20 (37)	34 (63)	54 (12.8)	
Orthopedics	13 (52)	12 (48)	25 (5.9)	
OPDs	2 (50)	29 (50)	58 (13.7)	
Pharmacy unit	12 (40)	18 (60)	30 (7.1)	
Laboratory unit	23 (65.7)	12 (34.3)	35 (8.3)	
Intensive Care Unit	16 (94.1)	1 (5.9)	17 (4)	<0.001
Total	**243 (56.7)**	**179 (42.3)**	**422(100)**	

Key: MD: General practitioner (Medical doctors), MD+: Specialist, OPDs: Outpatient departments

The overall practice of health care workers towards hand washing and disinfection of their fomites are illustrated in table 3. The majority (72%) of participants did not regularly wash their hands before touching a patient. Regular disinfecting of mobile phones and stethoscopes were not practiced by 361(85.5%) and 409 (96.9%) of participants, respectively. Moreover, 305 (72.3%) of participants were answered phones call while attending patients. The rate of fomites bacterial contamination was significantly higher in those HCWs who did not used laundry for white coat cleaning compared to their counter parts (P = 0.015). Moreover, the rate of contamination was higher in those HCWs who were used mobile phones at bed side and did not regularly wash their fomites. However, the difference was not statistical significance (P > 0.05) (**Table 4)**.

Table 4: Bacterial contamination of fomites, hand washing and disinfecting practices of HCWs at FHRH, Bahir Dar, 2017.

Characteristics	**Contaminated N (%)**	**Non-contaminated N (%)**	**Participants (422), N (%)**	**P-value**
Regular hand washing before touching a patient				
Yes	69 (58.5)	49 (42.5)	118 (28)	
No	174 (57.2)	130 (42.8)	304 (72)	0.817
Regular hand washing before clean/aseptic procedure				
Yes	159 (58.5)	113 (42.5)	272 (64.5)	
No	84 (56)	66 (44)	150 (35.5)	0.625
Regular hand washing after body fluid exposure				
Yes	223 (56.6)	171 (43.4)	394 (93.4)	
No	20 (71.4)	8 (28.6)	28 (6.6)	0.131
Regular hand washing after touching a patient				
Yes	144 (59)	100 (41)	244 (57.8)	
No	99 (55.6)	79 (44.4)	178 (42.2)	0.486
Regular disinfecting of mobile phone				
Yes	32 (52.5)	29 (47.5)	61 (14.5)	
No	211 (58.4)	150(41.5)	361 (85.5)	0.382

Use of mobile phone at bed side to medical information				
Yes	203 (59.5)	138 (39.5)	341 (80.8)	
No	40 (49.4)	41 (50.6)	81 (19.2)	0.098
Regular disinfecting of stethoscope				
Yes	7 (53.8)	6 (46.2)	13 (3.1)	
No	236 (57.7)	173 (42.3)	409 (96.9)	0. 782
Regular use of hand antiseptic				
Yes	207 (56.7)	158 (43.3)	364 (86.5)	
No	36 (63.2)	21 (36.8)	57 (13.5)	0.361
Regular cleaning of Stethoscope, Mobile phone and white coats				
Yes	45 (49.5)	46 (50.5)	91 (21.6)	
No	198 (59.8)	133 (40.2)	331 (78.4)	0.077
Answering phone calls while attending patients				
Yes	183 (60)	122 (40)	305 (72.3)	0.106
No	60 (51.3)	57 (49.7)	117 (27.7)	
Use of laundry white coat				
Yes	31 (44.3)	39 (55.7)	70 (16.6)	
No	212 (60.2)	140 (39.8)	352 (83.4)	0.015

6.2. **Bacterial isolates from HCWs fomites**

Out of 422 swab samples processed, 253 (60%) aerobic bacterial species were isolated. Of them, 231 (90%) were potential pathogens. *Coagulase negative Staphylococci spp*. were the most frequent isolate accounting 111 (44 %) followed by *S. aureus* 81 (32%) and *K.pneumoniae* 27 (10.2%) from all type of fomites. Isolation rate of *E.coli* (62.5%) followed by *S. aureus* (48.1%) were the leading isolates from white coats. On the other hand, *K. Pneumoniae* (59.2%) and CoNS (16.2%) were the predominant isolates from mobile phones and stethoscopes, respectively. The isolation rate of CoNS, *S.aureus* and *E.coli* were significantly higher from white coats compared to other fomites ($p<0.001$). However, the proportion of *K.pneumoniae* was significantly higher in mobile phones compared to other fomites ($p<0.001$) **(Table 5).**

Table 5: Isolation rate of bacteria in swabs collected from different HCW's fomites at FHRH, Bahir Dar, 2017.

Type of isolated organism	Types of fomites			P-value	Total (N=422)
	Stethoscope (n=63)	Mobiles phone (n=165)	White coat (n=194)		
CoNS	18 (16.2)	43 (38.7)	50 (45.0)	<0.001	111 (44)
S.aureus	11 (13.8)	31 (38.2)	39 (48.1)	<0.001	81 (32.2)
Bacillus spp.	2 (9.0)	10 (45.0)	10 (45.0)	<0.001	22 (8.6)
S.pyogens	1 (100)	0 (0.0)	0 (0.0)	NA	1 (0.3)
K. Pneumoniae	3 (11.1)	16 (59.2)	8 (29.6)	<0.001	27 (10.6)
E.coli	0 (0.0)	3 (37.5)	5 (62.5)	0.014	8 (3.1)
Citrobacter spp.	2 (100)	0 (0.0)	0 (0.0)	NA	2 (0.7)
P.aeruginosa	1 (100)	0 (0.0)	0 (0.0)	NA	1 (0.3)
Total	**38 (60.3%)**	**103 (62.4%)**	**112 (57.7%)**	**0.006**	**253 (60%)**

Key: NA: Not applicable

The distribution of *S.aureus and CoNs* were significantly higher in fomites from Nurses (43.2%) compared to other professionals (P<0.001)

Table 6:Distribution of pathogenic bacterial isolates among HCWs fomites at FHRH, Bahir Dar, 2017.

