Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Xinyue Wang Date

Assessing Environmental Contamination in the Maternity Wards of Two National Hospitals in Phnom Penh, Cambodia

By

Xinyue Wang
Master of Public Health

Global Environmental Health

_____
Christine L. Moe, PhD
Committee Chair

_____
Paige Tolbert, PhD
Committee Member

Assessing Environmental Contamination in the Maternity Wards of Two National Hospitals in Phnom Penh, Cambodia

By

Xinyue Wang

B.A. Global Studies
University of North Carolina at Chapel Hill
2015

Thesis Committee Chair: Christine L. Moe, PhD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Environmental Health
2017

Abstract

Assessing Environmental Contamination in the Maternity Wards of Two National Hospitals in Phnom Penh, Cambodia

By Xinyue Wang

Background: The burden of healthcare-associated infections in low- and middle-income countries is substantial, in part due to limited resources, lack of adequate infrastructure, insufficient healthcare services, and inadequate training on infection prevention and control. Poor water, sanitation and hygiene (WASH) infrastructure in the maternity ward is likely to increase the risks of infections among mothers and neonates. Cambodia has one of the highest maternal and infant mortality rates worldwide, and some of these deaths may be due to inadequate WASH provision.

Objectives: The goal of this mixed-method study was to examine the associations between WASH conditions and environmental contamination in the maternity wards of two national hospitals in Phnom Penh, Cambodia.

Methods: Four types of environmental samples (surfaces, medical equipment, tap water, and hand rinses) were collected over an eight-week period between June and August 2016. Samples were analyzed for *Escherichia coli*, Total coliforms, and *Staphylococcus aureus* by the membrane filtration method with Compact Dry plates. Information on WASH conditions were collected through observations and interviews with the hospital directors.

Results: Hospital A was observed to have better access to, and quality of, WASH infrastructure compared to Hospital B. Hand rinses collected in Hospital B had a higher incidence of microbial contamination compared to those collected in Hospital A (*S. aureus* OR: 21.43, 95% CI: 4.30-104.60). The odds of a tap water sample meeting the WHO drinking water guideline was significantly higher for Hospital A compared to Hospital B ($p < 0.05$). The odds of detecting any of the three target microorganisms on one of the high-touch surfaces was 1.9 times higher in Hospital B compared to Hospital A ($p < 0.05$).

Conclusions: The study suggested that inadequate WASH infrastructure may increase the likelihood of environmental contamination in the maternity ward. Interdisciplinary studies are needed to fully understand the burden of HAIs caused by inadequate and unsafe WASH infrastructure in maternity wards in resource-limited settings.

Assessing Environmental Contamination in the Maternity Wards of Two National Hospitals in Phnom Penh, Cambodia

By

Xinyue Wang

B.A. Global Studies
University of North Carolina at Chapel Hill
2015

Thesis Committee Chair: Christine L. Moe, PhD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Environmental Health
2017

Acknowledgements

First, I would like to thank all of the study participants for their time, understanding, and patience. I wish to thank the staff from Calmette Hospital and Preah Kossamak Hospital, and the clinical technicians from the Bacteriology Laboratory for their kind collaborations.

I am grateful to my colleagues at WaterAid Cambodia, for hosting me and treating me like family.

I would like to express my sincere gratitude to my thesis advisor, Dr. Christine Moe, for her continued support and guidance throughout the past two years.

I want to give special thanks to my practicum supervisor and my lovely mentor, Ms. Lindsay Denny, for being a role model.

I would also like to thank Dr. Joanne McGriff and Dr. Amy Kirby, for their valuable advice.

Thank you to everyone at the Emory University Center for Global WASH, I am honored to be part of the team.

Thank you to all my friends at Rollins for sharing this amazing journey with me.

Finally, I dedicate this thesis:

To my parents, for their unconditional love and support.

And to my best friend, Michael, for bringing joy and beauty to my life.

Table of Contents

INTRODUCTION

Globally, there is growing attention toward healthcare-associated infections (HAIs). Each year, millions of patients acquire new infections while receiving treatment for other medical conditions at healthcare facilities (HCFs) [1]. Approximately, five to ten percent of patients in high-income countries (HICs) are infected at HCFs, and the burden of HAIs is substantial in low- and middle-income countries (LMICs) [2]. The risk of HAIs in LMICs is approximately two to twenty times the risk reported in HICs, in part due to limited resources, lack of adequate infrastructure, staff shortage, insufficient healthcare services, and inadequate training on infection prevention and control (IPC) [2, 3]. In addition to the threat HAIs pose to the physical well-being of a patient, such infections may often cause financial and emotional burdens [4, 5].

Inadequate water, sanitation and hygiene (WASH) infrastructure, especially in maternity and neonatal wards, is likely to increase the risks of HAIs among mothers and newborns [5]. The World Health Organization (WHO) estimated that 56 percent of all neonatal deaths among facility-born babies in LMICs were caused by HAIs, and 10.7 percent of those deaths may be associated with "unhygienic conditions" [2]. In 2015, the United Nations proposed the *Sustainable Development Goals* that offer guidance for global development through 2030. The sixth goal, "Ensure access to water and sanitation for all", explicitly highlights the need for WASH provision in healthcare settings. In response to that call, a global initiative is underway. In 2016, WHO and UNICEF jointly announced a *Global Action Plan* for WASH in HCFs that emphasizes four areas: advocacy and leadership,

monitoring, evidence and research, and facility-based improvements [6]. Understanding the current state of WASH in HCFs is crucial for developing effective interventions, especially in countries where HAIs are widespread.

Cambodia has one of the highest infant mortality rates worldwide, and some of these deaths may be due to inadequate WASH provision and ineffective IPC practices in the maternity and neonatal wards [7]. Some studies have reported that about 40 percent of maternal and neonatal deaths in Cambodia were associated with HAIs [8]. Developed by the Cambodian Ministry of Health, the *National Strategic Plan for Infection Prevention and Control in Healthcare Facilities 2016-2020* included adequate WASH as a necessary condition to achieve sustainable IPC practices and better health outcomes [7]. To decrease the incidence of HAIs, it is necessary to identify areas of environmental contamination in HCFs and target interventions to interrupt disease transmission.

The goal of this mixed-method study was to assess WASH conditions in the maternity wards of two Cambodian national hospitals and examine the associations between WASH conditions and environmental contamination. This study had four specific objectives: 1) investigate access to, and quality of, water, sanitation, and handwashing facilities using unstructured observations and the WASH Conditions Assessment Tool, 2) characterize the microbial contamination on common surfaces and medical instruments by assessing the magnitude, frequency, and variability of the detection of key microorganisms that have been frequently associated with HAIs, 3) characterize the microbiological quality of tap water at the point of use based on the WHO and Cambodian drinking water guidelines, 4)

evaluate the hand hygiene of healthcare workers. Ultimately, this study aimed to provide recommendations for WASH infrastructure and practices in the maternity wards in resource-limited settings.

LITERATURE REVIEW

Definitions and Types of HAIs

The WHO defines an HAI as "an infection occurring in a patient during the process of care in a hospital or other health facilities, which was not present or incubating at the time of admission" [9]. Symptoms of infection appear either during or after discharge [9]. The term "HAIs" is used to exclusively indicate infections acquired by patients in hospitals or acute healthcare settings. These were previously referred to as "nosocomial infections" [10]. The definition has now evolved to include occupational infections among healthcare workers, as well as infections that occur in places where patients receive any medical treatment or care, including long-term care facilities, outpatient, ambulatory care, and home care [6, 10]. In general, HAIs arise 48 hours or more following contact with a care service [10]. The most common definition of HAIs used in the literature depends on certain inclusion criteria such as, receiving intravenous therapy within 30 days of infection or being hospitalized for at least two days in the previous three months [11]. Descriptions of HAIs vary across socio-cultural contexts, as a result, achieving an early and accurate diagnosis of these infections has been challenging [1, 10]. Therefore, having a standardized classification of HAIs is crucial for both developing surveillance guidelines and assessing healthcare performance [10].

The United States Centers for Disease Control and Prevention (CDC), together with the National Healthcare Safety Network (NHSN) published a list of criteria for each type of infection [12]. Those criteria have been widely used for diagnosis and public reporting in the U.S. [12]. The four most frequent types of HAIs in the U.S. are: central line-associated bloodstream infections (CLABSI), catheter-associated urinary tract infections (CAUTI), ventilator-associated pneumonia (VAP), and surgical site infections (SSI) [13-15]. In the past decade, there has been an alarming increase in the occurrence of device-related HAIs that cause morbidity and mortality in hospitalized populations [16]. The use of modern medical technology, such as catheters and ventilators, has greatly improved the efficacy of healthcare delivery. However, these invasive procedures involve high risks of HAIs due to the colonization of pathogens on the device surfaces [13, 17-20]. While CAUTI is reported as the most prevalent infection in HICs, SSI is the leading cause of infection in LMICs. The use of invasive devices during unsafe surgical practice puts critically ill patients at a higher risk of HAIs [14, 21]. Up to 30 percent of surgical patients suffer from HAIs in LMICs, which is approximately nine times higher than the proportion in HICs [14]. It is noteworthy that many HAIs are preventable if good prevention practices and monitoring efforts are established and followed [10, 18].

Global Burden of HAIs

Characterized as the "most frequent adverse event" for patients during healthcare delivery, HAIs have multi-dimensional impacts [1]. Developing unexpected infections during treatment often results in a prolonged hospital stay, additional medical interventions, and

increased risks of morbidity and mortality [10]. A multi-national study conducted in 17 Western European countries estimated that approximately 16 million extra hospital days and 150,000 deaths are attributable to HAIs annually [1, 18]. In addition to causing patient safety concerns, HAIs lead to increased costs to payers (patients) and providers (insurance companies), disturbing the efficiency and stability of healthcare systems [1, 10]. The CDC predicted that the U.S. spends upwards of $5 billion on HAIs each year, excluding the costs of externalities (e.g., costs of additional infections or disabilities) [10]. The estimated financial loss from HAIs in Brazil was nearly $18 million in 1992; in Mexico, the estimated average cost was approximately $13,000 per infection case [1, 14]. Furthermore, the occurrence of antimicrobial-resistant microorganisms continues to increase, bringing long-term, negative consequences for both patients and healthcare systems [15, 21]. Unfortunately, infected patients are not the only victims in a case of HAI, healthcare workers who are tasked to maintain the effectiveness of hospital operations are also prone to occupational health infections [10, 11]. The WHO estimated that over three million healthcare workers are exposed to blood borne pathogens each year [22]. The infection rate of HAI outbreaks in healthcare workers could range from 15 to 40 percent [23].

