MICROBIAL CONTAMINATIONS ON HANDS AMONG VISITORS AT CRITICAL UNITS OF HOSPITAL UNIVERSITI SAINS MALAYSIA (HUSM)

by

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Dissertation submitted in partial fulfillment of the requirements for the Degree of Bachelor of Health Sciences (Honours) (Environmental and Occupational Health)

June 2017

ACKNOWLEDGEMENT

I would like to take this opportunity to express my biggest gratitude to Allah (S.W.T) for granted me with patience and strength to complete the final year research study project. A special thanks to my supervisor, Dr. Siti Marwanis binti Anua for her knowledge, experiences and her precious time invested in giving me valuable advices, guidance, encouragements, recommendations and continuous supports throughout this project. I would like to extend my sincere thanks to my co-supervisor, Dr. Nurzafirah binti Mazlan for her kind cooperation throughout the research study.

I would also like to thank and give my deepest appreciation to the former Director of Hospital Universiti Sains Malaysia (HUSM), Professor Dato' Dr. Mafauzy Mohamed and the Senior Assistant Director of Nursing Unit, Puan Raudzah binti Hj. Mohd Ariffin. My heartful thanks to the laboratories officers who helped and teach me throughout the completion process of this dissertation.

A special thanks to my laboratory partner, Siti Nur Anis binti Mohd Sharom and my final year project teammates, Nurul Atikah binti Mohd Yunos, Nur Fasihah binti Mat Koris and Mumtaz Akmal binti Mohd Mabror for their kindness, concerns, moral supports and motivation during my study. Thanks for the friendship and memories. Last but not least, my deepest gratitude goes to my beloved parents for their prayers, financial support and encouragement throughout my studies in USM. Thank you very much.

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LISTS OF SYMBOLS AND ABBREVIATIONS

-	To/until
±	Plus and Minus
%	Percentage
/	Over to
=	Equal to
>	More than
°C	Degree Celsius
SD	Standard Deviation
CFU	Colony Forming Units
HCAI	Healthcare Associated Infection
E. coli	*Escherichia coli*
MRSA	*Methicilin-resistant Staphylococcus aureus*
HCW	Healthcare Workers
HUSM	Hospital Universiti Sains Malaysia
ICU	Intensive Care Unit
CICU	Coronary Intensive Care Unit
SICU	Surgical Intensive Care Unit
CCU	Critical Care Unit
NICU	Neuro Intensive Care Unit
BHIB	Brain Heart Infusion Broth
MAC	MacConkey Agar
NA	Nutrient Agar

EMB	Eosin Methylene Blue Agar
MHB	Mueller-Hinton Broth
MHA	Mueller-Hinton Agar
TNTC	Too Numerous To Count
mL	milliliter
L	Liter

PENCEMARAN MIKROORGANISMA PADA TANGAN PELAWAT DI UNIT KRITIKAL, HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRAK

Tangan pelawat boleh dicemari mikroorganisma patogen yang merbahaya kepada pesakit unit kritikal yang lemah dan kurang daya imuniti. Pembersihan tangan yang tidak sempurna boleh membawa organisma patogen dan menyebarkan penyakit kepada diri sendiri serta pesakit yang dilawati. Kajian ini dijalankan bagi mengenalpasti kehadiran *Escherichia coli (E. coli)* dan Methicilin-resistant *Staphylococcus aureus* (MRSA) pada tangan pelawat di unit kritikal dengan hubungkait amalan kebersihan tangan mereka. Seramai 52 orang pelawat dipilih ke dalam kajian ini dengan menggunakan persampelan secara rawak. Borang soal selidik yang mengumpulkan maklumat tentang kebersihan tangan telah diedarkan dalam kalangan pelawat sebelum sapuan tangan dominan mereka dijalankan. Pengenalpastian mikroorganisma telah dilaksanakan menggunakan kaedah pewarnaan gram, ujian biokimia, ujian penyerapan cakera dan media agar terpilih. Seramai 98.1% (n=51) sampel tangan mereka adalah dicemari mikroorganisma ($3.87x10^{14}\pm1.65x10^{15}$ CFU/ml). *E. coli* ditemui dari tangan 10 (19.2%) orang pelawat (0.81±0.398 CFU/ml) dan tiada MRSA yang dijumpai. Hanya 40.4% (n = 21) pelawat melakukan pembersihan tangan sebelum bertemu dengan pesakit. Terdapat sedikit hubungan yang signifikan antara mencuci tangan sebelum berjumpa pesakit dengan kehadiran *E. coli* (p=0.052). Terdapat perbezaan yang signifikan bagi pencemaran *Staphylococcus aureus* dalam kalangan pelawat yang membasuh tangan sebelum berjumpa dengan pesakit (p=0.014).