Type of isolated organism	Type of profession							Total N (%)	P-value
	Nurse (n=146)	Med. Laboratory (n=35)	Midwives (n =22)	Pharmacy (n=30)	Medical Doctor (n =86)	Intern students (n=87)	Anesthetist (n=16)		
K.pneumoniae	9 (33.3)	10 (37.0)	0 (0.0)	0 (0.0)	3 (11.1)	5 (18.5)	0 (0.0)	27 (11.1)	0.056
E.coli	2 (25.0)	2 (25.0)	1 (12.5)	0 (0.0)	2 (25.0)	1 (12.5)	0 (0.0)	8 (3.2)	0.682

CoNS	36 (32.4)	7 (6.3)	6 (5.4)	6 (5.4)	27 (24.3)	27 (24.3)	2 (1.8)	111 (45.6)	<0.001
S.aureus	35 (43.2)	4 (4.9)	6 (7.4)	4 (4.9)	12 (14.8)	18 (22.2)	2 (2.4)	81 (33.3)	<0.001
Pseudomonas spp.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0)	1 (100)	0 (0.0)	1 (0.4)	NA
Citrobacter spp.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	2 (1.6)	NA
S.pyogens	0(0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (0.4)	NA
Total	**82 (33.7)**	**23 (9.4)**	**13 (5.3)**	**10 (4.1)**	**46 (19.0)**	**53 (21.8)**	**4 (1.7)**	**231 (90)**	**0.023**

Key: NA: Not applicable

6.3. Antimicrobial susceptibility pattern of the isolates

Gram positive bacteria isolates revealed high rate of resistance to Penicillin (80%), Erythromycin (54.7%) and Cotrimoxazole (53.1%). However, low percentages of resistance were noticed against Ciprofloxacin (4.1%) and Clindamycin (5.2%). As indicated in Table 6, 12.3% of the *S. aureus* and 10.8% of CoNS isolates were methicillin resistant. *S.aureus* and CoNS showed high percentages of resistance to penicillin at 82.7% and 77.5%, respectively.

Overall, gram negative bacteria were resistant to Ampicillin (94.8%), Cotrimoxazole (72%) and Tetracycline (61.5%). Majority of gram negative isolates were sensitive to Ciprofloxacin (97.5%) and Nalidixic acid (77.0%). Majority (96.3%) of *K.pneumoniae* isolates were sensitive to Ciprofloxacin and 100% resistant to Ampicillin. *E. coli* was resistant to Ampicillin (87.5%) and Cotrimoxazole (87.5%). The overall resistance profiles of the isolates depicted in **Table 7**.

Table 7: Antimicrobial resistances (R) patterns of the bacterial isolates from HCWs fomites at FHRH, Bahir Dar, 2017.

Bacterial isolates	**Antimicrobials tested N (%) of resistance rate**										
Gram positives	**C**	**NOR**	**P**	**CIP**	**TE**	**GEN**	**FOX**	**CD**	**E**	**DOX**	**TS**
***S.aureus* (no = 81)**	16 (20)	10 (12.3)	67 (82.7)	1 (1.2)	40 (49.4)	14(17)	10 (12.3)	2 (2.5)	49 (60.5)	28(34.6)	43 (53.1)
***CoNS* (no=111)**	28 (25)	18 (16.2)	86 (77.5)	7 (6.3)	61 (55)	17 (15)	12 (10.8)	8 (7.2)	56 (50.5)	29 (26.1)	58 (15.3)
***S.pyogen* (no = 1)**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0 (0)	1 (100)
Total N (%) GP: 192	**44 (23)**	**28 (15)**	**153 (80)**	**8 (4.1)**	**101 (52.6)**	**31 (16)**	**22 (11.4)**	**10 (5.2)**	**105 (54.7)**	**57 (30)**	**102 (53)**
Gram negatives	**AUG**	**AMP**	**CRO**	**C**	**NOR**	**CIP**	**TE**	**GEN**	**NAL**	**DOX**	**TS**
***K.pneumoniae* (no=27)**	3 (11.1)	27 (100)	5 (18.5)	13 (48.1)	2 (7.4)	1 (3.7)	15 (56)	8 (29.6)	3 (11.1)	7 (26)	18 (67)
***E.coli* (no = 8)**	0 (0)	7 (87.5)	0 (0)	4 (50)	1 (12)	0 (0)	6 (75)	3 (37.5)	0 (0)	2 (25)	7 (87.5)
***Citrobacter* (no =2)**	0 (0)	2 (100)	0 (0)	(0)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)
***P.arogunosa* (no =1)**	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Total N (%) GN:39	**3 (7.6)**	**37 (94.8)**	**5 (12.8)**	**18 (46.1)**	**3 (7.6)**	**1 (2.5)**	**24 (61.5)**	**12 (30.7)**	**4 (10.2)**	**10 (25.6)**	**28 (72)**

Key: AMP: Ampicillin, CRO: Ceftriaxone, C: Chloramphenicol, NOR: Norfloxacin, P: Penicillin, CIP: Ciprofloxacin, TE: Tetracycline, GEN: Gentamicin, FOX: Cefoxitin, TS: Cotrimoxazole, CD: Clindamycin, E: Erythromycin, DOX: Doxycycline and AUG: Augmentin, NA: Nalidixic acid, NA: Not applicable

Multidrug resistance profiles of the isolates:

Overall, 9 (4%) of the isolates were susceptible to all drugs tested. Resistance to two or more different class of antibiotics were found in 204 (88.3%) of the isolates. The overall MDR rate among gram positive and gram negative bacteria isolates were168 (87.5%) and 36 (94.7%) respectively. The proportion of MDR S.aureus and CoNs isolates were 88.9% and 86.4%, respectively. Among MDR gram negative bacteria, *K.pneumoniae* (92.6%) and *E.coli* (100%) were the principal MDR species **(Table 8).**

Table 8: Multidrug resistance patterns of bacterial isolates from HCWs fomites to commonly used antibiotic classes at FHRH, Bahir Dar, 2017.

Organism isolated (n)	**Degree of resistance**										**Overall, MDR**
	R0 (%)	R1 (%)	R2 (%)	R3 (%)	R4 (%)	R5 (%)	R6 (%)	R7 (%)	R8 (%)	R9 (%)	
CoNS (111)	6 (5.4)	9 (8.1)	13 (12)	11 (10)	17 (15.3)	15 (13.5)	15 (13.5)	11(10)	9 (8.1)	5 (4.5)	96 (86.4)
S.*aureus* (81)	3 (3.7)	6 (7.4)	8 (10)	13 (16)	12 (14)	9 (11.1)	13 (16)	8 (9.8)	4 (4.9)	5 (6)	72 (88.9)
***K.pneumoniae* (27)**	-	2 (7.4)	3 (11.1)	3 (11.1)	5 (18.5)	2 (7.4)	3 (11.1)	2 (7.4)	4 (15)	3(11)	25 (92.6)
***E.coli* (8)**	-	-	-	1 (12.5)	1 (12.5)	3 (37.5)	2 (25)	1(12.5)	-	-	8 (100)
***Citrobacter spp*.(2)**	-	-	-	-	-	1 (50)	1 (50)	-	-	-	2 (100)
***P.aeruginosa* (1)**	-	-	-	-	-	-	-	-	-	1 (100)	1(100)
***S.pyogens* (1)**	-	1(100)	-	-	-	-	-	-	-	-	0 (0)
Total n = 231	**9 (4)**	**18 (8)**	**24 (10)**	**28 (12.1)**	**35 (15)**	**30 (13)**	**34 (15)**	**22 (9.5)**	**17 (7.3)**	**12 (5)**	**204 (88.3)**