Despite being an endemic global health problem, HAIs have received negligible public attention until recent years [1]. The fact that hospital stays may be shorter than the incubation period (the period between infection and onset of clinical symptoms) makes the burden of HAIs underreported and underestimated [10]. In order to monitor the burden of HAIs, many HICs have set up national surveillance systems and require periodic reports on the occurrence of HAIs from major HCFs [1]. Multi-national studies conducted in 14

HICs revealed that the prevalence of HAIs in hospitalized populations ranged from 3.6 to 12 percent over the past decade [1]. Establishing a comprehensive surveillance system to track the prevalence and incidence of HAIs not only requires time and funding, but also calls for trained experts and patient uptake, which is highly challenging in LMICs [21]. Consequently, few LMICs have HAIs reporting schemes, and only a limited number of studies of HAIs in LMICs were well-conducted and published [1, 11]. Based on available data, it has been estimated that the prevalence of HAIs in LMICs is much higher than HICs, and ranges from 5.4 to 19.1 percent [1]. Multiple meta-analysis studies have attempted to assess the burden of HAIs in both HICs and LMICs [21]. A review of 220 studies reported that the pooled prevalence of HAIs in the U.S. and Europe was 4.5 and 7.1 per 100 patients respectively, compared to 15.5 per 100 patients in LMICs [1, 21].

Common Etiologic Agents of HAIs

Understanding the features and pathogenicity of the microorganisms that cause HAIs is necessary for developing interventions to interrupt disease transmission, and creating better prevention and treatment guidelines [24]. Bacterial pathogens are the major causes of HAIs [10, 24]. Of 81,139 pathogens isolated from 69,475 HAIs in the U.S. between 2009 and 2010, the CDC reported that 90 percent of those pathogens were bacteria [25]. HAI-causing bacteria are mainly classified into two groups: bacteria present in the endogenous flora of patients, including skin surfaces, respiratory or gastrointestinal tract (commensal bacteria), and bacteria found in the natural environment that have entered the hospital environment (saprophytic bacteria) [10, 24]. Due to their ubiquity, persistence, and pathogenic nature, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus* spp. are the most common

commensal bacteria involved in HAIs: together they are responsible for 70 to 80 percent of infections [24, 26]. Saprophytic bacteria, such as *Legionella* and *Enterobacter* spp., can colonize patients by the use of invasive devices or via environmental pathways (e.g., water, air, soil) [24]. Furthermore, the antimicrobial-resistant mechanisms of certain bacteria (e.g., *Staphylococcus aureus*) have contributed to large, complicated HAI outbreaks, mostly in ICU settings, making the treatment of these infections increasingly challenging [10, 24, 27, 28].

A study conducted in western France reported that *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa* were the primary bacteria responsible for ICU-acquired infections [29]. Another French study detected pervasive colonization of the ICU environment by *Klebsiella* spp., *Staphylococcus aureus,* and *Escherichia coli* [30]. The researchers then concluded that methicillin-resistant *Staphylococcus aureus* resulted in approximately 80 percent of all HAIs in ICU [30]. Prospective epidemiological studies in Italy and Germany had similar findings: the most frequently isolated microorganism in ICU was methicillin-resistant *Staphylococcus aureus* [31, 32]. Those conclusions were further supported in a multi-national study of 17 European countries [30, 33]. Among 2,064 ICU-infected patients, 85 percent showed positive microbiological cultures: *Staphylococcus aureus* (30 percent) and *Pseudomonas aeruginosa* (29 percent) were the most identified pathogens, followed by coagulase-negative staphylococci (19 percent) and *Escherichia coli* (13 percent) [33].

Besides causing ICU-acquired infections, *Staphylococcus aureus* and *Escherichia coli* are among the top microorganisms most commonly associated with HAIs in both HICs and LMICs [21, 25]. With available microbiological information, the distribution of pathogens associated with HAIs display similar patterns across continents [34]. The report from NHSN highlighted *Staphylococcus aureus* as the primary cause of VAP and SSI, and *Escherichia coli* was found to be predominantly associated with CAUTI in the U.S. [25]. Consistent with the results obtained from HICs, *Staphylococcus aureus* appeared to be the most common microorganism in hospitalized populations and was a leading cause of surgical-site and bloodstream infections in LMICs [21]. A systematic review of 19 studies of the burden of HAIs in ten African countries reported that *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* were among the most commonly isolated pathogens in both general and surgical patients [34].

Maternal and Child Health (MCH)

The burden of HAIs falls disproportionally on mothers and newborns because of their inherent vulnerabilities and distinct health behaviors [35]. An estimate of global maternal mortality prevalence in 2013 indicated that approximately 10 percent of deaths were attributable to infections in healthcare settings [36]. Puerperal sepsis is closely related to maternal illness because of unsanitary delivery practices [37-39]. Furthermore, up to 60 percent of the neonatal mortality in facility-born infants is linked to HAIs [40]. Newborns with low birth weight are at the highest risk. Studies conducted in Canada and Germany reported that about 12 to 24 percent of very-low-birth-weight newborns developed HAIs while receiving neonatal care in the ICU [41, 42]. Bloodstream-associated infections are

the most common HAIs among children under the age of two, mainly due to the use of contaminated devices in either the ICU or neonatal wards [43]. A systematic review by Zaidi *et al.* reported that the rate of HAIs among newborns in LMICs can be three to twenty times higher than in HICs [40]. In LMICs, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp. and *Acinetobacter* spp. are the leading HAI pathogens in neonates [40]. *Staphylococcus aureus* is responsible for up to 22 percent of bloodstream infections in neonatal populations [40]. The lack of surveillance and reporting systems for neonatal infections in local community health centers where most births occur results in unknown or underestimates of the burden of HAIs among newborns in many LMICs [43].

The WHO has pointed out that water, sanitation, and hygiene (WASH) in HCFs is a "prerequisite for effective and safe care, especially during childbirth," and has highlighted the importance of WASH provision for MCH [44]. Women can become infected during pregnancy, at the time of delivery, and following birth from poor hygiene conditions and practices, such as the use of contaminated devices [38, 45]. A systematic review by Benova *et al.* reported that inadequate provision of water and sanitation was associated with an increased maternal morbidity and mortality rate [38]. Mothers who did not have access to safe water and sanitation were 1.5 times more likely to become infected or die compared to those with adequate WASH access [38]. Both observational and prospective cohort studies have found that handwashing by healthcare workers, especially by birth attendants, functions as a protective measure against maternal and neonatal illness [21].

Because of the links between WASH and healthcare delivery, the availability and quality of WASH infrastructure in HCFs should be included in strategies for enhancing MCH [38]. However, integrating WASH interventions with healthcare service improvement has been challenging, in part due to poor communication and divided interests among stakeholders [46]. Collaboration between the healthcare and WASH sectors requires efforts from policy-makers, health providers, public health workers, engineers, and researchers [38, 46]. Educational programs focusing on clean and safe birthing practices, together with legislation focusing on WASH provision for HCFs should be priorities for health authorities [45]. Achieving universal access to WASH is a basic step to ensure the quality and equity of healthcare services, which will ultimately lead to better health outcomes [45, 46]. Rigorous studies to further assess the relationship between WASH and MCH, with a focus on the socio-cultural determinants of health, may provide additional evidence for advocacy [45].

WASH in HCFs

WASH is a collective terminology that often refers to access to adequate quantity and quality of water, presence of sanitation facilities that provide safety, privacy and dignity, and quality hygiene practices, such as handwashing with soap and water [47]. Given its interrelated nature, work done in each field is dependent on the outcomes of other sectors: to ensure effective handwashing behaviors, provision of adequate water is essential [47]. The long-lasting socio-economic impacts of WASH have been well-recognized by the global community [47-49]. WASH is considered to be a fundamental factor for national development and human health [48, 49]. The *Joint Monitoring Programme for Water*

Supply and Sanitation (JMP), conducted by WHO and UNICEF since 1990, has been monitoring the global progress on WASH coverage [49]. To date, the majority of the information collected by this program has primarily been household-oriented [49]. In 2010, an international *Call to Action for WASH in Schools* was launched [49, 50]. Two years later, the United Nations proposed that providing accessible, safe, and sufficient WASH services in non-household settings is a central step in realizing basic human rights, while eliminating discrimination and inequity toward vulnerable populations [51, 52]. The focus of the WASH sector has now gradually shifted to include non-household settings (e.g., schools, healthcare facilities, workplaces, prisons) [49]. WASH in HCFs has since been recognized as one of JMP's top priorities in the post-2015 era [49].

Many HCFs in LMICs have limited access to basic WASH infrastructure, making the provision of quality healthcare to patients more difficult to achieve [53]. In 2015, WHO and UNICEF published the first multi-national review of WASH conditions in HCFs in Sub-Saharan Africa, Latin America, the Caribbean, and Southeast Asia [53]. A total of 66,101 HCFs in 54 LMICs were included in the assessment, and information on the availability and quality of WASH infrastructure was evaluated using available data from three common healthcare surveys [49, 53]. Major findings from this review indicated that 38 percent of the facilities surveyed did not have access to an improved water source, 19 percent did not have any improved sanitation facilities, and 35 percent lacked rudimentary conditions (water and soap) for handwashing [6, 53]. Similar conclusions have been made in national studies [6, 38, 46]. Research conducted in Tanzania, Malawi, India, and Bangladesh reported that over 80 percent of mothers gave birth in places where WASH

infrastructure was absent [46]. Access to improved WASH was disproportionally provided among health facilities, and there was limited monitoring of WASH in HCFs [6, 54]. Water safety and access is a key concern in many HCFs [55]. National surveys in Rwanda and Uganda reported that more than 60 percent of HCFs did not have an improved water source within 500 m of a facility [55]. Moreover, an improved water source does not necessarily guarantee adequate water quality and quantity; water provided at those facilities was often found to be contaminated and not suitable for use [52, 55]. Some studies have reported that despite the presence of sanitation facilities, many toilets in HCFs were locked and unavailable for patient use [6, 53].

In 2008, the WHO published *Essential Environmental Health Standards in Health Care* to provide guidance on water facilities and access to sanitation in HCFs [56]. However, WASH in HCFs has been a blind spot for most policy-makers in LMICs [44, 52]. Results from the 2014 *United Nations Water Global Analysis and Assessment of Sanitation and Drinking-Water* (GLAAS) report showed that only a few countries (less than 28 out of 94 countries) had national financing and regulations for WASH services in HCFs [44, 52, 53]. Countries with clear national plans to advance WASH in HCFs were found to have better WASH coverage, indicating the importance of developing strategies on a country level [44]. The 2015 multi-national report by WHO and UNICEF highlighted the need for establishing guidelines and political collaboration, assuring proper implementation and monitoring of WASH services in HCFs, and providing evidence-based recommendations for WASH interventions [53]. The *Global Action Plan* launched by WHO and UNICEF aims to achieve “universal access to WASH in health care facilities by 2030,” and has

formulated a detailed framework for improving WASH conditions in HCFs [57]. By engaging policy-makers, researchers, health specialists, and donors, WASH in HCFs will no longer be a forgotten issue [6].