Kesimpulannya, pencemaran bakteria yang tinggi pada tangan mungkin disumbangkan oleh amalan pembersihan tangan yang tidak betul kerana ianya sangat rendah (kurang daripada 50%) walaupun tiada MRSA dijumpai. Oleh itu, tingkah laku dan sikap mengenai kebersihan tangan dalam kalangan pelawat perlu dipertingkatkan dengan pendekatan disiplin yang pelbagai.

MICROBIAL CONTAMINATIONS ON HANDS AMONG VISITORS AT CRITICAL UNITS OF HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRACT

Visitors' hands can be contaminated with pathogenic microorganisms that are dangerous to the vulnerable and immunocompromised critical unit patients. Inadequate cleaning of hands could carry the pathogenic organisms and transmit disease to one self as well as to the visited patients. This study was done to determine the presence of *Escherichia coli (E. coli)* and Methicilin-resistant *Staphylococcus aureus* (MRSA) contamination on hands among visitors at critical units with respect to their hand hygiene practices. A total of 52 visitors were selected into this study using grab sampling. Questionnaire gathering information on hand hygiene were distributed among the visitors prior their dominant hand swabbing. Identification of the microorganisms were conducted by gram staining, biochemical tests, disc diffusion test and selective agar medium. It was found that 98.1% (n=51) of their hands were contaminated by microorganisms ($3.87x10^{14}\pm1.65x10^{15}$ CFU/ml). *E. coli* was isolated from the hands of 10 (19.2%) visitors (0.81±0.398 CFU/ml) and there was no isolated MRSA found. Only 40.4% (n=21) visitors performed hand hygiene before meeting with patients. There was marginally significant association between washing hands before meeting patients and the presence of *E. coli* (p=0.052). A significant difference was obtained in *Staphylococcus aureus* contamination among those who perform hand washing

before patient contact (p=0.014). As a conclusion, a higher bacterial contamination on hands might be contributed by inadequacy of proper hand hygiene practices as it was unacceptably low (less than 50%), despites no MRSA was found. Thus, behaviour and attitudes regarding hand hygiene among visitors need to be improved by multidisciplinary approach.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Hand is inseparable from being contaminated by pathogenic microorganisms. Pathogenic microorganisms can be transferred to hands from contaminated surfaces that people come into contact in their daily life. Pathogenic microorganisms are harmful microorganisms that can cause serious harm, disease or even immediate death. There are several types of pathogenic microorganisms that are capable of causing life-threatening infections which are Methicilin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli (E. coli), Enterobacteriaceae, proteus and K. pneumoniae* (David et al., 2015). The vulnerable intensive care units (ICU) patients are highly susceptible to infection of pathogenic microorganisms that can cause a risk to them (David et al., 2015). A contaminated hand can transmit the disease to oneself and others.

Patients of ICU needs to be observed, managed and monitored in the highest level of care and treatment because they have life-threatening illness, injuries and complications and potentially life-threatening conditions (The Faculty of Intensive Care Medicine/The Intensive Care Society, 2013). In a previous study conducted by Bartley and Streifel (2010), the involvements of visitors in patient-centered care ICU give major implications for healthcare associated infections (HCAI). The ICU visitors' hands can be colonised with pathogens that are dangerous and immunocompromised to patients (Zimring et al., 2013).

Common diseases related to the HCAI are pneumonia, gastrointestinal infection, urinary tract infection, and primary bloodstream infection.

Failure to perform hand hygiene is the leading cause of HCAI and the spreading of multiresistant organisms has been recognised as a significant contributor to the outbreaks of infectious diseases (World Health Organisation, 2009). Hands are the most common medium of transmission of microorganisms and therefore visitors should wash their hands before coming in direct contact with patients. It has been reported that Gram-negative and Gram-positive bacteria are able to survive up to months and a dry inanimate surfaces with a longer persistence under humid and lower-temperature conditions.

Hand hygiene is considered the most effective and least expensive way to prevent the transmission of microorganisms (World Health Organisation, 2009). Thus, hand hygiene alone can significantly reduce the contamination of pathogenic microorganisms (Purva, 2011). This study was conducted to determine the presence of pathogenic microorganisms on hands among critical units' visitors with respect to their hand hygiene practices as hands is one of the medium of bacteria transmission from visitors to patients and via versa.