Key: R0: susceptible to all antibiotics, R1-9: resistance to1, 2, 3, 4, 5, 6, 7, 8 and 9 antibiotics, ≥R2: resistance to 2 or more antibiotics

7. DISCUSSION

This study shows the bacterial contamination of HCWs fomites at FHRH which is first in its type. The finding would be important for the future specific intervention to tackle HAIs and spreading of drug resistance pathogens even at the community level**.** Contamination of health care worker's fomites might be considered as the most relevant hazard **(Wiener-well *et al.,* 2011; Maryam *et al*., 2016).**

The overall rate of bacterial contamination of HCWs fomites (55.6% to 59.2%) in this study was coherent with a report in Uganda (57.59%) **(Segujja *el al*., 2016).** However, it was lower than previous reports in Egypt (100%) (**Selim *et al*., 2015**), Iran **(90%) (Parhizgari *et al*., 2014)** and Ethiopia **(**71.2%, 97.4%) (**Misgna *et al.,* 2015*;* Daka *et al*., 2015).** This variation might be due to difference in proper handling of fomites, sampling technique and study area. Moreover, variation in service years, lack of regular hand washing, use of mobile phone at bedside for medical information and answering phones calls while attending patients might contribute for the presence of significant rate of bacterial contamination of fomites in the present study.

The proportion of bacterial contamination of HCWs stethoscopes in this study was lower compared to study in Jimma, Ethiopia at 85.8% **(Shiferaw *et al*., 2013)**.This lower level of bacterial contamination might be associated with variation in regular disinfecting of the stethoscope**.**

In the present study, the level of bacterial contamination of white coats was lower than studied in India at 69% **(Banu *et al*., 2012**), Ethiopia at 70.7% **(Gashaw *et al*., 2014),** Tanzania at 73.3% **(Qaday *et al*., 2015)** and Nigeria at 65.7% **(Maryam *et al.,* 2016).** However, it is higher than a study conducted in America (0-16%) **(Haun *et al;* 2016).** This might be due to variation in cleaning practice of white coats and working wards.

The proportion of bacterial contamination of mobile phones of HCWs in the present study is higher compared to previous studies in Iran at 32% (**Sepehri *et al*., 2009),** Saudi Arabia at 38.3% **(Asmari *et al.,* 2015**) and in America at 0-20% (**Haun *et al;* 2016**). This might be due to non-restriction use of mobile phones at bed side and poor in disinfecting practice and hand hygiene problem in the study area as indicated in table 4.

Other studies have also suggested that a patient's skin as well as hand can be a source of contamination for the HCWs fomites **(Mbim *et al*., 2016).**

The level of bacterial contamination of fomites at medical doctors such as specialists (53.1%), general practitioners (65.6%) and intern students (60.9%) in this study was lower than a study conducted in Jimma, Ethiopia **(Shiferaw *et al*., 2013**) with contaminated stethoscope of medical doctors (100%). However, it was higher than previous reports in Tanzania (35%) **(Qaday *et al*., 2015)** and Iran (50%) (**Sepehri *et al*., 2009).** This variation might be due to differences in hand washing and fomites disinfection practices of HCWs in different health care settings.

In this study, the bacterial contamination was significantly higher in fomites from medical laboratory professionals, midwives, nurses and medical intern students, respectively. It was higher compared to reports in Iran (**Sepehri *et al*., 2009)** and Tnzania **(Qaday *et al*., 2015)** nurses and medical intern students. However; this study was lower in Jimma, Ethiopia **(Shiferaw *et al*., 2013**) from diferrent field of specializations. Moreover, this was lower than study in Egypt **(Selim *et al*., 2015)** medical doctors, laboratory personells and nurses. This varaition might be due to working area and habit of hand practice.

In this study, highest bacterial contaminations of fomites were found in those professionals working at ICU (94.1%) compared to other working wards. This is consistent with previous studies conducted in Jimma, Ethiopia **(Shiferaw *et al*., 2013).** However, highest number of bacterial isolates was found at othopedics ward in Nigeria **(Chikre *et al*.2008),** internal medicine in Iran **(Askari *et al*., 2013)** and laboratory unit in Egypt **(Selim *et al*., 2015).** This might be due to be the presence of favorable temperature to bacteria in ICU, environments of ICU beds and medical equipment's is crowded, and the frequency of hand touching to give patient care makes it higher level of bacterial contamination as indicated by **(Smith *et al*.,** 2012**).**

In the present study, majority (90.3%) of the isolates were potential pathogens .This is higher than previous studies in Saudi Arabia (38.3%) **(Asmari *et al*., 2015)** and 52% in Ethiopia (**Shiferaw *et al., 2013*).** However, it was lower than reports in Iran (94%) (**Askari *et al***., 2013). This variation might be associated with poor hand hygiene practice and no regular hand washing with antiseptic solutions in clinical setting.

In this study, the frequency of gram-positive isolates was higher than gram negative isolates. This is consistent with previous studies in Gondar (**Gashaw *et al*., 2014),** Jimma (**Shiferaw *et al., 2013*; Misgana *et al*., 2015)** and Hawassa (**Daka *et al*., 2015),** Ethiopia**.** Moreover, the study was also similar compared to studies done in Iran (**Sepehri *et al*., 2009),** Saudi Arabia **(Asmari *et al.;* 2013)** and Egypt **(Eldegla *et al.,* 2016*).*** The reason for high isolating rate of gram positive organisms might be due to the direct contact of the fomites to human skin flora which contains mostly gram positive bacteria. Moreover, gram positive bacteria have a longer life span in vitro compared to gram negative bacteria. This is the fact that they can tolerate in animate objects for a long period of time. Genus staphylococci were the leading isolates during the study period, which was also found in studies conducted in Ethiopia (**Shiferaw *et al., 2013*; Gashaw *et al*., 2014; Misgana *et al*., *2015),*** India (**Banu *et al*.,2012**), Iran (**Sepehri *et al*., 2009; Parhizgari *et al*., 2014).**

Coagulase negative staphylococci were the most frequent isolates in the study. However, its proportion (44%) was lower than earlier studies in Gondar (47.5%) (**Gashaw *et al*., 2014;** Jimma **(**58.8%) **Misgana *et al*., 2015),** Hawassa **(**84.2%) (**Daka *et al*., 2015),** Ethiopia and Iran (77.1%) (**Sepehri *et al*., 2009).** On the other hand, its proportion was higher than other studies done in India (10.3%) **(Banu *et al*., 2012).** The dominancy of CoNS might be due to the direct contact of the stethoscope, mobile phones and white coats to the skin. Moreover, it can be tolerate medical device and clothes in the hospital environment.