WASH as a Risk Factor for HAIs

Risk factors for HAIs can be categorized into three major groups: organism factors (the nature of microorganisms dictates pathophysiology and transmission route), host factors (the nature of patients determines their susceptibility to infection), and environmental factors (the nature of environment determines the distribution, persistence and transmission causal pathways) [10, 58]. In addition, the risks of HAIs can be influenced by the behaviors of healthcare workers and their interactions with patients [58, 59]. The effect of household-level WASH on nutritional status, diarrheal disease and some neglected tropical diseases has been examined by several studies [6, 60-62]. Studies assessing the impact of WASH provision in HCFs on the burden of HAIs are ongoing [6, 38]. Although, a causal association between poor WASH and HAIs has not yet been established, insufficient WASH infrastructure has been identified as a potential risk factor for HAI [6, 38]. Improvements in WASH conditions in HCFs are likely to reduce the risk of HAIs and other health problems [6, 21, 40]. Adequate access to WASH in HCFs remains a cornerstone in the provision of quality healthcare and is assuming greater importance as the incidence of HAIs increases on a global scale [53].

Water and HAIs

Although the burden of HAIs attributed to water remains unclear, numerous studies have identified water as a source of infections in healthcare settings [63, 64]. There is a wide range of water usage in HCFs, including drinking, bathing, preparing food, washing clothes, sterilizing equipment, as well as for medical devices such as, oxygen concentrators and ventilators [59, 64]. Exposures to water during treatment and hospital stays are nearly inevitable, and any water contamination can pose a risk to the life of patients, as well as jeopardize the well-being of healthcare workers and family members [1, 59]. In the late 1960s, Moffet and Williams documented the survival and recovery of microorganisms from hospital water storage tanks [65]. Unprotected water sources, old distribution systems, poor premise plumbing design and maintenance, together with biofilm formation can serve as natural reservoirs for pathogens to multiply and disseminate [59].

Exposure to unsafe water at the point of use (e.g., sink, shower) is the most common cause of HAIs [59]. Patients can get infected through direct contact, inhalation, or ingestion of contaminated water [59, 64, 66]. An outbreak occurred in cancer patients where the pipes, sinks, and faucets were found to be colonized by *Stenotrophomonas maltophilia* [66]. Water can also function as an indirect source of HAIs by contaminating surfaces and medical instruments [59, 67]. In 2006, an outbreak occurred in ten hospitals in Madagascar, due to the use of aspiration tubes, which had been rinsed by *Klebsiella pneumoniae*-contaminated water [59, 67]. *Legionella pneumophila* is one of the most prevalent bacteria found in hospital water supplies and has been associated with increased morbidity and mortality in patients [63]. Drug-resistant bacteria, such as *Pseudomonas aeruginosa*, have prolonged survival rates in water and can cause persistent outbreaks in ICU and pediatric

wards [64, 68]. A review of epidemiological studies recorded that antimicrobial-resistant bacteria found in water were responsible for 76 percent of all the waterborne disease outbreaks in HCFs [63].

Sanitation, Hygiene and HAIs

The impact of inadequate sanitation and hygiene on health has been well-documented in the literature [1, 69]. It has been stated that ten percent of the global disease burden is associated with a lack of sanitation [70]. In addition to causing diarrheal diseases, poor sanitation is linked to neglected tropical diseases and malnutrition [1]. Pathogens excreted in human and animal feces can spread to new hosts via multiple pathways, known as the "fecal-oral routes" demonstrated in the "F-Diagram" [69]. Sanitation provision and handwashing practices function as the first, and most effective barriers to interrupt disease transmission [69]. However, many HCFs in LMICs do not provide proper sanitation facilities for patients, staff, and visitors and do not have safe disposal of human waste [71].

Along with sanitation, clean hands aid to reduce the risk of HAIs [1, 56]. The causal relationship between hand hygiene and HAIs has been well-demonstrated in the literature [6, 72, 73]. As early as the mid-19th century, an Austrian physician, Ignaz Semmelweis, explored the source of puerperal sepsis -- a common reproductive tract infection in women due to unhygienic birthing practices [74]. Semmelweis concluded that the hands of healthcare workers served as the carriers of microorganisms and caused cross-infection among the patients in the maternity ward [72, 74]. With that discovery, Semmelweis requested healthcare workers to wash hands with chlorinated lime solution after each

patient contact [72, 74]. The rate of puerperal sepsis decreased significantly upon the implementation of that intervention [72, 74]. The work Semmelweis laid the very foundation for hand hygiene promotion and infection controls in HCFs [72].

Since the time of Semmelweis, there is abundant evidence to support handwashing as an effective strategy for HAIs prevention [73, 75, 76]. In London, Casewell and Phillips reported that approximately 17 percent of healthcare workers in ICU had *Klebsiella* spp. contamination on their hands, and handwashing with chlorhexidine cleanser eliminated 98 to 100 percent of the pathogens [73]. In Seattle, the rate of hospital-associated rotavirus disease in pediatric patients was reduced by two-fold when a handwashing campaign was put in place [75]. Proper use of water and soap or alcohol-based hand rubs can effectively remove pathogens from hands [10]. However, a lack of compliance has been identified as a critical issue [10]. In the University of North Carolina Hospitals, a longitudinal study analyzing over 140,000 patients concluded that when the compliance of handwashing by health providers was higher, a reduced rate of healthcare-associated *Clostridium difficile* infection was observed [76]. The *Clean Care is Safer Care* program launched by the WHO has adopted handwashing as the first step to reduce HAIs in HCFs; efforts are needed to strengthen education about handwashing and assess compliance [72].

In summary, WASH is fundamental to the prevention of HAIs and the broader aspects of quality care [6]. Unfortunately, WASH tends to be an overlooked issue [63]. Disease transmission in HCFs due to lack of proper WASH provision can cause outbreaks in particularly vulnerable populations [77]. Adequate water, sanitation facilities, and hand

hygiene infrastructure in HCFs can protect patients, healthcare workers, and visitors from infections and disease transmission and ensure quality healthcare delivery to vulnerable groups, including the disabled, immuno-compromised, elderly, mothers, and newborns [6].

Environmental Contamination and Monitoring in HCFs

Environmental contamination may contribute to the occurrence and transmission of HAIs [10, 78]. The possible role of the hospital environment in HAIs has been extensively investigated [79]. A meta-analysis of HAI outbreaks concluded that contaminated surfaces (e.g., sinks, tables), medical equipment (e.g., endoscope, scissors), water, and hands of healthcare workers are potential reservoirs for microorganisms [1, 21, 63]. Monitoring the presence and concentrations of pathogens in the hospital environment helps to determine the focus of IPC practices, which is imperative for ensuring the quality of healthcare delivery [4, 28]. Yet, in many resource-limited settings, microbial assessment of environmental cleanliness and systematic cleaning protocols are absent [4].

Surface Contamination

Environmental hygiene is typically evaluated based on the presence and concentrations of bacteria detected in the environment [4]. Visibly clean surfaces are not necessarily free of HAI-causing microorganisms [80]. There are few studies in LMICs that have assessed surface contamination in hospital environment. Traditional microbiological sampling techniques for surfaces are swabs and sponges [4, 28, 81]. With the use of enrichment, selective, and/or differential media, researchers can grow and isolate microorganisms from swabs or sponges onto cell culture plates and try to identify them [4, 81]. Additional

bacterial sampling methods, including contact plates and dip slides, which involve direct attachment to the surfaces, have been proven to have better sensitivity and reproducibility on dry areas [4, 82]. Dancer *et al.* proposed that the detection of one or more colony forming units (CFU) per cm^2 of target indicator microorganisms (e.g., *Staphylococcus aureus*, *Clostridium difficile*, vancomycin-resistant enterococci, and *Salmonella* spp.) should be used as a trigger for immediate disinfection procedures [80]. In recent years, adenosine triphosphate (ATP) bioluminescence has emerged as a common approach for assessing the efficacy of environmental cleaning in healthcare settings [81, 83]. Compared to standard microbiological methods, ATP bioluminescence indicates the total number of aerobic colony counts (ACC) in relative light units (RLU) within a short period [81, 82]. In Taiwan, a prospective study led by Huang *et al.* demonstrated no significant difference between the classic microbiological tests and ATP bioluminescence tests in their ability to measure microbial concentrations on surfaces [84]. Further studies should determine an ideal ATP benchmark for evaluating the hospital environment and associated risks of HAIs [84].

Commonly contaminated surfaces in HCFs include the wall, floor, bedside rails, bed linens, sinks, countertops, computer keyboards, toilet seats, door and faucet handles [79, 83, 85, 86]. Certain bacteria can survive on dry surfaces for months [28]. *Staphylococcus aureus* is one of the predominant causes of HAI that has been well-documented in the literature [78, 87]. In a tertiary hospital in Taiwan, Chen *et al.* concluded that medical charts used to record patient clinical data were a major source of cross-contamination, and *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* were identified from

125 samples [88]. In the U.K., moist mattress padding and wet mops in the HCF environment can serve as harbors for *Staphylococcus aureus* and other microorganisms and have been confirmed as sources of HAI outbreaks [89].

The recent appearance of multidrug-resistant strains of *Staphylococcus aureus* has increased the burden of diagnosis and treatment [78, 86, 87]. Methicillin-resistant *Staphylococcus aureus* has been detected on floors, beds, and lockers in several U.S. health facilities [89]. Using pulsed-field gel electrophoresis (PFGE), Bures *et al.*, discovered that the strains of methicillin-resistant *Staphylococcus aureus* isolated from computer keyboards and faucet handles were identical to those identified in infected patients in a Hawaiian medical center [79]. Similar observations were made by Layton and colleagues while investigating an outbreak in the Yale New Haven Hospital [85]. By applying PFGE, Layton *et al.* confirmed that the mupirocin-resistant *Staphylococcus aureus* detected from blood pressure cuffs, communal showers, and nine infected patients had indistinguishable DNA patterns [85]. Characterization of bacterial isolates from the environment and patients has demonstrated that surfaces may indeed serve as reservoirs for HAI-related pathogens [79]. Without properly cleaning contaminated fomites, there can be a high risk of infections through either direct or indirect contact [89].

Medical Equipment

Patients are exposed to a number of devices during medical procedures, and the widespread use or reuse of non-sterile medical equipment has become a potential source of HAIs [90]. In the absence of sterilization, injections with contaminated needles or syringes in

healthcare settings may be responsible for 32 to 40 percent of global hepatitis B virus and hepatitis C virus infections [91]. A surveillance study by Agodi *et al.* established the relationship between *Pseudomonas aeruginosa* infections and invasive devices (e.g., ventilators) in ICU patients [19]. Similarly, an assessment by Sui *et al.* demonstrated that the Y-pieces and water traps on ventilators were the most contaminated parts, and pathogens were detected in all the samples [20]. A high concentration of *Staphylococcus aureus* was detected on the Y-pieces, and *Pseudomonas aeruginosa* was more prevalent on the water traps [20]. Note that both Y-pieces and water traps are part of the breathing circuits, and pathogen detection on these points may result in a high risk of HAIs [19, 20].

Cotton swabs rinsed by sterile saline or broth and inoculated on culture plates are the most common method to assess microbial contamination of medical equipment [65, 92]. One study in London reported that swabs from seven out of twenty-four bedside stethoscopes were contaminated with *Acinetobacter* spp., *Pseudomonas* spp., and methicillin-resistant *Staphylococcus aureus* [92]. Another sampling approach is direct placement of equipment on agar plates or rinsing equipment in broth culture [93, 94]. By directly plating ultra-sonographic probes on blood agar plates, Frazee and colleagues detected methicillin-resistant *Staphylococcus aureus* on the surface of probes [93]. Using a different approach, Muradali *et al.* employed culture media to detect bacterial growth: they inserted ultra-sonographic probes into a nutrient broth, inoculated that broth on agar plates, and then measured the concentration of methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* [94]. Although different sampling and processing techniques

were used, both studies demonstrated that medical instruments were contaminated with pathogens and could be sources of HAIs [90].