1.2 Problem Statement

In mid-1800s, a Hungarian physician in Vienna, Austria, Ignaz Semmelweis showed that hospital-acquired diseases were transmitted via the hands of healthcare workers (HCWs) and the community. A hand hygiene concept was introduced by Ignaz after he found that when a physician at a hospital in Vienna, Austria practiced hand washing before delivering

babies, it prevented deaths in postpartum women. The incidence of puerperal fever among women was as high as 40% and the death rate due to a puerperal fever varied from 2% to 9.2% in Vienna General Hospital, Austria. Throughout the study, he stated that hand washing is an effective way to prevent HCAIs (World Health Organisation, 2009).

Kanitha et al. (2005) also stated that, hand hygiene is the most important and effective measure to prevent HCAI in hospitals. Their study reported that a non-compliance of hand hygiene practices among visitors are due to several reasons which are the patients' needs are given a priority (51.2%), forgetfulness (35.7%) and skin allergy, irritation or dryness caused by hand hygiene agents (15.5%). There are three components route of transmission of microbial infection within healthcare setting which is the reservoir, susceptible host and mode of transmission. Patients and visitors are the susceptible host while the mode of transmission is varying according to the type of microorganisms (Siegel, Rhinehart, Jackson & Chiarello, 2007).

Multidrug-resistant pathogens are commonly involved in HCAI and the pathogenic microorganisms may be carried by visitors on their hands. This may happen when they do not practice proper hand hygiene before entering the ICU and the vulnerable patient population might be at increased risk for infection (Randle, Arthur & Vaughan, 2010). Rozaidi, Sukro and Dan (2001) found that, the organisms responsible for nosocomial infections in ICU are *Acinetobacter, Pseudomonas, Pseudomonas Aeruginosa, Enterobacter,* MRSA, *Enterococcus,* MRSE and others. The level and type of bacterial infection depends on the clinical and geographical area.

Several studies in the United States of America (USA) had determined that the rate of bacterial counts on dominant hands of visitors who did not perform hand hygiene is higher compared to those hands who did perform hand hygiene and more likely to have positive cultures (100% versus 30%) (David et al., 2015). According to Fahriye et al. (2010), the rate of microbial contamination on hands is higher in which from the 154 specimens, 148 (96.1%) showed the microbial growth and others were isolated as single or multiple members of resident flora in Turkey.

Thus, hand hygiene is important to avoid the contamination of pathogenic microorganisms. Therefore, this study aims to determine microbial contamination on hands among critical units' visitors with respect to their hand hygiene practices. In Malaysia, there is limited study which states about on the presence of microbial contamination on hands among visitors and it is still lacking compared to other developed countries such as Thailand, Japan, United States and United Kingdom.

1.3 Research Objectives

1.3.1 General Objectives

To study the presence of *E. coli* and MRSA contamination on hands among visitors at critical units of HUSM with respect to their hand hygiene practices.

1.3.2 Specific Objectives

i. To determine the total number of colony forming units (CFU/ml) of bacterial contamination on visitors' dominant hands.

ii. To determine the presence of *E. coli* and MRSA on hands among visitors at critical units of HUSM.

iii. To determine the hand hygiene practices among visitors at critical units of HUSM.

iv. To determine the association between the presence of *E. coli* and MRSA on hands among visitors at critical units and their hand hygiene practices.

v. To determine the mean difference of bacterial contaminations (*E. coli* and MRSA) on hands of visitors between hygiene practices.

1.4 Hypothesis

Null Hypothesis 1: There is no significant association in the presence of *E. coli* and MRSA contamination on hands among visitors at critical units of HUSM with respect to their hand hygiene practices.

Alternative Hypothesis 1: There is a significant association in the presence of *E. coli* and MRSA contamination on hands among visitors at critical units of HUSM with respect to their hand hygiene practices.

Null Hypothesis 2: There is no significant difference for bacterial contaminations (*E. coli* and MRSA) on hands of visitors between hygiene practices.

Alternative Hypothesis 2: There is a significant difference for bacterial contaminations (*E. coli* and MRSA) on hands of visitors between hygiene practices.

1.5 Significant of Study

Multidrug-resistant organisms such as *Staphylococcus aureus*, *Acinetobacter sp.*, vancomycin-resistant *Enterococcus* and carbapenem-resistant *Enterobacteriaceae* are the organisms that are present as a significant challenge in the current healthcare environment (Pogorzelska, Stone & Larson, 2012). The infectious agents can transmit to patients from the hands of visitors and can have major implications for HCAI (Bartley & Streifel, 2010). Visitors are rarely evaluated or enforced to use the alcohol-based hand sanitiser even though it is readily available throughout the hospital.