This study revealed that *E.coli* (62.5%) was the leading isolate from white coats. This was coherent with a study conducted in Nigeria **(Chikere *et al*., 2008).** However, it differs from a study done in Iran **(Askari *et al*., 2013)** where *Bacillus spp* was the most frequent. This study also differs from findings in India (**Banu *et al*., 2012)** where *S.aureus* was the most prevalent. On the other hand, *K. pneumoniae* (59.2%) and CoNS (16.2%) were found to be the predominant isolates from stethoscopes and mobiles phones, respectively. This was consistent with previous studies done in Ethiopia **(Shiferaw *et al*., 2013)** and Egypt (**Selim *et al*., 2013),** Uganda **(Segujja *el al*., 2016)** and India (**Banu *et al*., 2012)**. The predominancy of *E.coli* and *K. Pneumoniae* might be associated with their long time survival in the wet environment, contamination from patient wounds and HCWs hands **(Chikere *et al*., 2008; Parhizgari *et al*., 2014).**

In this study gram positive isolates showed high level of resistance to penicillin (80%). This is consistent with previous reports in India **(Banu *et al*., 2012)** and Saudi Arabia **(Asmari *et al*., 2015).** However, it is higher than a report in Hawassa, Ethiopia **(Daka *et al*., 2015)**; and lower than a report in Jimma, Ethiopia **(Misgana *et al*., 2015).**

In this study, gram positive isolates showed least resistance to ciprofloxacin and clindamycin which are consistent with similar studies in Iran (**Parhizgari *et al*., 2014)** and Jimma, Ethiopia (**Shiferaw *et al*., 2013).** However**,** in Egypt gentamycin was the least resisted antibiotic **(Eldegla *et al*., 2016).**

In the present study, all gram-negative bacteria isolates were susceptible to ciprofloxacin except *K.pneumoniae.* This is inconsistent to other studies in Iran **(Parhizgari *et al*., 2014),** Ethiopia **(Gashaw *et al*.,2014)** and Egypt **(Eldegla *et al*., 2016).** However, gentamicin was the most susceptible antimicrobial for gram negative bacteria studied in Nigeria **(Maryam *et al*., 2016).** In this study, ampicillin (94.8%) was the most resistant antibiotics for gram negative bacteria. This was similar compared to studies in Egypt **(Eldegla *et al*., 2016)** and Nigeria **(Maryam *et al,* 2016).**

In this study, the overall MDR rate among gram positive and gram negative bacteria isolates were 88.3%. This study was higher compared to earlier studies in Gondar, Ethiopia **(Gashaw *et al*., 2016)** and Egypt **(Eldegla *et al*., 2016).** In terms of MDR, *E.coli* (100%), *K.pneumoniae* (92.6%) and S.aureus (88.9%) were the principal MDR species in this study. These finding were higher compared to a study in Saudi Arabia 71.8% (**Asmari *et al*., 2015).**

The higher multidrug resistance in both gram positive and gram negative bacteria reported in the present study might be due to indiscriminate of antibiotics, mis-use of antibiotics such as using antibiotics for inappropriate conditions, empirical treatments, use of broad spectrum antibiotics and use of leftover antibiotic prescription and taking inappropriate dose for inappropriate duration. Most of the antibiotic classes were used as treatment alternatives in the study area. This might be challenged to the therapeutic medicine as the spread of these isolates goes to in this direction and if intervention is not considered.

8. LIMITATIONS OF THE STUDY

The limitations of this study were

- This study did not check for extended spectrum beta lactamase producing *E.coli* and *K.pneumoniae* isolates.
- Use of non-probability sampling technique might affect taking representative fomites from HCWs.
- Multivariate analysis was not computed to identify the risk factors associated with bacterial contamination of HCWs

9. CONCLUSIONS

The rate of bacterial contamination of HCWs fomites obtained in this study is high that remain carries potential pathogens. Relatively higher rate of pathogenic bacteria species isolated as compared to earlier studies. Hence, fomites of HCWs are a major source of infections to hospital clients, health care workers itself and the community. The present study also indicated those not regularly cleaning fomites and those taken from intensive care units most likely to be contaminated. Moreover, the rate of contamination varies with field of specialization. The highest burden of bacterial contamination was occurred in mobile phones. Over all, the skin flora *Staphylococcal species* are the most frequent contaminants of HCWs fomites. Moreover, *Klebsiella pneumoniae*, *E.coli* and CoNs were found to be the most frequent isolates from stethoscopes, white coats and mobile phones, respectively. Gram positive and Gram negative isolates showed high level of resistant to single and multiple classes of the commonly prescribed antimicrobial agents. Moreover, *K.pneumoniae* and *E.coli* were the principal MDR isolates. Ciprofloxacin and Clindamycin were found to be the least resisted drugs by *Staphylococcus aureus* and CoNs. On the other hand, Ciprofloxacin was found to be the most effective drugs against gram negative isolates.

10. RECOMMENDATIONS

Based on the finding of this study, the following recommendations are made:

- Proper handling of HCWs fomites should be promoted.
- Frequent hand washing before and after touching a patient and contaminated environmental sources must be promoted in order to reduce HCWs fomites contamination by transient flora and hospital pathogens.
- Education should be given on proper handling and disinfection of HCWs fomites.
- Focus should be given to the conservative and wise use of antibiotics to minimize the spreading of multi-drug resistant pathogens.
- Further studies in other hospitals of Ethiopia is recommended to have multi-centered national data.

11. REFERENCES

Allegranzi B., Storr J., Dziekan G., Leotsakos A., Donaldson L. & Pittet D.(2007). The first global patient safety challenge "clean care is safer care": From launch to current progress and achievements. *Journal of Hospital Infection;* 65: 115-123.

Alp E.and Damani M. (2015). Healthcare-associated infections in Intensive Care Units: epidemiology and infection control in low-to-middle income countries. *Journal of Infection Developing Countries;* 9(10):1040-1045.

Askari E., Zahra M., Yasaman F., Mahboubeh N (2013). Study on Bacteria Associated with White Coats of Healthcare Worker'sin Two Tertiary Hospitals, Mashhad, Iran. Journal of Med Bacteriology; 2(4): 17-25.

Asmari A., Mahfoud M., Barrak A., Babair Y., Elkhizzi N. & Omani S.(2015). antimicrobial resistance pattern of pathogens isolated from mobile phone at tertiary care hospital in saudi arabia. *World Journal of Pharmaceutical Research;* 4(11):1744-61.

Banu A., Anand M. & Nagi N.(2012). White coats as a vehicle for bacterial dissemination. *Journal of Clinical and Diagnostic Research;* 6(8): 1381-4.