To date, there has not been sufficient evidence to conclusively prove a causal relationship between environmental contamination and HAIs [89]. Inconsistencies in study results could be explained by differences in study design, measurement, and potential confounders [89]. Future research should focus on the temporality between exposure to the contaminated environment and the incidence of infections. Evaluating the effectiveness of hospital cleaning also requires long-term efforts and collaborations from all stakeholders.

Water Quality

Waterborne pathogens are major causes of HAI, and ensuring microbiological water quality is fundamental to the prevention of infection [95]. Microbial indicator organisms, including total coliforms, fecal coliforms, and *Escherichia coli*, are typically used to measure water quality [96]. The detection of fecal coliforms and *Escherichia coli* indicate fecal contamination and the potential presence of pathogens in the water [97]. *Escherichia coli* is generally found in human and animal feces and is sensitive to disinfection [97]. The WHO guideline for *Escherichia coli* in drinking water is <1 per 100 mL [96]. Alternatively, the group of total coliforms includes bacteria that can survive and multiply in the environment, thus they are not ideal fecal contamination indicators [96]. Instead, total coliforms have been used to evaluate the efficacy of disinfection procedures [96]. Concentrations of total coliforms in treated drinking water should be <1 per 100 mL, which indicates effective disinfection [96]. Chlorine is the most commonly used disinfectant in

drinking water treatment [96]. The WHO guideline for drinking water recommends 5 mg/L as the maximum chlorine level in water, and water with a free chlorine residual between 0.2 and 1 mg/L is considered sufficiently disinfected [96]. The absence of free chlorine in water implies the possible presence of pathogens, indicating that the water may not be safe to drink [96].

Monitoring water quality in HCFs is essential to design timely interventions for infection controls [97]. Direct colony counting and most probable number (MPN) are the most common approaches to quantify indicator microorganisms in water [98]. Direct colony counting relies on filtering water samples through membranes and placing them on culture plates (e.g., membrane filtration). A MPN test (e.g., IDEXX) utilizes statistical method to estimate microbial concentrations in water samples based on the number of replicate wells with visual signs of bacterial growth (color change, fluorescence) in specific media [98]. Both techniques have been used in healthcare settings to assess water quality [99, 100]. Using membrane filtration, Bhalchandra *et al.* found that the concentration of *Pseudomonas aeruginosa* in an Indian hospital's water supply exceeded the acceptable limit of <1 CFU per 250 mL, and the free chlorine level was below the WHO recommendation [99]. Huttinger and colleagues used the MPN method to measure water quality in ten health centers in Rwanda [100]. They concluded that when the water treatment systems (ultrafiltration and chlorination) were functional, 98 percent of the water samples met the WHO drinking water guidelines for microbiological water quality [100]. In recent years, new water quality testing systems have been developed (e.g., Compartment

Bag Test) [97]. Further investigation is needed to compare the available tests and determine the appropriate method to measure water quality in specific settings [98].

Hand Hygiene

While providing patient care, healthcare workers are not only susceptible to colonization and infection, but may also serve as sources of HAIs transmission [79]. Previous literature has mostly focused on detecting *Staphylococcus aureus* contamination on the hands of healthcare workers, primarily because of its potential resistance to antibiotics and increased risk of morbidity and mortality in patients [101-104]. An outbreak investigation by Weber *et al.* discovered that three children acquired methicillin-resistant *Staphylococcus aureus* infections following surgical procedures [104]. Molecular typing was performed, and investigators found that 31 out of 212 (15 percent) healthcare workers had the same epidemic strain on their hands, and two of them had close contact with the infected children [104].

Multiple studies have explored *Staphylococcus aureus* contamination by culturing hand samples on selective media [101-103]. Tammelin and colleagues collected hand imprint samples from 133 healthcare workers in a Swedish hospital and cultured the samples on Blood agar plates [101]. They found 14 (10.5 percent) of the participants had *Staphylococcus aureus* contamination on hands [101]. Other researchers have reported higher detection rates of *Staphylococcus aureus* on the hands of healthcare workers [102, 103]. Horn *et al.* requested nurses and physicians to rinse their hands in sterile bags containing 50 mL of sterile phosphate buffer [102]. Mannitol salt agar plates were used to

isolate *Staphylococcus aureus*, and the microorganism was detected in 22 of 93 (24 percent) samples from healthcare workers [102]. Similarly, Bauer *et al.* used sterile bags containing 100 mL supplemented saline to collect 328 hand rinse samples from 39 healthcare workers [103]. The frequency of *Staphylococcus aureus* isolation on Blood agar plates was 21 percent [103]. One plausible explanation for the discrepancies in the detection frequencies is the different sampling techniques (hand imprint versus hand rinse) [101].

Infection Prevention and Control (IPC)

Between 1970 and 1975, the Study of the Efficacy of Nosocomial Infection Control (SENIC) was performed in U.S. hospitals to evaluate the cost-benefit of IPC programs [10, 105]. Hospitals with effective IPC programs (HAI surveillance systems, performance evaluations, dedicated infection control physicians and nurses) witnessed a 32 percent decrease in the rate of HAIs, while hospitals without those interventions experienced an 18 percent increase in the rate of HAIs [10, 105]. Findings from SENIC indicated that having an operational facility-wide IPC program is essential to prevent infections among patients and healthcare workers [10]. In general, IPC has numerous components, including monitoring activities, trained infection control personnel, use of protective equipment, careful use of antibiotics, safe disposal of medical wastes, isolation of infectious patients, and disinfection of medical equipment [106]. Many IPC approaches emphasize environmental cleanliness and adequate hand hygiene [2, 10, 107].

Disinfection of commonly touched surfaces is important to lower the risk of direct or indirect disease transmission [10]. However, because some microorganisms can survive in the environment even after room sterilization, the risks of cross-contamination and infection are increasing [108, 109]. Therefore, environmental cleaning procedures require comprehensive guidelines, training, supervision, and adherence [10]. Additionally, the appropriate practice of handwashing has been recognized as the most effective, inexpensive method to ensure patient safety and reduce the incidence of HAIs [10]. Nevertheless, difficulty in individual behavior change, lack of monitoring, shortage of healthcare staff, insufficient or broken handwashing stations, lack of soap and hand sanitizer, and patient overcrowding have together led to a low compliance to hand hygiene, which has resulted in this being a common cause of hospital outbreaks in both HICs and LMICs [10, 110, 111]. Launched by the WHO in 2014, the *World Alliance for Patient Safety* program focuses on education and raising awareness about HAIs by engaging governmental leaders and individual hospitals in IPC campaigns [2]. Promotion of hand hygiene is at the center of these discussions [2]. Successful IPC practices require community efforts, and "a more systematic, multidisciplinary approach" to achieve better health outcomes [10].

HAIs and WASH in Cambodian HCFs

The health systems in Cambodia are primarily financed by non-governmental organizations and private donors [112]. The genocide in the late 1970s resulted in a desperate shortage of teachers, trainers, and healthcare workers [112]. Since 1991, Cambodia has begun to restore its healthcare environment and make maternal health one

of its top priorities [112]. Over the past two decades, there has been increasing use of HCFs and professional health personnel for childbirth [113]. The maternal mortality rate decreased from 4.7 to 1.7 per 1,000 livebirths between 2005 and 2014 [8]. However, the rate of neonatal mortality (18 per 1,000 livebirths) remains one of the highest in the world and accounts for nearly half of all deaths among children under five [8, 114]. In 2009, approximately 40 percent of maternal and neonatal deaths were associated with HAIs [8]. The prevalence of sepsis and hepatitis B virus infections associated with healthcare settings in Cambodia is higher than in other LMICs [115].

Various approaches have been used to assess the burden of HAIs in the neonatal population of Cambodia [116, 117]. A study monitoring health outcomes following caesarean delivery observed that 11 of 176 mothers (6.25 percent) developed a SSI while staying in a provincial hospital [118]. Khun *et al.* reported that the prevalence of HAIs was 13.2 per 100 patients, and Hearn *et al.* calculated an incidence rate of 4.6 per 1,000 patient-days [116, 117]. Current surveillance efforts are limited to individual hospitals, and results from those studies are not nationally representative [116]. Establishing a national monitoring system of HAIs would guide the promotion and enforcement of effective IPC interventions [116].

In 2014, the WHO partnered with WaterAid and RainWater Cambodia to assess WASH conditions in 12 HCFs [3]. They discovered that two hospitals relied on untreated, unprotected surface water as their major water supply, which could be harmful, even life-threatening to patient health [3, 38]. Drinking water was not provided in any of the

facilities, which not only increases the financial burden for patients, but can also have detrimental effects on the behavior and performance of healthcare workers [3]. In delivery rooms, functional sinks with water and soap were available for handwashing, and delivery beds were visibly clean [3]. Yet, less than 40 percent of the rooms in the maternity wards had access to water and soap, which is below the global average of 65 percent [3]. Moreover, patient beds in the maternity wards appeared to be in poor condition [3, 49]. Other studies have reported similar problems with WASH infrastructure and services across Cambodian HCFs [8, 112, 115]. Hospitals usually placed alcohol-based hand sanitizers throughout the hallway of the maternity wards, but hand washing facilities were less frequent [8]. Toilets were often not clean or accessible to both healthcare staff and patients [8, 112]. Few hospitals had running tap water, and water stored in outside containers was not covered properly and observed to be turbid [8]. These infrastructure problems, and the lack of resources and supportive working environment can make it challenging to practice recommended IPC measures and good hand hygiene [112, 115].

While some studies have provided evidence of the association between WASH and MCH, remaining knowledge gaps need to be filled to advance the quality of healthcare services and reduce HAIs [8]. The *National Strategic Plan for Infection Prevention and Control in Healthcare Facilities 2016-2020* by the Cambodian Ministry of Health includes adequate WASH infrastructure as a necessary condition for sustainable IPC practices [7]. It is noteworthy that the existing information about HAIs in Cambodia was mostly derived from either qualitative or anthropological studies with a small sample size [3, 115]. Systematic assessment of Cambodian HCFs is necessary to further characterize HAI burdens and

WASH provisions. Improved understanding of WASH and environmental conditions in HCFs can enhance our ability to reduce the risks of HAIs by designing effective IPC interventions and evaluation programs.

METHODS

Study Sites

Major public hospitals with maternity wards that were within 1-2 hours of the collaborating laboratory in the city of Phnom Penh were considered for inclusion in this study. Two hospitals were selected based on these inclusion criteria. Known for its high-quality healthcare services and well-maintained infrastructure, Hospital A is one of the most reputable public hospitals in Cambodia. In contrast, Hospital B has relatively less financial support, poor human resources, and outdated infrastructure.