Thus, it is important to conduct this study in determining the presence of *E. coli* and MRSA contamination on hands among visitors at critical units of HUSM which can give risks to the vulnerable critical unit patients. Besides, the study can give exposure and awareness on the importance of using provided hand sanitiser before and after visiting a patient to critical units' visitors which it can reduce the microbial contamination on their hands. The data of the study represented the status of microbial contamination levels on hands among visitors at critical units of HUSM.

CHAPTER 2

LITERATURE REVIEW

2.1 Critical units

Critical units is a hospital unit in which it provides the facility for provision of intensive nursing and medical care of seriously ill patients. It is characterised by the high quality and quantity of continuous nursing and medical supervision and use of sophisticated monitoring and resuscitative equipment for the care of critically ill patients that require immediate and continuous attention. The units are organised for the care of specific patient groups such as Intensive Care Unit (ICU), Coronary Intensive Care Unit (CICU), Surgical Intensive Care Unit (SICU), Critical Care Unit (CCU), Neuro Intensive Care Unit (NICU) and others.

Intensive care is a service for patients that have potentially recoverable conditions who needs more observation and invasive treatment in an ordinary ward or high dependency area. Intensive care is reserved for the patients with a threatening organ failure, complication from an acute illness or trauma. It represents the highest level of patient care and treatment because the patients admitted to the critical units are more susceptible to get the HCAI infection due to involvement with invasive machine or procedure. An ICU is a designated area offering facilities for the prevention, diagnosis and treatment of multiple organ failure and other chronic ill persons. ICU is also called or known as critical care units (CCU) or intensive therapy unit (ITU).

Annually, more than 5.7 million patients are admitted in ICU in the United States for an intensive monitoring, support of airway, breathing or circulation, stabilisation of acute or life-threatening medical problems and others (Wunsch et al., 2011). According to World Health Organisation (2009), the concerns towards infections are 5% to 15% of hospitalised patients and it can affect patients who are admitted about 9% to 37% in ICUs'. Thus, infection control is taken seriously in the ICU.

2.2 Modes of transmission

Pathogens can be transferred to humans in many ways. The modes of transmission are classified as follow which are by a direct contact, respiratory droplets, fomites and airborne transmission. Some pathogens are spread by direct contact between the body of a person and another such as by kissing, touching and sexual intercourse. These pathogens are too weak to survive outside human in a short period of time so they need to be deposited directly onto the body of a new host. A transmission of pathogens through a direct contact is the most frequent in healthcare setting. MRSA can be transmitted directly by a contact from person to person or indirect contact such as contaminated healthcare settings.

The respiratory droplets secretions are formed and expelled when a person cough, sneeze, laugh or talk. They transmit many pathogens directly from one host to another. Respiratory droplets is one of the efficient mode of transmission from one person where he or she exhaling the infected droplets, it can be transmitted to every susceptible person in the environment. It also can be spread rapidly where people live in crowded condition or within

the same area. Respiratory droplet can transmit diseases such as pharyngitis, meningitis, and pneumonia (Burke, 2009).

Fomite is the condition where some pathogens remain on inanimate objects such as cups, towels, bedding and handkerchieves. The object that serves as a fomite for a particular pathogens depends on the pathogen's portal entry. Fomite does not constitute a reservoir because pathogens do not survive on them in a very long time. They usually transmit disease among people who come into fairly close contact with one another. Airborne transmission occurs when small size pathogens and viable being suspended in air are inhaled into the respiratory system and spread to the patients. Those pathogens can withstand prolonged drying unlike the pathogens transmitted by respiratory droplets and as a result they can be transmitted greater than a meter distance between people that had no close contact with one another.

2.3 Hand hygiene and microbial contamination

Human hand can be the single most important of all disease transmission due to its regular contact with the surrounding environment. A variety of pathogens can be transferred from one person to another through direct contact with an unclean hand. Hand hygiene is an effective measure to prevent cross infection in hospitals especially in critical units because it is the place where serious ill persons are admitted and they need to be observed and monitored in the highest level of care and treatment.

Pathogenic microorganisms can cause a risk to the vulnerable ICU patients and they are highly susceptible to the infection (David et al., 2015). Visitors that did not perform hand hygiene can be colonised with pathogens that are dangerous to those patients. The pathogens include MRSA, *E. coli, Proteus, Enterobacter* sp. and *K. pneumoniae* in which all of the pathogens can cause life threatening infections towards the ICU's patients. A study conducted by David et al. (2015), found that nine (26%) out of the 35 ICU visitors who did not perform hand hygiene had cultures that were positive for 12 pathogenic organisms (eight had gram-negative rods and four *S. aureus* (one positive for MRSA)). Thus, hand hygiene is very important to prevent a pathogenic microorganism's contamination towards the ICU patients.