Cheesbourgh M (2006). District laboratory practice in tropical countries part II, 2nd edition. Cambridge University Press, Newyork.

Chikere C.,Omoni V.& Chikere B.(2008). Distribution of potential nosocomial pathogens in a hospital environment. *African Journal of Biotechnology;*7(20): 3535-39.

Clinical and Laboratory Standards Institute (2016). Performance standards for antimicrobial susceptibility testing. CLSI document (M100-S26), Wayne (USA)

Daka D., Yihdego, D. & Tadesse, E.(2015). Level of Contamination and Antibiotic Resistance of Bacterial Isolates from Mobile Phone of HCW's in Hawassa Referral Hospital. *Asian Journal of Medical Sciences;*7 (3): 30-35.

Eldegla H., Eldars W., Mesbah m. R., Farag R. E.-S., Mousa N., Abdel-Aziz M. & Elrazik A. A. (2016). Healthcare Associated Infections and Patterns of Antibiotic Resistance in Tropical Medicine Department in Egypt. *International Journal of Current Microbiololgy and Applied Science;*5(6): 1-15.

Endalafer N., Gebre-Selassie S. & Kotiso B. (2011). Nosocomial bacterial infections in a tertiary hospital in Ethiopia. *Journal of Infection Prevention*;12(1):38-43.

Gashaw M., Abtew D. & Addis Z. (2014). Prevalence and Antimicrobial Susceptibility Pattern of Bacteria Isolated from Mobile Phones of Health Care Professionals Working in Gondar Town Health Centers. *ISRN Public Health;* 2014(1).

Harley-Prescott (2002), Laboratory Exercises in Microbiology, Fifth Edition. McGraw-Hill, USA

Haun N., Hooper-Lane C. & Safdar N. (2016). Healthcare Personnel Attire and Devices as Fomites: A Systematic Review. *Infection Control & Hospit Epidemiology;* 37: 1367-1373.

Jorgensen J. H. & Turnidge J. D. (2015). Susceptibility test methods: dilution and disk diffusion methods. *Manual of Clinical Microbiology Eleventh Edition.* American Society of Microbiology.

Lopez G. U., Gerba C. P., Tamimi A. H., Kitajima M., Maxwell S. L. & ROSE J. B. (2013). Transfer efficiency of bacteria and viruses from porous and nonporous fomites to fingers under different relative humidity conditions. *Applied and Environmental Microbiology;* 79(18): 5728-5734.

Loveday H., Wilson J., Pratt R., Golsorkhi M., Tingle A., Bak A., Browne J., Prieto J. & Wilcox M. (2014).National evidence-basedguidelines for preventing healthcare-associated infections in NHS hospitals in England. *Journal of Hospital Infection;*86: S1-S70.

Magill S. S., Edwards J. R., Bamberg W., Beldavs Z. G., Dumyati G., Kainer M. A., Lynfield R., Maloney M., Mcallister-Hollod L. & Nadle J.(2014). Multistate point-prevalence survey of health care–associated infections. *New England Journal of Medicine;* 370: 1198-1208.

Maryam A., Hadiza U.-S. & Aminu U. M. (2014). Characterization anddetermination of antibiotic susceptibility pattern of bacteria isolated from some fomites in a teaching hospital in northern Nigeria. *African Journal of Microbiology Research;* 8(8): 814-818.

Mbim e. N., Mboto C. I. & Agbo B. E.(2016). A Review of Nosocomial Infections in Sub-Saharan Africa.British Microbial Research Journal;15:1-11

Melaku S., Gebre-Selassie S., Damtie M. & Alamrew K. (2012). Hospital acquired infections among surgical, gynaecology and obstetrics patients in Felege-Hiwot referral hospital, Bahir Dar,Northwest Ethiopia. *Ethiopian Medical Journal;* 50: 135-144.

Misgana G. M., Abdissa K. & Abebe G.(2015). Bacterial contamination of mobile phones of health care worker'sat Jimma, South West Ethiopia. *International Journal of Infection Control;*11:1-8

Parhizgari N., Farajzadeh Sheikh A. & Sadeghi P.(2014). Identification of bacteria isolated from mobile phones of three medical and teaching hospitals administrative and medical staff in Ahvaz. *Jentashapir Journal of Health Research;* 4(5): 397-403.

Qaday J., Sariko M., Mwakyoma A., Kifaro E., Mosha D., Tarimo R.,Nyombi B. & Shao E.(2015). Bacterial Contamination of Medical Doctors and Students White Coats at Kilimanjaro Christian Medical Centre, Moshi, Tanzania. *International Journal of Bacteriology;1-5 volume*

Rhomberg P.R., Fritsche T.R., Sader H.S. & Jones R.N.(2006). Antimicrobial susceptibility pattern comparisons among intensive care unit and general ward Gram-negative isolates from the Meropenem Yearly Susceptibility Test Information Collection Program (USA).*Diagnostic Microbiology and Infectious Disease;* 56:57-62.

Rosenthal V. D., Lynch P., Jarvis W., Khader I. A., Richtmann R., Jaballah N. B., Aygun C.,Villamil-Gómez W., Duenas L.&Atencio-Espinoza T.(2011). Socioeconomic impact on device-associated infections in limited-resource neonatal intensive care units: findings of the INICC. *Infection;* 39(5): 439-450.

Samuel S., Kayode O., Musa O., Nwigwe G., Aboderin A., Salami T. Taiwo S. (2010). Nosocomial infections and the challenges of control in developing countries. *African Journal of Clinical and Experiment Microbiology;* 11(2):102-110

Segujja F., Mwambi B., Drago C. K., Lubowa N. M., Mugambwa J. & Wabuyi P.(2016). Characterization and Antimicrobial Susceptibility Patterns of Isolates from Ward Fomites. *British Biotechnology Journal; 14(4): 1-16*

Selim S. H. and Abaza F.(2015). Microbial contamination of mobile phones in a health care setting in Alexandria, Egypt. GMS Hygiene and Infection Control; 10(1): ISSN 2196-5226: Doc03.

Sepehri G., Talebizadeh N., Mirzazadeh A., Mir-Shekari T.-R. & Sepehri E. (2009). Bacterial contamination and resistance to commonly used antimicrobials of healthcare workers' mobile phones in teaching hospitals, Kerman, Iran. *American Journal of Applied Sciences;* 6(5): 806-810.

Shiferaw T., Beyene G., Kassa T. & Sewunet T.(2013).Bacterial contamination, bacterial profile and antimicrobial susceptibility pattern of isolates from stethoscopes at Jimma University Specialized Hospital. *Annals of Clinical Microbiology and Antimicrobials;* 12: 1-9

Smith S.,Young V., Robertson C. & Dancer S. (2012).Where do hands go? An audit of sequential hand-touch events on a hospital ward. *Journal of Hospital Infection;* 80(3): 206-211.