WASH Conditions Assessment

Accessibility and quality of WASH infrastructure in both hospitals were evaluated by field staff at WaterAid Cambodia and Emory University using the WASH Conditions Assessment Tool (*Appendix A*) developed by the Center for Global Safe WASH at Emory University. Information on WASH conditions, infrastructure, and resources in the labor and delivery wards were compared through observations and interviews with the hospital directors.

Target Population

The target study population included mothers who gave birth through normal delivery and received post-delivery healthcare services at one of these two hospitals and infants born in either hospital that stayed with their mothers in post-delivery rooms. Their contacts with environmental surfaces and medical instruments during hospital stays were observed. Furthermore, behaviors and practices of healthcare workers and cleaning staff were recorded to understand context information.

Sample Types

Prior to the beginning of the study, another Emory University student spent two weeks observing the behaviors of mothers, newborns, and healthcare workers in the same labor and delivery areas. Routine cleaning procedures at both hospitals and frequently touched surfaces by mothers and newborns were recorded. Analysis of these structured observations, together with discussion with team members, guided the location of environmental sample collection.

In total, there were four different sample types: surfaces, medical equipment, tap water, and hands of healthcare workers. The most commonly touched surfaces, including delivery beds, patient bed covers, patient bedside rails, door handles, and faucet handles, were swabbed. Surgical instruments used for delivery were rinsed. The microbiological quality of tap water was measured weekly. Water in these two hospitals was not only used for medical uses and cleaning, but also for food preparation, bathing, washing hands and clothes. Finally, hand rinses of doctors, nurses, and midwives, were collected due to the frequent contact between staff and mothers and newborns during and after delivery.

Sample Size

Sampling was conducted over an eight-week period between June and August 2016. Each hospital was visited two days a week, every other week. Sampling times varied between 9 am to 3 pm on each day.

Sample Collection

Parallel samples were collected from both hospitals to compare frequency and magnitude of microbiological contamination. In order to gather samples that were representative of the dynamic conditions in the maternity ward, multiple environmental samples were collected at different times of the day to reflect the conditions that a mother or an infant would encounter at different times of the day.

Surface Swabs

Surfaces were sampled using EnviroTrans^{TM} swabs with 5 mL neutralizing buffer (Hardy Diagnostics, USA). When the target surface was large and flat, an estimated area of at least 5 cm^2 to 10 cm^2 was swabbed. If the target surface was round or irregular, then the entire surface was swabbed.

Equipment Rinses

Instruments that had been sterilized following each hospital's autoclave protocol were tested for target microorganisms. Each instrument was picked up using sterile forceps and slowly inserted into a 1000 mL Whirl-Pak bag containing 500 mL sterile water. The

instrument was completely submerged into the water and rinsed for 1 minute. Then, it was dried with a clean paper towel before it was returned to the staff.

Tap Water

Tap water samples were collected from multiple points of use in delivery rooms, post-delivery rooms, bathrooms, and offices. 1000 mL Whirl-Pak bags were used to collect tap water samples. The first flow of tap water was captured, and each bag was filled to slightly above the 500 mL mark, without touching the tap or inside of the bag.

Hand Rinses

Healthcare workers on duty were randomly asked to participate in hand rinses. Consent was obtained from each participant every time before collecting the sample. Each selected healthcare worker was asked to place one hand in a 1000 mL Whirl-Pak bag containing 500 mL deionized water and rinse the hand for 30 seconds. Then, the healthcare worker repeated that step with the other hand in the same bag.

Microbiological Testing Technique

Samples were analyzed for *E. coli*, total coliforms, and *S. aureus* by the membrane filtration method with Compact Dry EC Plates and X-SA Plates (Hardy Diagnostics, USA). Membrane filtration is commonly used to analyze environmental samples and provides quantitative results of the concentrations of target microorganisms in the sample in colony-forming units (CFU). Compact Dry Plates contain dehydrated chromogenic culture media for microorganism identification, which are ready-to-use and convenient for field research.

Compact Dry EC is designed to enumerate and differentiate *E. coli* from other members of the coliform group. *E. coli* appears as blue-colored colonies while other coliforms appear as pink-purple colonies; the two counted together is the number of total coliforms. Bacteria other than coliforms also grew and produced yellow to colorless colonies, which were not counted. Compact Dry X-SA is used to select *S. aureus*, which produces light blue to blue colonies. Other bacteria may produce white or red-purple colonies, which were not counted. *Bacillus spp*. produces large, flat, and matte blue-colored colonies that could be easily differentiated from *S. aureus* and were not included in the total count.

Sample Processing

After collection, samples were sealed and transported in a cooler to the Bacteriology Laboratory at Hospital A for processing the same day. Travel time from Hospital B to the laboratory was about 40 minutes. Each swab tube was vortexed for about 1 minute and 1 mL of the sample was directly inoculated on each plate, colonies were counted in CFU per swab. For equipment rinses, tap water samples, and hand rinses, 1 mL of deionized water was inoculated on each plate to rehydrate the media. Undiluted 100 mL samples were filtered through the membrane, which was then placed on the plate with media. For equipment and hand rinses, the results were expressed as number of CFU per equipment and CFU per pair of hands, respectively. Colonies detected in tap water samples were expressed as CFU per 100 mL.

Plates were incubated upside down at 35°C for 24 hours and read against a white paper as background. Each sample was tested in duplicate to account for variation within the assay and generate reproducible quantitative results. When the colony counts of both plates were within the countable range (10-200 colonies) the total colony count was divided by the total volume filtered to determine the CFU concentration in the sample. Plates that had colonies exceeding 200 were categorized as too numerous to count (TNTC) and recorded as 201 for analysis.

In the last two weeks of sample collection, free and total chlorine residuals in tap water samples were measured at both hospitals. 100 mL sterile water was collected in a Whirl-Pak bag and processed using the membrane filtration method as a negative control. The membrane filter was inoculated on the agar plate to detect any bacterial growth. The negative control was performed every day that samples were analyzed, which examined the sterility of the testing environment and validated the experimental procedure.

Data Analysis

Samples processed at the time when the negative control showed contamination were excluded from analysis. Surface samples that were positive for any target microorganism were described in proportions, and unadjusted odds ratios were analyzed to compare the results between the two hospitals. Detection frequencies of each target microorganism were examined for each sample type stratified by the two study sites. Unadjusted odds ratios were calculated for the presence of total coliforms and *E. coli* in water samples. The Chi-square test was performed to compare the proportions of hand rinse samples that were

positive for the target microorganisms between the two hospitals. All analyses were performed using Microsoft Excel (Redmond, WA) and SAS (Cary, NC).

Ethics Approval

This study protocol was reviewed and approved by the Institutional Review Board of Emory University (IRB00078907) and the Cambodian Ministry of Health National Ethic Committee for Health Research (NECHR). The director generals and chiefs of maternal services at both hospitals gave permission to conduct the study and interact with their healthcare workers and patients. All participants provided oral consent and no personal identification information was collected.

RESULTS

WASH Conditions Assessment

Baseline administrative information from interviews with each hospital's chief of maternal services demonstrated the different service capacity and patient flows in the maternity wards of the two study sites (*Table 1*). Both hospitals provided prenatal examination and consultation services. Neither of the hospitals had separate neonatal wards; newborns always stayed with their mothers and family in the post-delivery rooms. Hospital A had approximately six times the number of inpatient beds than Hospital B. Even though inpatient bed occupancy rates were not available, the maternity ward of Hospital A was usually busy throughout the day. Whereas, only a few patients came into Hospital B after the daily morning rounds. The estimated numbers of monthly natural births and cesarean sections in Hospital A were 900 and 200, respectively, which were considerably higher

compared to 60 and 20 in Hospital B. According to the directors, around 120 healthcare personnel worked in Hospital A on a typical weekday, while Hospital B usually had 32 staff on duty. More than half of the clinical staff in Hospital A were licensed doctors and trained midwives, while over 70% of healthcare workers in Hospital B were student interns from local medical schools.

The access to, and quality of, WASH infrastructure were documented and compared between the two hospitals. Both healthcare facilities received water from the municipal water utility, which drew its water from the Tonle Sap River. The city-wide Phum Prek water treatment plant processed the surface water following standard practice (flocculation, coagulation, sedimentation, filtration, and chlorination) before distribution. While both hospitals provided piped water services, Hospital A installed a private water treatment plant on-site to further chlorinate the municipal water source. Hospital B used a 100,000 L underground water storage tank, which had neither been inspected nor cleaned in recent decades according to the hospital director. Though the chief of maternal services claimed that Hospital B chlorinated its water on-site, water treatment plant was not observed. In both hospitals, most people relied on bottled water as their primary drinking water source. Some healthcare workers, patients, and family members were observed to drink boiled water.

In addition to surveys, unstructured observations were conducted to understand WASH conditions in both hospitals. The two sites differed in the quantity, quality, and access to sanitation and hygiene facilities. In Hospital A, there were a total of 24 functional sinks in

the maternity ward. Of the 24 sinks, 19 were in patient care areas. Soap was not provided at each handwashing station, except in the three delivery rooms. Alcohol-based hand rub was provided in bottles and attached to the wall throughout the hallway in the maternity ward. Each mother and her family were housed in a private post-delivery room equipped with a bathroom, which included a functional handwashing sink, a flush toilet designed for sitting, and a shower. In Hospital B, five out of six sinks were functional in the maternity ward. Two of the five functional sinks were in the patient care areas. Soap was provided next to the sink in the delivery room, and alcohol-based hand rub was not available. A total of three post-delivery rooms were shared by all patients and their family. Each room could accommodate four to six patients. Visitors were observed to sit on the floor, where they would eat, drink, and sleep. Two dry squat toilets outside of the post-delivery rooms were available for patient use. Water used for washing, bathing, and toilet flushing was usually stored in large buckets next to the squat toilets. The post-delivery rooms opened on to a single outdoor balcony area where a tap was installed to provide patients and families with access to piped water.

Surface Swabs

A total of 74 surface swabs were collected. Three high-touch surfaces, including handles (door and faucet handles), patient bedside rails, and bed covers (surfaces of delivery beds and covers of patient beds), were sampled. Due to the variability in the size and shape of the sampled surfaces, the results were expressed as the number of CFU per swab. In Hospital A, the proportions of surface samples that were positive for any target microorganism were: 5 of 14 (36%) handles, 4 of 8 (50%) bedside rails, and 6 of 15 (40%)

bed covers. In Hospital B, the proportions of surface samples that were positive for any target microorganism were: 6 of 16 (38%) handles, 5 of 7 (71%) bedside rails, and 10 of 14 (71%) bed covers. The odds of detecting any target microorganism on one of the high-touch surfaces was 1.9 times higher in Hospital B compared to Hospital A ($p < 0.05$).