2.4 Normal bacterial flora on hands

Normal microflora is the mixture of microorganisms that are regularly found at or within any anatomical site body of a healthy person. Some of the microorganisms are found in association with humans or animals only. Bacteria covered on hands can be divided into two categories which are namely as resident and transient. The resident microflora consists of microorganisms that stay under the superficial cells of the stratum corneum and the surface of the skin. *Staphylococcus epidermis* is the dominant species, other resident bacteria include *S. homis* and other coagulase-negative staphylococci. Resident microflora is less likely to be associated with infections but it may cause infections in sterile body cavities, the eyes, or non-intact skin (Masumeh & Akbar, 2015).

Transient microflora colonised on the superficial layers of the skin are more effectively removed by routine hand hygiene. Transient microflora does not multiply on the skin but it continually survives and multiplies on skin surface from time to time (Kampf & Kramer, 2004). The microflora can be obtained by HCWs during the direct contact with patients or contaminated environmental surfaces near to the patients and most of it can be associated with HCAIs. The transmissibility of transient depends on the species present, the numbers of microorganisms on the surface and the condition of the skin (moisture). The hands of HCWs, visitors and patients may be colonised by pathogenic microflora such as *S. aureus*, Gram-negative bacilli or yeast (Adams & Marrie, 1982).

A normal human skin is colonised by bacteria ranging from more than $1x10^6$ colony forming units CFU/cm^2 (Total aerobic bacterial counts) on the scalp, $5x10^5$ CFUs/cm^2 in the axilla, $4x10^4$ CFUs/cm^2 on the abdomen and $1x10^4$ CFU/cm^2 on the forearm (Selwyn, 1980). The number of bacteria on an individual's skin remains constant and the colonisation depends on the exposure of skin to a particular environment and bacterial activity on the skin. Gram-negative bacteria are ubiquitous and the most of Gram-negative bacteria infections in healthcare settings are caused by *Klebsiella, E. coli, P. aeruginosa* and *Acinetobacter* (Centers for Disease Control and Prevention, 2011).

2.5 Pathogens that can cause harm to human

2.5.1 Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA is the common bacterium that lives on the skin and it is a type of *S. aureus* (staph germs) that resistant to a number of different antibiotics. MRSA can cause harmful infections if it enters the body such as boils, carbuncles, wound infection, chest infection, deep abscesses and bloodstream infection especially among ICU patients. According to the Centers for Disease Control and Prevention (CDC) (2008), MRSA is the current cause (about 1%) of all *Staphylococcus* infections and more than 50% of it is the healthcare associated *Staphylococcus* infections. MRSA is the highest prevalence of healthcare associated infections occurs in intensive care units and in acute surgical and orthopaedic wards (National Clinical Effectiveness Committee, 2013).

The most staphylococcus germs, MRSA are spread by skin to skin contact from an individual to another individual. National Clinical Effectiveness Committee (2013), states that ICU patients are the vulnerable population and they can get MRSA that is spread by unclean hands of staff or visitors through the use of care equipment that is passed from one to another which is inadequately cleaned. The sign and symptoms for the patients who get an infection caused by MRSA are developing a high body temperature, wound becomes red and inflamed area on the skin, chest pain, cough or short of breath, fatigue, headache, rash and wounds that do not heal.

2.5.2 *Escherichia coli (E. coli)*

E. coli is a Gram-negative bacteria can be found outside the environment, digestive tracts of human and animals and food. *E. coli* is a rod shape bacteria and non-spore forming bacilli which are common inhabitants of the small intestine and large intestine of mammals. *E. coli* is one of the family of Enterobacteriaceae and most strains are harmful to people. Some of them are pathogenic and cause illness such as diarrhea, urinary tract infections, respiratory illness and pneumonia and other illness. The most common sources of the presence of *E. coli* are contaminated food, contaminated water, animals and their environment and faeces of infected person (Centre of Disease Control and Prevention, 2016).

According to the Centre of Disease Control and Prevention, (2016), the most common germs responsible for hospital-acquired infections are *S. aureus*, including MRSA (11%), *Klebsiella* (10%), *E. coli* (9%), Enterococcus (9%) and Pseudomonas (7%). *Klebsiella* and *E. coli* are the most worrisome bacterial infections because they have become increasingly resistant to an antibiotic which is carbapenems.