CDC (2013). Department of Human service Center for Disease Control and Prevention. Antibiotic resistance threats in the United States.

Whittington A., Whitlow G., Hewson D., Thomas C. & Brett S. (2009). Bacterial contamination of stethoscopes on the intensive care unit. *Anaesthesia;* 64: 620-624.

WHO (2014). Antimicrobial resistance global report on surveillance, Geneva

WHO (2009).Core components for infection prevention and control programmers, Geneva

Wiener-Well Y., Galuty M., Rudensky B.,Schlesinger Y., Attias D & Yinnon A. M.(2011). Nursing and physician attire as possible source of nosocomial infections. *American Journal of Infection Control;* 39: 555-559.

Yallew W W., Kumie A., Yehuala M. (2016). Point prevalence of hospital acquired infections in two teaching hospitals of Amhara region in Ethiopia. Drug, Healthcare and Patient Safety; 8:71–76.

Annex I: Information sheet and consent form

Study Title: Bacterial contamination and antimicrobial susceptibility pattern of isolates from HCWs at Felege Hiwot Referral Hospital, North West Ethiopia

Purpose:

I have planned and conducted a study with the objective of determining Pathogenic bacteria profile and antimicrobial susceptibility pattern isolates in HCW in Ethiopia. The knowledge gained from this work is believed to help program to reduce the morbidity and mortality associated with HAIs that comes from HCWs fomites.

Procedure:

For this study a structured questionnaire was used to interview to HCW with fomites suspected to pathogenic bacterial contamination and to collect their socio demographic data. Appropriate swab sample was collected and processed by different culture media as standard protocol procedure for isolation of bacteria and antimicrobial susceptibility pattern was identified by Kirby disk diffusion method. By saying you are selected as one of the study participants. If you are willing to participate you are kindly requested to give your true response and give swab sample from your fomites to the data and sample collectors during interview.

Risk and /or Discomfort; by participating in this research project you might fill discomfort due to wasting your time about less than 10 minute for questionnaire response and for sample collection and there is no risk in participating in this study project.

Benefits

If you participate in this research and if your fomites are found to have contaminated by pathogenic bacteria, your fomites may be able receive appropriate intervention. We will facilitate screening of your fomites free of charge. Moreover, your participation helps to determine load of bacterial contamination, antimicrobial susceptibility of isolates and its associated factors. This is used to design regular use of hand hygiene, infection prevention practice and use of restriction for HCWs fomite in clinical settings. Use of best practice in hospital may be reducing the morbidity and mortality health care worker's associated with HAIs.

Confidentiality: The information collected from you was kept confidential. It was stored in a file using codes without your name. And it will not be used only for this

particular research revealed to anyone except the principal investigator. In addition it was used any for this particular research but no other purpose.

Voluntary to participation (right to refusal)

You were full right to refuse from participating in this research you can refuse to give specimen and not to respond any or all the question.

Person to contacts

This research was reviewed and approved by the institutional reviewed board of Department of Medical Laboratory Sciences, college of medicine and health science, Bahir Dar University. If you want to know more information, you can contact the following individual and you may ask any you want.

Investigator: Workneh Ayalew, Mobile: +251-918781447/251-918164943

E-mail: workneh_ayalew@yahoo.com

Adonay_aman@gmail.com

Health care worker's consent form

He/she was read and/or listened to the description of the study and He/She understood. What the procedures was and what was happen to my materials that expected as fomites because of day to day contact with patients or clients in the study area. He/She know that his/her fomites can quit the study at any time. He/She agreed to allow him/her to participate.

_____ _____

Signature of health care provider Date

_____ _____

Signature of Investigator Date

Annex II: **Amharic Version Study Participant Information and Consent Form**

(የመረጃና የስምምነት ወል ቅጽ)

የጥናቱ ርእስ

በአማራ ብሔራዊ ክልላዊ መንግስት በባህር ዳር ጤና ጥበቃ ቢሮ ስር በፈለገ ህይወት ሪፈራል ሆስፒታል ለሚሰሩ ጤና ባለሙያዎችና በባህር ዳር ዩኒቨርስቲ ህክምና ተማሪዎች አገልግሎት ሲሰጡ የሚጠቀሙባቸዉ ቁሳቁሶች ላይ የተለያዩ ባክተርያዎች ሲገኙ መለየት፣ የንክኪ ክብደቱ/ ደረጃዉ እና ባክተርያዎቹ ለተለያዩ የፀረ-ባክተርያ መድሀኒቶች ያላቸው የመቋቋም ሀይል መለየት፡፡

የጥናቱ ዓላማ

የዚህ ጥናት አላማ በፈለገ ሕይወት ሪፈራል ሆስፒታል በሚሰሩ ለታካሚዎች ቅርበት ባለሙያዎች መገለገያ ቁሳቁሶች / fomites ምን ያህል ለተለያዩ ባክተርያ የተጋለጡ ናቸው እና ባክተርያውስ ለተለያዩ የፀረ-ባክተርያ መድሀኒቶች ያላቸው የመቋቋም ሀይል ምን ያህል ነው፡፡ ከጥናቱ የሚገኘዉ ዉጤት በባክተርያ የሚመጣን በሽታ እና ሞት ወይም ብክለት ለመቀነስ ወይም የሚረዳ ሲሆን በዚህ ዙሪያ መከላከያ ዘዴዎችን ለማስቀመጥ ከፍተገኛ ሚና ይጫወታል::

የአሰራር ሁኔታ (ሂደት) በዚህ ጥናት የሚሳተፍ የጤና ባለሙያ ለበሽታው /ለንኪኪዉ ሊጋለጡ ይችላሉ ብለን የምናስባቸው የባለሙያዎች መገልገያ ቁሳቁሶች ሲሆኑ ፤በአጠቃላይ ማህበራዊ ነክ መረጃዎች፤አጋላጭ ሁኔታወች እና በቂ ናሙና በመዉሰድ የተለያዩ ባክተርያዎች መኖራቸዉን ማረጋገጥ እና ባክተርያዎቹ ለተለያዩየፀረ-ባክተርያ መድሀኒቶች ያላቸው የመቋቋም ሀይል መለየት፡፡የእርስዎ ማለትም ለህክምና አገልግሎቱ ቁሳቁሶች (Fomites) ለጥናቱ ተመርጥዋል/ለች፡፡ ለመሳተፍ ፈቃደኛ ከሆኑ፤ ናሙናና ለመጠየቁ የእርስዎን እዉነተኛ መልስ በተዘጋጀ መጠይቅ መሰረት እንዲነግሩን እንጠይቃለን፡፡