The frequency of target microorganism detection on the three major surface samples was compared between the two hospitals (*Figure 1*). Ten of 37 (27%) surfaces swabbed in Hospital A were found to be contaminated with *E. coli*. Bedside rails had the highest median colony count of 14 CFU per swab (*Table 2*). Eleven of 37 (30%) surfaces swabbed in Hospital B had *E. coli* contamination. Bedside rails yielded the highest median colony count of 25.5 CFU per swab. Total coliforms were pervasive on bedside rails in both hospitals, 4 of 8 (50%) bedside rails in Hospital A, and 5 of 7 (71%) in Hospital B had positive total coliform detection. The median concentration obtained from bedside rails in hospital B (36 CFU per swab) was lower than that in Hospital A (more than 200 CFU per swab). The proportions of surface samples that were positive for *S. aureus* in both hospitals were lower than for the other two target microorganisms: 8 of 37 (22%) in Hospital A and 7 of 37 (19%) in Hospital B. Bedside rails sampled in Hospital B had the highest median *S. aureus* colony count of 12.5 CFU per swab.

Equipment Rinses

Due to limited access to hospital resources, only nine surgical instruments were tested for target microorganisms. The equipment sampled was primarily used for delivery, including needle holders, dressing forceps, surgical scissors, and vaginal speculum. Target

microorganisms were not detected from four pieces of equipment tested in Hospital A. Among five pieces of equipment tested in Hospital B, a pair of surgical scissors had one total coliform colony and one *S. aureus* colony in the 100 mL rinse.

Tap Water

In the eight weeks of water quality testing, water interruption or shortage never occurred during the time of sampling. A total of 58 tap water samples were collected. An equal number of water samples was collected at both hospitals from every week (*Table 3*). Chlorine residual monitoring did not begin until the sixth week. Eight of 29 water samples from Hospital A, and 11 of 29 water samples from Hospital B were examined for total and free chlorine level.

Among 29 water samples collected at Hospital A, 28 (97%) met the WHO drinking water guideline of less than one total coliform CFU per 100 mL. The remaining water sample (3%) had one total coliform CFU per 100 mL, and would be characterized as "low risk" by WHO. Concentrations of *E. coli* were below one CFU per 100 mL in all water samples from Hospital A, and met the WHO guideline for drinking water quality of less than one *E. coli* CFU per 100 mL. The mean free chlorine residual was 0.5 mg/L (range: 0.2-0.8). In Hospital B, total coliforms were detected in 14 of 29 (48%) water samples and *E. coli* in 5 of 29 (17%) samples. The median concentration of total coliforms in the 14 water samples was 1.25 CFU per 100 mL (range: 0.5-6.5), and the median concentration of *E. coli* was 1.0 CFU per 100 mL (range: 0.5-2.5). The average level of free chlorine was 0 mg/L (range: 0-0.1).

There was a significant difference ($p = 0.02$) in the proportion of tap water samples with more than one CFU *E. coli* per 100 mL between two hospitals. The odds of a tap water sample meeting the WHO drinking water guideline for total coliforms was 26 times higher for Hospital A compared to Hospital B (OR: 26.13, 95% CI: 3.13-218.50).

Hand Rinses

A total of 66 hand rinse samples were collected. In both hospitals combined, 36% of sampled healthcare workers (N=66) had *E. coli* present on their hands, 60% had total coliforms, and 73% had *S. aureus*. A significant difference ($p < 0.05$) was observed between the two hospitals in the proportion of hand samples with detection of target microorganisms among selected healthcare workers. Of 34 hand rinse samples collected in Hospital A, 15 (44%) had no target microorganisms detected. One of 32 (3%) hand rinse samples collected in Hospital B had no detectable target microorganisms. The odds of detecting target microorganisms on hands were significantly higher among healthcare workers in Hospital B compared with healthcare workers in Hospital A (*E. coli* OR: 6.00, 95% CI: 1.95-18.48; total coliforms OR: 8.72, 95% CI: 2.68-28.35; *S. aureus* OR: 21.43, 95% CI: 4.30-104.60). The wide confidence intervals of these associations could be due to small sample size (*Table 4*).

The number of target microorganisms per pair of hands was categorized into four levels (<1, 1-10, 11-200, and >200 CFU) and the distribution of the microbial load on hands was compared between the two hospitals (*Figure 2*). Around 90%, 70%, and 65% of hand rinse

samples from Hospital A had no detectable *E. coli*, total coliforms, and *S. aureus*, respectively. Two of 34 nurses (6%) had more than 200 CFU total coliforms and *S. aureus* on their hands, and both nurses were working in the pre-delivery consultation rooms and attending pregnant mothers without wearing gloves. In Hospital B, 47% of hand rinse samples had less than one *E. coli* CFU per pair of hands. Nearly 45% and 60% of samples had total coliforms and *S. aureus*, respectively, between 1 and 10 CFU per pair of hands. A nurse who was giving postnatal injections to a mother without wearing gloves had more than 200 CFU *E. coli* and total coliforms on both hands.

DISCUSSION

This study examined the potential associations between WASH conditions and environmental contamination in two hospital maternity wards in Cambodia. The goal of this study was to provide evidence on the importance of maintaining sufficient WASH infrastructure to interrupt disease transmission. Based on the analysis of the environmental samples collected, the findings from the present study suggested that inadequate WASH infrastructure may increase the likelihood of environmental contamination and drive the transmission of pathogens. The significant difference detected in water quality between the two study sites may be due to the availability of onsite water treatment plant. The difference in the microbial contamination levels on hands between the two maternity wards may be explained by the poor access to functional handwashing stations with soap and lack of hand rub in Hospital B.

Hand Hygiene

This study demonstrated that a hospital with accessible handwashing infrastructure had a greater proportion of healthcare workers with no detectable target microorganisms on their hands. Hand rinse samples collected in Hospital B had a higher incidence and a greater magnitude of contamination compared to the samples collected in Hospital A. Among the three target microorganisms, *S. aureus* was the most frequently isolated bacteria on hands and was detected in 14 of 34 (41%) hand rinse samples collected in Hospital A, and 30 of 32 (94%) samples collected in Hospital B. Among the 30 samples from Hospital B, the median count of *S. aureus* was 4.5 CFU per pair of hands, which was higher than the median count of *S. aureus* detected in the 14 hand rinse samples (2.5 CFU per pair of hands) from Hospital A.

In Hospital A, handwashing facilities were provided at the exit of all toilets. The functional handwashing stations and the available wall-mounted hand rub dispensers in the maternity ward offered healthcare workers an incentive and increased opportunity to wash and disinfect their hands. In Hospital B, the lack of access to sink, soap, and hand rub may have discouraged healthcare workers from practicing proper handwashing after defecation and before/after patient contact. Most of the healthcare workers from Hospital A were observed to carry alcohol-based hand rub in their pockets and apply it to their hands before and after interacting with patients. The same behavior patterns were not witnessed in Hospital B. These conditions probably explain the higher frequency of microbial contamination detection on the hands of healthcare workers in Hospital B. Other factors that could influence hand contamination levels include whether the selected healthcare workers

washed their hands before sample collection, the effectiveness of handwashing practices, the level of microbial contamination on regularly touched surfaces, and the quality and quantity of water used for handwashing.

Previous studies have described inadequate handwashing infrastructure as a barrier to hand hygiene adherence. A systematic review by Erasmus and colleagues showed that improved accessibility to handwashing materials (water, soap and/or alcohol-based hand rub) could trigger better hand hygiene compliance in health settings [119]. In Vietnam, researchers concluded that limited and dysfunctional sinks, together with a lack of soap in hospitals, became major barriers for healthcare workers to wash hands [120]. At the healthcare facility level, only a handful of studies have assessed the hand contamination of healthcare workers, and most of those studies merely focused on the proportions of workers with bacteria on their hands. In Sweden, 14 of 133 (11%) hand imprints of healthcare workers had *S. aureus* contamination [101]. In Argentina, fingerprint samples were collected from 100 healthcare workers, and *S. aureus* was isolated from 62 samples [121]. Neither of these hand impression studies quantify the amount of *S. aureus* detected on hands. Compared to hand imprint, hand rinse is a more sensitive method to quantify the bacterial contamination on hands [122]. Monistrol and colleagues collected 89 hand rinse samples from healthcare workers in a Spanish hospital, and found *S. aureus* contamination in 15 samples (17%), with a mean concentration of 150 CFU per pair of hands [123]. The hand samples from the present study had less microbial contamination compared to the samples from the Spanish study. Differences in the study design and sample collection need to be considered. These

two studies used somewhat different metrics and culture media to grow and select bacteria on hands.

Microbial contamination on hands may serve as a vehicle for the transmission of HAIs, putting patients at risk for infection. *S. aureus* was tested as an indicator of hand hygiene because it is a common cause of HAIs [10]. Singh *et al.* found that 95 of 200 (47.5%) hand imprint samples from healthcare workers in an Indian hospital were positive for *S. aureus*, and 50 of the 95 samples (50.2%) showed resistance to methicillin [124]. An outbreak investigation in the U.S. concluded that the methicillin-resistant strain of *S. aureus* detected on the hands of healthcare workers was the primary cause of infections among children in pediatric wards [104]. Even though this study did not analyze the antimicrobial resistant mechanisms, our detection of *S. aureus* on the hands of healthcare workers suggests that there may be an increased risk of HAIs for patients due to poor hand hygiene. Observational and prospective studies have established that handwashing or hand disinfection by healthcare workers is an effective, protective measure against maternal and neonatal illness [46]. Casewell *et al.* reported that handwashing with chlorhexidine cleanser effectively reduced the amount of bacteria on hands [73]. Rhee *et al.* reported a 41% decrease in the mortality rate in Nepali neonatal population when handwashing by caregivers was practiced [125]. Hand hygiene adherence and promotion require collaborative efforts from both individuals and facilities. Hand hygiene of healthcare workers in hospitals in LMICs requires further investigation to better understand the magnitude of the problem and identify the determinants of good hand hygiene and how to effectively promote it.

Water Quality

There was a significant difference in the water quality between the two study sites. Both hospitals provided municipal tap water in the maternity wards, and this water was accessible to patients and healthcare workers. Nearly all the water samples (28 out of 29) collected from Hospital A met the WHO drinking water guidelines for *E. coli* and total coliforms and had chlorine levels that were within the recommended range. In Hospital B, about half of the water samples (14 out of 29) were not safe for drinking as fecal contamination was detected. The concentrations of *E. coli* and total coliforms in the 14 water samples ranged between one and ten CFU per 100 mL and would be categorized as "low risk" according to the WHO guidelines. Most of the water samples from Hospital B had no detectable chlorine residuals.

As both facilities used the municipal water supply that received full conventional treatment, the significant difference in the water quality between the two study sites may be due to the use of onsite water treatment. In Hospital A, the private water treatment plant was fully functional, and the chlorination system was regularly monitored. Adequate levels of disinfectant in the water are critical for ensuring the quality, and chlorination is a necessary step to maintain the water quality at the point of distribution [100]. As a result, the microbiological water quality in Hospital A was usually good, and the free chlorine levels were consistently within the WHO recommendation. In Hospital B, the absence of onsite chlorination and reported inadequate cleaning of the water tank and the distribution system presented a risk of water re-contamination. It was likely that the piping network and/or the underground storage at Hospital B was re-contaminating the water.