Thus, regular hand washing is important to prevent spreading of the infection. *E. coli* can be spread by person-to-person contact from bacteria in homes, daycare center, nursing homes and hospitals. *E. coli* can spread from one person to another when the infected person does not wash his or her hands properly after had bowel movement. Exposure to *E. coli* may cause severe effect such as bloody diarrhea, severe abdominal cramps and pain, vomiting and can cause serious kidney damage and death to the vulnerable people (World Health Organisation, 2008).

CHAPTER 3

METHODOLOGY

3.1 Study Design

This was a cross-sectional study design.

3.2 Study Location

The study location was at the HUSM, Kubang Kerian, Kelantan which is located in the East Coast of Peninsular Malaysia.

3.3 Study Participants

The target populations were critical unit's visitors involving Critical Care Unit (CCU), Intensive Care Unit (ICU), Surgical Intensive Care Unit (Surgical ICU), Coronary Intensive Care Unit (CICU) and Neuro Intensive Care Unit (NICU). The inclusion and exclusion criteria are shown in the table:

Table 3.1: The lists of inclusion criteria and exclusion criteria

Inclusion criteria	Exclusion criteria
1) Age above 18 years old	1) Unwilling to consent or participate in the research
2)Visiting critical unit's patients (ICU, CICU, SICU, CCU, Neuro ICU)	
3) No duplication on different days	
4) Dominant hand	

3.4 Study Period

The study started from September 2016 until June 2017. The data collections were conducted simultaneously with sampling and laboratory analysis.

3.5 Sampling Method

Grab sampling method was used in recruiting the subjects. The subjects were selected from selected from critical units ward that placed the patient in need of highest level of care and treatment which are CCU, ICU, SICU, CICU and NICU. The observer was waiting at the entrance and the samples were collected once the visitors finished visiting the patients, at their free and available time. Each visitor was asked if he or she would like to participate in the study and having their hand being swabbed. The observer observed the condition of the visitor before approaching them to explain about the research study.

The visitors' hand that had been previously swabbed was excluded because to ensure that there was no duplication of information occurs. The same observer was responsible to make the observation along the eight week period of the study. A questionnaire was used to identify respondent's background information and their behaviour and attitudes towards hand hygiene practices. Microbial cultures were taken from the visitor's dominant hand because previous study has stated that the dominant hand tends to be more contaminated (Sharon et. al, 2013).

3.6 Sample Size Calculation

The sample size (N) was determined using a single proportion formula that was calculated based on the previous study by Fahriye, Aysen, Murat, Sadik & Iclal (2010). The study showed the prevalence of microbial flora on the hands of healthcare workers was 96.1% (n=154). Therefore, the sample size calculated as below.

$$N = \left[\frac{Z_{1-a/2}}{\blacktriangle} \right]^{2} * P\,(1-P)$$

$$N = \left[\frac{1.96}{0.05} \right]^{2} * 0.96\,(1-0.96)$$

N= 59

Total sample size after consider 10% dropout= 65

n=sample size

▲= precision

$Z_{1-a/2}$ = Z = 95% = 0.95 (1.96)

P= prevalence based on previous study (96.1%)

Desired precision (half desired CI width) = 5%

The sample size calculated is N=59 and with an additional 10% of the sample size need to be added to offset the missing or incomplete data, so the total sample, n is 59+(6)= 65 persons of visitors at critical units need to be observed and hand cultured.

3.7 Research Tools

3.7.1 Questionnaires

A set of questionnaire was developed according to previous research and it consists of 2 sections which are respondent's background information and behaviour and attitudes regarding hand hygiene of visitors in critical units at HUSM. The questionnaires had been piloted among seven visitors who were not included in this study. The Cronbach's Alpha results obtained for reliability testing was 0.671.

3.7.2 Materials for culture media

3.7.2.1 Brain Heart Infusion Medium (BHI) Broth

The compositions for the brain heart infusion medium (BHI) (Oxoid, CM 1135) broth were calf brain infusion solids (12.5 g/litre), beef heart infusion solids (5.0 g/litre), proteose peptone (10.0 g/litre), glucose (2.0 g/litre), sodium chloride, (NaCl) (5.0 g/litre), disodium phosphate powder (2.5 g/litre), pH 7.4 $\pm$ 0.2 at 25°C and distilled water (1 liter).