ሊከሰቱ የሚችሉ ችግሮችና የምቾት መጓደሎች

በዚህ ጥናት ምናልባት 10 ደቂቃ አካባቢ እና ከዚያ በታች ሊወስድ ይችላል እናም ለጥናቱ ከተፈለገዉ ጤና ባለሙያዉ መገልገያ ቁሳቁስ ላይ ናሙና ተወስዷል፡፡ ሌላ ምንም ችግር አልነበረም፡፡

ጥቅሞች

በዚህ ጥናት ጤና ባለሙያዉ ተሳታፊ ነበር፡፡ በስራ ምክንያት ንክኪ መኖሩ አለሞኖሩ ለመለየት በነጻ ተመርመሯል ወይም ተመርመራለች፡፡በሽታ አምጭ ተዋስያን ባክቴሪያ ከተለየ አስፈላጊውን መከላከያ ዘዴ ጤና ባለሙያዉ በሚሰራበት ተቋም መረጃ እንድጠቀም/እንዲጠቀም ይሆናል፡፡ በተጨማሪም ጤና ባለሙያዉ በጥናቱ ላይ መሳተፉ ከጥናቱ የሚገኘዉ ዉጤት በባክተርያው የሚመጣ ንክኪ እና ስርጭት ለመቀነስ የሚረዳ ሲሆን በዚህ ዙሪያ መከላከያ ዘዴዎችን ለማስቀመጥ ከፍተኛ ሚና ይጫወታል፡፡

የጥናቱ መረጃ ሚስጥራዊነት: ጤና ባለሙያዉ የተሰበሰበዉ መረጃ ሚስጥራዊነቱ የተጠበቀ ነዉ ፡፡ ለዚህ ጥናት የተሰበሰበዉ መረጃ በማህደር የሚተቀመጠ ሲሆን ማህደሩም ጤና ባለሙያዉ ስም ሳይሆን በተለየ ኮድ ሲቀመጥ ከዋናዉ ተመራማሪ በስተቀር ለማንም አይገለጽም፡፡

የመዉጣት (የማቋረጥ) መብት: ጤና ባለሙያዉ በጥናቱ ላይ ያለመሳተፍ የተጠበቀ ነዉ፡፡ ናሙና ያለመስጠት፤ ማነኛዉንም ያልፈለጉትን ጥያቄ ተጠብቋል፡፡

ሊገኙአቸዉ የሚችሉ ሰዎች:ይህ ጥናት በባህርዳር ዩኒቨርሲቲ የስነምግባር ፤ የምርምር ኮሚቴና ሕክምናና ጤና ሳይነንስ ኮሌጅ የሜዲካል ላቦራቶሪ ሳይንስ ትምህርት ክፍል በመላክ የሚጸድቅ ይሆናል፡፡ ጤና ባለሙያዉ ጥያቄ ካለዉ እና ተጨማሪ መረጃ ከፈለገ/ች በማነኛዉም ጊዜ ከዚህ በታች የተጠቀሰዉን አድራሻ ተጠብቋል፡፡

ዋና ተመራማሪ ወርቅነህ አያሌዉ

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የጥናቱ ተሳታፊ የጤና ባለሙያዎች የስምምነት ማረጋገጫ ፊርማ

ጤና ባለሙያዉ የጥናቱን ገለጻ ፤የጥናቱን ሂደት እና በጥናቱ ጊዜ ከጤና ባለሙያዎች fomites ንክኪ ጋር ተያይዞ ስለሚያጋጥሙት በሽታዎች/ነገሮች/ምክንያቶች በሚገባ ተረድቷል ፡፡ ከጥናቱም በማነኛዉም ደረጃ ለንክኪ አጋላጭ ሊሆኑ የሚችሉ የመገልገያ ቁሳቁሶች መሆናቸዉንም ተረድቶ በጥናቱ እንዲሳተፍ ተስማምቷል፡፡

የዋና ተመራማሪ ፊርማ ----------ቀን----------

Annex III: English Version of Questionnaire

Bahir Dar University of College of Medicine and Health Sciences, Department of Medical Laboratory Sciences.

Topic: Bacterial contamination, antimicrobial susceptibility pattern of isolates from HCWs fomites at Felege Hiwot Referral Hospital, North West Ethiopia.

Date ----------2009 E.C

Data collection form

A. Sample from HCW

1. Data collection form number----------
2. Code number----------
3. Fomite code/type (stethoscope swabs (01), Mobile phone swabs (02), white coat swabs (03)
4. Qualification ----------
5. Field of specialization ----------
6. Age ----------sex----------
7. Year of service ----------
8. Currently working ward ----------
9. Do you regularly wash your hand before touching a patient? A. No B. Yes
10. Do you regularly wash your hand before clean/aseptic procedure? A. No B. Yes
11. Do you regularly wash your hand after body fluid exposure risks? A. No B. Yes
12. Do you regularly wash your hand after touching a patient? A. No B. Yes
13. Do you regularly disinfect your mobile phone? A. Yes B. No.
14. Do you use of your mobile phone at bed side to medical information A. Yes B. No
15. Do you regularly disinfect your stethoscope? A. Yes, B. No
16. Do you regularly use antiseptic for your hands? A. Yes B. No
17. Do you clean your Stethoscope, Mobile phone and white coat regularly?
    A. Yes B. No
18. Do you answer phone calls while attending patients? A. Yes B. No
19. Do you use laundry for your white coat? A. Yes B. No (Home wash)

B. Laboratory

A. Media used ----------.

B. Organism isolated ----------.

C. Colony number ----------

1. *K. pneumonia* 2. *E.coli*

3. Coagulase negative staphylococci 4. *S. aureus*

5. *Pseudomonas* 6. *Bacillus spp.*

7. *Proteus spp.* 8. Others….specify……

C. Biochemical test ----------

D. Gram reaction result from culture ----------

E. Other remarks ----------

F. Drug susceptibility pattern

Antibiotic	**Sensitive(s)**	**Resistant(r)**
1. Amoxy/clav	-------	-------
2. Ciprofloxacin	--------	--------
3. Tetracycline	--------	--------
4. Cotrimoxazole	--------	--------
5. Nalnidixic acid	--------	--------
6. Cefoxitin	-------	--------
7. Ceftriaxone	--------	-------
8. Erythromycin	--------	------
9. Penicillin	--------	--------
10. Chlorampenicol	-------	--------
11. Gentamycin	-------	--------
12. Clindamycin	-------	--------
13. Norfloxacin	--------	--------
14. Ampicillin	---------	-------
15. Doxycycline	---------	--------

Appendix I: Procedures

A. To prepare the culture media

1. Read the label on a bottle of dehydrated agar media. It specifies the amount of dehydrated powder required to make 1 liter (1,000 ml) of medium. Calculate the amount needed for 1/2 liter and weigh out this quantity.
2. Place 500 ml of distilled water in an Erlenmeyer flask. Add the weighed, dehydrated agar while stirring with a glass rod to prevent lumping.
3. Set the flask on a stand over an asbestos mat.
4. When the agar mixture is completely dissolved, remove the flask from the flame or hot plate, close it with the cotton plug or cap, and it has to be sterilized in the autoclave.
5. When the flask of sterilized agar is returned to you, allow it to cool to about 50°C (the agar should be warm and melted, but not too hot to handle in its flask). Remove the plug or cap with the little finger of your right hand and continue to hold it until you are sure it won't have to be returned to the flask. Quickly pour the melted, sterile agar into a series of petri dishes. The petri dish tops are lifted with the left hand and the bottoms are filled to about one-third capacity with melted agar.
6. Replace each petri dish top as the plate is poured. When the plates are cool (agar solidified), invert them to prevent condensing moisture from accumulating on the agar surfaces.