Previous studies have reported that the water provided at HCFs in LMICs was often contaminated and not suitable for use [52]. A systematic review and meta-analysis by Bain *et al.* showed that more than 25% of improved water sources in hospitals in LMICs had fecal contamination [55]. Unsafe and untreated water may pose a health threat to those who use that water for work and basic needs. Although the burden of HAIs attributed to water quality remains unclear, numerous studies have identified water as a source of pathogens [63, 64]. A review of epidemiological studies reported that antimicrobial-resistant bacteria found in the water were responsible for 76 percent of all the waterborne disease outbreaks in HCFs [63]. To prevent the occurrence of water-related HAIs and achieve better health outcomes, the WHO has recommended that each hospital adopt a water safety plan that measures the microbiological quality of water, assesses the water distribution system, and provides education and surveillance on water facility maintenance [64].

Surface Contamination

A significant difference in the frequency of surface contamination was observed between the two maternity wards. The odds of detecting microbial contamination on any of the high-touch surfaces were 1.9 times higher in Hospital B compared to Hospital A. Bedside rails were found to be the most contaminated location. These were places where mothers and family hung food and other personal items. In both maternity wards, microbial contamination was detected on the covers of the delivery beds and patient beds even after disinfection. Door and faucet handles were the least contaminated surfaces, probably due to the small sampling area. The inadequate cleaning and disinfection practices by healthcare workers in both hospitals could be a major contributing factor to environmental

contamination. A lack of adequate WASH infrastructure in Hospital B may negatively influence the cleaning behaviors of healthcare workers and may explain the higher rate of environmental contamination on surfaces. In both hospitals, there was a lack of promotional materials and a lack of monitoring efforts on infection control. Routine cleaning was unstructured, and the schedule was unpredictable.

The sampling areas in the present study have been described as among the most frequently contaminated surfaces in HCFs, where regular and sufficient disinfection procedures are necessary [83, 89]. Boyce *et al.* isolated methicillin-resistant *S. aureus* from 12 of 20 (60%) bedside rails in a university-affiliated hospital [83]. Hota *et al.* indicated that bedside rails and patient bed covers had the highest likelihood of being colonized by HAI-causing bacteria [89]. Previous studies have provided evidence to support the importance of hospital cleaning as a key infection control intervention [89]. Griffith *et al.* evaluated the effectiveness of cleaning regimes in the surgical ward of a British hospital and found a significant decrease in the amount of bacterial counts on commonly touched surfaces after cleaning [81].

In addition to the exposures to contaminated hands and unsafe water supplies, exposures to unclean environmental surfaces could increase the risk of HAIs. Layton and colleagues investigated an outbreak of mupirocin-resistant *S. aureus* in a U.S. hospital, and they identified the communal shower handles in the patient care areas as the environmental reservoirs and likely transmission route of the pathogen [85]. Bures *et al.* identified the same strain of methicillin-resistant *S. aureus* from faucet handles and infected patients in

a U.S. medical center [79]. Moreover, the inappropriate use of contaminated equipment during delivery may lead to an unavoidable infection risk for mothers and newborns [38]. Randrianirina *et al.* concluded that the reason for the *Klebsiella pneumoniae* outbreak in ten healthcare facilities in Madagascar was because of the use of contaminated aspiration tubes [67].

Sanitation Facilities

Both hospitals had access to improved sanitation based on the JMP definition. Flush or pour flush toilets were provided for patients and healthcare workers in both maternity wards to safely dispose human waste. Doors and locks were available to guarantee safety and privacy. The WHO *Essential Environmental Health Standards in Health Care* recommends that toilets should be designed as gender-specific and accessible to vulnerable populations, including children, pregnant women, and people with disabilities. However, none of those requirements were met in the two maternity wards in this study. Facilities for changing and disposal of menstrual hygiene management materials were also lacking. Existing studies on the evaluation of sanitation conditions in HCFs have primarily been focused on the access to improved toilets [54]. Other important aspects, including the quality of the sanitation environments and the issues related to gender and socio-cultural factors, require close attention and further investigation.

Strengths and Limitations

This study was designed as a pilot project. It is one of the few studies conducted in Cambodia that focused on environmental contamination in clinical settings. It was also one

of the first studies to explore the associations between WASH conditions and environmental cleanliness in hospitals in LMICs. The microbiological sampling and processing techniques used in the study allowed for the quantification of the bacterial counts on environmental surfaces, medical instruments, hands of healthcare workers, and in water. The findings from the current study provide a first estimate of the microbial environmental conditions in maternity wards of two national hospitals in Cambodia. To better understand the microbiological results from the environmental sampling, this study also collected qualitative data through unstructured observations of the hospital conditions and the behaviors of healthcare workers over several weeks.

Certain limitations should be taken into consideration when interpreting the results. First, the WASH Conditions Assessment surveys collected data through in-person interviews with the directors of each maternity ward at a single point in time. It was difficult to evaluate the reliability and validity of information reported in interviews. Recall bias and social desirability bias could occur and influence some of the responses about cleaning and hygiene practices. For example, the director from Hospital B claimed that the hospital chlorinated the municipal water onsite. However, chlorine residuals were not detected in the water samples. Moreover, pilot testing of the environmental sampling and microbiological processing technique was limited due to the short time frame. A lack of familiarity with the use of the Compact Dry Plates may have affected the quality of the microbiological results at the beginning of the study. Restricted resources, time, and manpower limited the number of samples collected. Because only two hospitals were included in the study, the findings from this study may not be generalizable and nationally

representative of hospital maternity ward conditions in Cambodia. The concentrations of target microorganisms detected were estimates, and the isolated colonies need further bacteriology identification. It is important to note that this study did not attempt to measure HAIs among the patients in these maternity wards, and there is no well-defined association between the WASH conditions, environmental contamination, and the risk of infections. Despite these limitations, this study is a useful starting point to identify future research priorities.

CONCLUSIONS AND RECOMMENDATIONS

Inadequate WASH provision is one of the potential determinants of the high burden of HAIs in LMICs [53]. This study showed the possible link between poor WASH infrastructure and environmental contamination in the maternity ward. Without adequate access to clean water, safe sanitation and hygiene facilities, there may be a high frequency and magnitude of environmental contamination, which could affect the health outcomes of patients.

Hand hygiene is a protective, cost-effective method for preventing disease transmission but is often neglected in both high- and low-resource settings [21]. In this study, the difference in the hand contamination of healthcare workers between the two hospitals may reflect a lack of consistent handwashing habits, limited access to handwashing stations with soap and water, and a lack of promotional handwashing messaging [126]. Hospitals need to identify perceived barriers to good hand hygiene practices and establish training and

monitoring guidelines on hand hygiene. By improving the enabling environment, such as maintaining functional handwashing stations, and providing materials for handwashing, there would be a lower incidence of microbial contamination on hands. Educating healthcare workers and patients about personal hygiene and the procedures of preventing infections related to appropriate behaviors is also a practical approach to reducing the transmission of pathogens.

During the study, several practices were observed that may contribute to environmental contamination in the maternity wards. In both hospitals, medical waste was observed to be segregated inappropriately and disposed of unsafely. Unorganized and used surgical instruments and cleaning equipment were left on counters or sinks without proper labels. Disinfection procedures by the hospital staff were neither sufficient nor closely monitored. On numerous occasions, delivery beds that were supposedly cleaned and disinfected were found to be contaminated. It is likely that most of the cleaners have never received any proper IPC training. Both hospitals need to establish a systematic disinfection strategy for environmental surfaces and medical instruments to interrupt the transmission of pathogens.

In general, there is a strong need for healthcare facilities to implement and monitor IPC measures, which should include the routine testing of: 1) microbiological water quality at point of use, 2) hand hygiene of health providers, 3) disinfection of room and regularly touched surfaces, and 4) effectiveness of equipment sterilization. WASH is fundamental to HAIs prevention and to the wider aspects of quality care [6]. To maintain adequate WASH

infrastructure and achieve optimal healthcare delivery requires long-term efforts and collaborations from all stakeholders.

For the future, interdisciplinary studies are needed to fully understand the burden of HAIs caused by inadequate and unsafe WASH infrastructure. Standardized sampling and processing methods should be developed to help identify sources of environmental contamination and enable reliable comparison of results among facilities. Further investigation is recommended to explore the routes of infection transmission in the hospital environment in resource-limited settings and determine the critical contamination points to target interventions. A study of WASH conditions and individual behaviors that includes infection surveillance and environmental microbiology would be an important step toward defining the risks and the transmission routes of HAIs-related pathogens in healthcare facilities. Researchers should incorporate qualitative methodologies and choose the most appropriate target microorganisms and microbiological assays for environmental samples in this setting. As antimicrobial-resistance is becoming a growing public health concern, identifying antimicrobial-resistant microorganisms from environmental surfaces would be an effective approach to designing hospital disinfection interventions. It would be useful to characterize the sensitivity and specificity of different growth media, as well as explore the efficacy of different techniques for environmental sampling. Further research is needed to better understand the needs and barriers of improving water quality in hospitals in LMICs. There is also a need to investigate the impact of adequate WASH on patient satisfaction and utilization of health care services.

REFERENCES

1. WHO, *Report on the burden of endemic health care-associated infection worldwide*, in *Clean Care is Safer Care*. 2011, A World Alliance for Safter Health Care.
2. Morris, K., *Global control of health-care associated infections.* Lancet, 2008. **372**(9654): p. 1941-2.
3. WaterAid, *Safer health care facilities in Cambodia*, in *Health care facility assessment report*. 2015.
4. Galvin, S., et al., *Microbial monitoring of the hospital environment: why and how?* J Hosp Infect, 2012. **82**(3): p. 143-51.
5. Stone, P.W., *Economic burden of healthcare-associated infections: an American perspective.* Expert Rev Pharmacoecon Outcomes Res, 2009. **9**(5): p. 417-22.
6. WHO/UNICEF, *Water, sanitation and hygiene in health care facilities: global strategy, burden of disease, and evidence and action priorities*. 2016.
7. MoH, *Cambodia National Strategic Plan for Infection Prevention and Control in Health Care Facilities 2016-2020*. 2015.
8. Bazzano, A.N., et al., *Environmental factors and WASH practices in the perinatal period in Cambodia: implications for newborn health.* Int J Environ Res Public Health, 2015. **12**(3): p. 2392-410.
9. WHO. *The burden of health care-associated infection worldwide*. Clean Care is Safer Care 2017 [cited 2017 January 17]; Available from: http://www.who.int/gpsc/country_work/burden_hcai/en/.
10. Collins, A.S., *Preventing health care-associated infectiosn*, in *Patient safety and quality: an evidence-based handbook for nurses*, H. RG, Editor. 2008, Agency for Healthcare Research and Quality: Rockville (MD).
11. Cardoso, T., et al., *Classification of healthcare-associated infection: a systematic review 10 years after the first proposal.* BMC Med, 2014. **12**: p. 40.
12. Horan, T.C., M. Andrus, and M.A. Dudeck, *CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting.* Am J Infect Control, 2008. **36**(5): p. 309-32.
13. CDC. *Types of healthcare-assocaited infections*. Healthcare-associated infections 2014 [cited 2017 January 17]; Available from: https://www.cdc.gov/hai/infectiontypes.html.
14. WHO. *Health care-associated infections FACT SHEET*. Patient Safety [cited 2017 January 17]; Available from: http://www.who.int/gpsc/country_work/gpsc_ccisc_fact_sheet_en.pdf.
15. Burke, J.P., *Infection control - a problem for patient safety.* N Engl J Med, 2003. **348**(7): p. 651-6.
16. Septimus, E.J. and J. Moody, *Prevention of Device-Related Healthcare-Associated Infections.* F1000Res, 2016. **5**.
17. Percival, S.L., et al., *Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control.* J Med Microbiol, 2015. **64**(Pt 4): p. 323-34.
18. Guggenbichler, J.P., et al., *Incidence and clinical implication of nosocomial infections associated with implantable biomaterials - catheters, ventilator-*