3.7.2.2 MacConkey agar No.3 (MAC)

The compositions for the MacConkey agar (MAC) (Oxoid, CM 0115) were peptone (20.0 g/litre), lactose (10.0 g/litre), bile salts no.3 (1.5 g/litre), sodium chloride, (NaCl) (5.0

g/litre), neutral red (0.03 g/litre), crystal violet (0.001 g/litre), agar (15.0 g/litre), pH 7.1 ± 0.2 and distilled water (1 liter).

3.7.2.3 Nutrient agar

The compositions for the nutrient agar (Oxoid, CM 0003) were peptone (5.0 g/litre), 'Lab-Lemco' powder (1.0 g/litre), yeast extract (2.0 g/litre), sodium chloride, (NaCl), (5.0 g/litre), agar (15.0 g/litre), pH 7.4 ± 0.2 at 25°C and distilled water (1 liter).

3.7.2.4 Eosin Methylene Blue (EMB) agar

The compositions for Eosin Methylene Blue (Oxoid, CM0069) agar were peptone (10.0 g/litre), lactose (10.0 g/litre), dipotassium hydrogen phosphate (2.0 g/litre), eosin Y (0.4 g/litre), methylene blue (0.065 g/litre), agar (15.0 g/litre), pH 6.8 ± 0.2 at 25°C and distilled water (1 litre).

3.7.2.5 Peptone Water

The compositions for peptone water (CM0009) were peptone (10.0 g/litre), sodium chloride, (NaCl) (5.0 g/litre), pH 7.2 ± 0.2 at 25°C and distilled water (1 litre).

3.7.2.6 Identification Test (Gram Positive Bacteria)

3.7.2.6.1 Mueller-Hinton Broth

The compositions for the Mueller-Hinton broth (Oxoid, CM 0405) were beef, dehydrated infusion from (300.0 g/litre), casein hydrolysate (17.5 g/litre), starch (1.5 g/litre), pH 7.3 ± 0.1 at 25°C and distilled water (1 litre).

3.7.2.6.2 Mueller-Hinton agar (dics diffusion test)

The compositions for the Mueller-Hinton agar (CM0337) were beef, dehydrated infusion from (300.0 g/litre), casein hydrolysate (17.5 g/litre), starch (1.5 g/litre), agar (17.0 g/litre), pH 7.3 ± 0.1 at 25°C and distilled water (1 litre).

3.7.3 Materials for sampling (hand swab)

The materials for visitor's hand swab procedures were sterile cotton swabs, test tubes and stopper (BHIB), sterile distilled water, cooler boxes (filled with crushed ice), gloves, marker and sampling sheet or stickers.

3.7.4 Materials for gram staining

The materials required to conduct gram staining procedures were glass slides, normal saline, inoculating loop, staining tray, wash bottles, dropper, gloves, forceps, sampling

sheet or stickers, marker, crystal violet (purple color dye), gram's iodine solution, acetone, safranin, immersion oil and light microscope.

3.7.5 Materials for processing the samples

The materials required for samples processing were alcohol swab, autoclave machine, Duran bottles, sterile agar plates, micropipette (1000µl), micropipette (100µl), pipette tips (blue and yellow), electronic weighing balance, inoculating loop, bunsen burner, 70% alcohol, vortex, test tubes and caps, test tubes rack, incubator (Temperatures: 37° and 44.5°), autoclave machine, and L-shaped spreader (hockey stick).

3.8 Preparation of Culture Media

3.8.1 The preparation of Brain Heart Infusion Medium (BHI) Broth

Brain Heart Infusion Broth (BHIB) (Oxoid, CM 1135) was a highly nutritious medium recommended for cultivation of streptococci, *Neisseria* and other fastidious organisms. The preparation of BHIB was conducted by dissolving 37g of BHIB formula in 1 liter of distilled water. Then, it was autoclaved for 15 minutes at 121°C.

3.8.2 The preparation of MacConkey agar No.3 (MAC)

MacConkey No.3 (MAC) (Oxoid, CM 0115) agar was used for the selective isolation, cultivation, and differentiation of enteric pathogens, especially *Salmonella* in clinical

specimens. For the preparation of MAC, 52g of MAC formula were suspended in 1 liter of distilled water. The medium were mixed thoroughly. Then, the mixture was heated awhile with frequent agitation and boiled for 1 minute to dissolve the powder. The dissolved mixture was autoclaved for 15 minutes at 121°C. After that, MAC was allowed to cool at 45-50°C. The mixture was dispensed into sterile plates while it still liquids and air bubbles were avoided.