Place inverted agar plates in the 35°C incubator. They should be incubated for at least 24 hours to ensure they are sterile (free of contaminating bacteria) before you use.

B. Collection and processing of specimen from health care worker's

1. The swab specimen was collected by experienced personal from health care provider aseptically.
2. Label the sample as soon as possible with the health profession code number.
3. Inoculate the sample swabs in to normal saline
4. After 24 hours the samples was sub cultured on blood agar base, MacConkey agar and Manitol salt agar plates and incubated at 35°C overnight.
5. Examine and report the culture; look for colony characteristics and it used to perform biochemical test and determine drug susceptibility pattern of the isolated organism after colony counting.

C. The pour-plate techniques

1. With a wax pencil, label sterile saline, sterile cotton tips.
2. Prepare sterile tryptic soy broth in a sterile test tube and cool in a 2 to 8° C or room temperature for at least 10 to 15 minutes.
3. With a wax pencil, label the bottom of the broth medium tubes with the name of the fomites, wards, professions, code number, and date.
4. Tryptic soy broth tube (1ml) with 9ml normal saline using aseptic technique and mix thoroughly. This represents a 10^{-1} dilution
5. Using aseptic technique, immediately pours (1ml) to blood agar plate which was mixed from 1ml of TSB and 9ml normal saline(from tube1 to tube 2 ; 1:10 dilution)
6. Gently circular motion while keeping the plate flat on the bench top. Do not allow any agar to splash over the side of the plate. Set the plate aside to cool and harden.
7. Incubate the plates at 30° to 37°C for 24 hours in an inverted position or Invert the plates and incubate for 24 hours at room temperature.
8. Examine the pour plates and record your results after incubation, measure some representative colonies and carefully observe their morphology.

D. Gram stain procedures

1. Prepare a thin smear of the culture or specimen was observed.
2. Allow to air-dry and fix the smear.
3. Cover the fixed smear with crystal violet for 1 min.
4. Rinse with clean water and tip off all the water.
5. Cover the smear with Lugol's iodine for 1 min.
6. Wash off the iodine with clean water.
7. Add acetone-alcohol for 30 sec.
8. Wash the smear immediately with clean water.
9. Cover the smear with safranin for 1-2 minutes.
10. Rinse with clean water.
11. Wipe the back of the slide and place in a draining rack for the smear to air-dry.
12. Examine microscopically, first with the 40x objective and then with the oil immersion objective for white cells, bacteria and other structures.

    Gram- positive bacteria ----------dark purple

    Gram- negative bacteria ----------pale to dark red.

E. Antimicrobial susceptibility testing procedure

1. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth
2. Much the turbidity of suspension with turbidity standard
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
4. Spread the inoculum evenly over the Muller-Hinton agar plate with the swab
5. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate
6. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours
7. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.
8. Interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard.

   Sensitive: zone of radius is wider or equal to the control,

   Intermediate: zone of radius is more than three mm smaller than the control and

   Resistance: no zone of inhibition.

F. Biochemical testing procedures

Identification of Gram positive bacteria: Gram-positive cocci were identified based on their gram reaction, catalase and coagulase test results.

Catalase test: This test was used to differentiate *staphylococci* (positive) from *streptococci (*negative)

Procedure

1. Pour 2-3 ml of 3% hydrogen peroxide to a test tube
2. Using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution
3. Look for immediate bubbling
4. Interpretation :Active bubbling--positive test and no release of bubbles-negative test

Coagulase test: This test is used to differentiate *S. aureus* from other *Staphylococcus* spp.

Procedure:

1. Place a drop of physiological saline on two separate slides

2. Emulsify the test organism in each of the drop to make thick suspension
3. Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the organism within 10 seconds
4. Interpretation

   Clumping within 10 seconds ----------*S.aureus*

   No clumping within 10 seconds ----------other staphylococcus species

G. **Identification of gram negative bacteria procedure:** This was based on their test result with a series of biochemical tests.

1. Prepare a suspension of the test organism with nutrient broth. 3-4 colony of test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, manitol, urea agar and motility medium.
3. Incubate at 35-37^{o} c for 18-24 hours.
4. Look for color change (turbidity for motility) of the medium
5. Identify the test organism by considering the result of the six biochemical tests.

Appendix II: Overall procedural workflow sample swab from HCW fomites of data collection

1. Sterile cotton swab moistened with sterile normal saline swab was collected using Tryptic soy broth, and transport to FHRH Microbiology laboratory for analysis

2. Mix 1ml of Tryptic soy broth with 9ml of normal saline and makes 1:10 (10^{-1} dilution)

3. Streak on Plate count agar and Incubate at 37^0c for 24-48 hours

4. Then the growth is inspecting to identify the bacteria and Count the colony using colony counter and naked eye

5. Sub culture the discreet colony on pure Blood Agar, MacCkonkey and MSA Incubate at 37^0c for 24-48 hours &

6. Identification of bacteria isolates was based on colony characteristics, gram reaction and series of biochemical tests

7. Antimicrobial susceptibility test on Muller Hinton agar

8. Measure the complete inhibition zone by using ruler including the diameter of the disk, and expressed as susceptible/intermediate/resist

Annex IV. Declaration

The research work in this thesis entitled **"Bacterial contamination and antimicrobial susceptibility pattern of isolates from heath care worker's fomites at Felege Hiwot Referral Hospital, North West Ethiopia."** was carried out by me under the supervision of Wondemagegn Mulu and Dr. Fantahun Biadgelegne at Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Microbiology, for the award of MSc Degree in Medical Microbiology. I declare that this work is original and has not been submitted to any other University or institution.

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Sign_____ Date: _____

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2. Dr. Fantahun Biadgelegne	_____	_____

Examiners	Sign	Date
1. Prof. Feleke Moges	_____	_____
2. Mrs. Fetlework Bereded	_____	_____