3.8.3 The preparation of Nutrient agar (NA)

Nutrient agar (NA) (Oxoid, CM 0271) was used for the cultivation and maintenance of a wide variety microorganism. For the preparation of NA, 40g of NA formula was weighed and suspended in 1 liter of distilled water. The mixture was heated while stirring to fully dissolve all components. Then, the dissolved mixture was autoclaved for 15 minutes at 121°C. After that, the nutrient agar was allowed to cool to 45- 50°C but not solidify once it has been autoclaved. The mixture was dispensed into a sterile plate while it was still liquids and air bubbles were avoided.

3.8.4 The preparation of Eosin Methylene Blue (EMB) agar

Eosin Methylene Blue (EMB) (Oxoid, CM0069) agar was used for the isolation, cultivation, and differentiation of gram-negative enteric bacteria based on lactose fermentation. Bacteria that ferment lactose, especially the coliform bacterium *E. coli,* appears as colonies with a green metallic sheen or blue-black to brown color. Bacteria that do not ferment lactose, appear as colourless or transparent, light purple colonies.

For the preparation of EMB agar, 37.5g of EMB agar was weighed and dissolved in distilled water. The medium was mixed thoroughly and boiled to dissolve the powder completely. Then, it was sterilised by autoclaving at 121°C for 15 minutes. The mixture was dispensed into a sterile plate while it was still liquids and air bubbles were avoided.

3.8.5 The preparation of Peptone Water

Peptone water (CM0009) was used for the cultivation of nonfastidious microorganisms and for carbohydrate fermentation tests. The preparation of peptone water was conducted by dissolving 15g of peptone water formula in 1 liter of distilled water. The mixture was mixed well and the dissolved mixture was autoclaved for 15 minutes at 121°C. Then, 9 mL of peptone water was pipette into each test tube.

3.8.6 The preparation of Identification Test (Gram-positive Bacteria)

3.8.6.1 The preparation of Mueller-Hinton Broth

Mueller-Hinton broth (Oxoid, CM 0405) was used for the cultivation of a wide variety of microorganisms and for the microbial susceptibility testing. The preparation of MHB was done by dissolving 21g of MHB formula in 1 litre of distilled water and it was mixed well to dissolve. Then, it was autoclaved for 15 minutes at 121°C.

3.8.6.2 The preparation of Mueller-Hinton agar (MHA) (disc diffusion test)

Mueller-Hinton Agar (MHA) (Oxoid, CM0337) was an antimicrobial susceptibility testing medium which was used internationally as recognised standard procedures. To prepare MHA, 38g of MHA formula was dissolved in 1 litre of distilled water. The medium was mixed thoroughly. Then, the mixture was heated and boiled for 1 minute to dissolve the powder. The dissolved mixture was autoclaved for 15 minutes at 121°C. The mixture was dispensed into a sterile plate while it still liquids and air bubbles were avoided.

3.9 Data Collection

3.9.1 Questionnaires

A set of questionnaire and informed consent form were given before the volunteered visitor's hand been swabbed.

3.9.2 Hand swabbing

A sterile cotton swab moistened with sterile water was used to wipe the palm of critical unit's visitor's dominant hand. The hand palm area was swabbed horizontally, vertically and diagonally. The swabbed sample was immediately transferred into sterile test tubes that contain 5ml of BHIB and screw-capped. The collected samples were transported to the laboratory in a cooler boxes filled with ice-packs. The samples were incubated at 37°C for

48 hours upon arrival at Culture Laboratory, Health Campus Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan.

3.10 Bacteriological cultures

3.10.1 Obtaining bacteria sample from sterile cotton swabs and making serial dilution for bacterial plate count

After incubation for 48 hours, the BHIB solution was shaken vigorously for 10 seconds to evenly spread the bacteria in the solution. Then, the BHIB solution was taken for a serial dilution of bacterial plate count. A serial dilution of tenfold were made which started from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. 1 mL of BHIB solution was added into 9 mL of peptone water. Then, the solutions were mixed by vigorously flicking the tube for a few seconds to evenly spread the bacteria throughout the tube and break up the bacterial clumps. From the 10^{-1} dilution, further dilutions of 10^{-2} until 10^{-7} were prepared. Spread plate of 0.1 ml from the 10^{-6} and 10^{-7} dilutions were each dropped onto the center of both MAC and NA plates, each, in triplicate using 100µl micropipette. L-shape spreader was used to spread the dilution evenly throughout the plates. Then, it was incubated at 37°C (NA) and 44.5°C (MAC) for 48 hours. After the incubation period, the colonies growth on the plate were counted for the colony forming units (CFU) by using the formula as shown below and it represents the bacteria that were present in the diluted sample.

$$\text{CFU/ml: } \frac{\text{Average Colony Count}}{\text{Dilution Factor}}$$