associated pneumonia, urinary tract infections. GMS Krankenhhyg Interdiszip, 2011. **6**(1): p. Doc18.
19. Agodi, A., et al., *Pseudomonas aeruginosa carriage, colonization, and infection in ICU patients.* Intensive Care Med, 2007. **33**(7): p. 1155-61.
20. Sui, Y.S., et al., *Effectiveness of bacterial disinfectants on surfaces of mechanical ventilator systems.* Respir Care, 2012. **57**(2): p. 250-6.
21. Allegranzi, B., et al., *Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis.* Lancet, 2011. **377**(9761): p. 228-41.
22. WHO, *The world health report: reducing risks, promoting healthy life*. 2002, World Health Organization: Geneva, Switzerland.
23. Sepkowitz, K.A., *Occupationally acquired infections in health care workers. Part I.* Ann Intern Med, 1996. **125**(10): p. 826-34.
24. Marcel, J.P., et al., *Healthcare-associated infections: think globally, act locally.* Clin Microbiol Infect, 2008. **14**(10): p. 895-907.
25. Sievert, D.M., et al., *Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010.* Infect Control Hosp Epidemiol, 2013. **34**(1): p. 1-14.
26. Kovacs, C.S., et al., *Hospital-acquired Staphylococcus aureus primary bloodstream infection: A comparison of events that do and do not meet the central line-associated bloodstream infection definition.* Am J Infect Control, 2016. **44**(11): p. 1252-1255.
27. Fournier, P.E. and H. Richet, *The epidemiology and control of Acinetobacter baumannii in health care facilities.* Clin Infect Dis, 2006. **42**(5): p. 692-9.
28. Poza, M., et al., *Exploring bacterial diversity in hospital environments by GS-FLX Titanium pyrosequencing.* PLoS One, 2012. **7**(8): p. e44105.
29. Legras, A., et al., *Nosocomial infections: prospective survey of incidence in five French intensive care units.* Intensive Care Med, 1998. **24**(10): p. 1040-6.
30. De Rosa, F.G., et al., *SPIR01 and SPIR02: a two-year 1-day point prevalence multicenter study of infections in intensive care units in Piedmont, Italy.* New Microbiol, 2008. **31**(1): p. 81-7.
31. Gastmeier, P., et al., *Risk factors for death due to nosocomial infection in intensive care unit patients: findings from the Krankenhaus Infektions Surveillance System.* Infect Control Hosp Epidemiol, 2007. **28**(4): p. 466-72.
32. Malacarne, P., et al., *Epidemiology of nosocomial infection in 125 Italian intensive care units.* Minerva Anestesiol, 2010. **76**(1): p. 13-23.
33. Spencer, R.C., *Predominant pathogens found in the European Prevalence of Infection in Intensive Care Study.* Eur J Clin Microbiol Infect Dis, 1996. **15**(4): p. 281-5.
34. Bagheri Nejad, S., et al., *Health-care-associated infection in Africa: a systematic review.* Bull World Health Organ, 2011. **89**(10): p. 757-65.
35. Posfay-Barbe, K.M., D.M. Zerr, and D. Pittet, *Infection control in paediatrics.* Lancet Infect Dis, 2008. **8**(1): p. 19-31.

36. Kassebaum, N.J., et al., *Global, regional, and national levels and causes of maternal mortality during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.* Lancet, 2014. **384**(9947): p. 980-1004.
37. Brooker, S., P.J. Hotez, and D.A. Bundy, *Hookworm-related anaemia among pregnant women: a systematic review.* PLoS Negl Trop Dis, 2008. **2**(9): p. e291.
38. Benova, L., O. Cumming, and O.M. Campbell, *Systematic review and meta-analysis: association between water and sanitation environment and maternal mortality.* Trop Med Int Health, 2014. **19**(4): p. 368-87.
39. Gould, I.M., *Alexander Gordon, puerperal sepsis, and modern theories of infection control--Semmelweis in perspective.* Lancet Infect Dis, 2010. **10**(4): p. 275-8.
40. Zaidi, A.K., et al., *Hospital-acquired neonatal infections in developing countries.* Lancet, 2005. **365**(9465): p. 1175-88.
41. Aziz, K., et al., *Variations in rates of nosocomial infection among Canadian neonatal intensive care units may be practice-related.* BMC Pediatr, 2005. **5**: p. 22.
42. Geffers, C., et al., *Use of central venous catheter and peripheral venous catheter as risk factors for nosocomial bloodstream infection in very-low-birth-weight infants.* Infect Control Hosp Epidemiol, 2010. **31**(4): p. 395-401.
43. Richards, M.J., et al., *Nosocomial infections in pediatric intensive care units in the United States. National Nosocomial Infections Surveillance System.* Pediatrics, 1999. **103**(4): p. e39.
44. WHO/UN-Water, *UN-water global analysis and assessment of sanitation and drinking-water (GLAAS) 2014 - report*. Investing in water and sanitation: increasing access, reducing inequalities. 2014.
45. Shordt, K., Smet, E. and Herschderfer, K., *Getting it Right: Improving Maternal Health Through Water, Sanitation & Hygiene.* Haarlem: Simavi, 2012.
46. Velleman, Y., et al., *From joint thinking to joint action: a call to action on improving water, sanitation, and hygiene for maternal and newborn health.* PLoS Med, 2014. **11**(12): p. e1001771.
47. UNICEF. *Water, sanitation and hygiene*. 2016 [cited 2017 January 17]; Available from: https://www.unicef.org/wash/3942_3952.html.
48. Eid, U., *The importance of water, sanitation, and hygiene as keys to national development*, in *Johns Hopkins Water Magazine*. 2015.
49. WHO/UNICEF, *Progress on sanitation and drinking water - 2015 update and MDG assessment*. 2015.
50. UNICEF, *Equity of Access to WASH in Schools- a comparative study of policy and service delivery in Krygyzstan, Malawi, the Phillippines, Timor-Leste, Uganda and Uzbekistan*, in *Equity of Access Study*. 2011.
51. UN, *UN special rapporteur on the human right to safe drinking water and sanitation*, in *Report to the General Assembly, Integrating Non-discrimination and Equality into the Post-2015 Development Agenda for Water, Sanitation and Hygiene*. 2012: UN Doc. A/67/270.
52. Cronk, R., T. Slaymaker, and J. Bartram, *Monitoring drinking water, sanitation, and hygiene in non-household settings: Priorities for policy and practice.* Int J Hyg Environ Health, 2015. **218**(8): p. 694-703.

53. WHO/UNICEF, *Water, Sanitation and Hygiene in Health Care Facilities: Status in low- and middle-income countries and way forward*, in *WHO*. 2015, WHO: Geneva.
54. Benova, L., et al., *Where there is no toilet: water and sanitation environments of domestic and facility births in Tanzania.* PLoS One, 2014. **9**(9): p. e106738.
55. Bain, R., et al., *Fecal contamination of drinking-water in low- and middle-income countries: a systematic review and meta-analysis.* PLoS Med, 2014. **11**(5): p. e1001644.
56. WHO, *Essential environmental health standards in health care*. 2008, WHO.
57. WHO/UNICEF, *Water, Sanitation and Hygiene (WASH) in Health Care Facilities Global Action Plan*. 2016.
58. Hughes, R.G., *Patient Safety and Quality: An Evidence-Based Handbook for Nurses*. 2008, Rockville, MD: U.S. Department of Health and Human Services.
59. Ferranti, G., et al., *Aetiology, source and prevention of waterborne healthcare-associated infections: a review.* J Med Microbiol, 2014. **63**(Pt 10): p. 1247-59.
60. WHO/UNICEF/USAID, *Improving nutrition outcomes with better water, sanitation and hygiene: practical solutions for policy and programmes*. 2015: WHO. 58.
61. Cairncross, S., et al., *Water, sanitation and hygiene for the prevention of diarrhoea.* Int J Epidemiol, 2010. **39 Suppl 1**: p. i193-205.
62. Freeman, M.C., et al., *Integration of water, sanitation, and hygiene for the prevention and control of neglected tropical diseases: a rationale for inter-sectoral collaboration.* PLoS Negl Trop Dis, 2013. **7**(9): p. e2439.
63. Anaissie, E.J., S.R. Penzak, and M.C. Dignani, *The hospital water supply as a source of nosocomial infections: a plea for action.* Arch Intern Med, 2002. **162**(13): p. 1483-92.
64. Williams, M.M., C.R. Armbruster, and M.J. Arduino, *Plumbing of hospital premises is a reservoir for opportunistically pathogenic microorganisms: a review.* Biofouling, 2013. **29**(2): p. 147-62.
65. Moffet, H.L. and T. Williams, *Bacteria recovered from distilled water and inhalation therapy equipment.* Am J Dis Child, 1967. **114**(1): p. 7-12.
66. Cervia, J.S., G.A. Ortolano, and F.P. Canonica, *Hospital tap water as a source of stenotrophomonas maltophilia infection.* Clin Infect Dis, 2008. **46**(9): p. 1485-7.
67. Randrianirina, F., et al., *Role of contaminated aspiration tubes in nosocomial outbreak of Klebsiella pneumoniae producing SHV-2 and CTX-M-15 extended-spectrum beta-lactamases.* J Hosp Infect, 2009. **72**(1): p. 23-9.
68. Buttery, J.P., et al., *Multiresistant Pseudomonas aeruginosa outbreak in a pediatric oncology ward related to bath toys.* Pediatr Infect Dis J, 1998. **17**(6): p. 509-13.
69. Wagner, E.G. and J.N. Lanoix, *Excreta disposal for rural areas and small communities.* Monogr Ser World Health Organ, 1958. **39**: p. 1-182.
70. Mara, D., et al., *Sanitation and health.* PLoS Med, 2010. **7**(11): p. e1000363.
71. WHO/UNICEF, *Poor sanitation threatens public health*, in *WHO Media centre*. 2008: WHO.
72. WHO, *WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Satety Challenge Clean Care is Safer Care*. 2009, Geneva: WHO.

73. Casewell, M. and I. Phillips, *Hands as route of transmission for Klebsiella species.* Br Med J, 1977. **2**(6098): p. 1315-7.
74. Ataman, A.D., E.E. Vatanoglu-Lutz, and G. Yildirim, *Medicine in stamps-Ignaz Semmelweis and Puerperal Fever.* J Turk Ger Gynecol Assoc, 2013. **14**(1): p. 35-9.
75. Zerr, D.M., et al., *Decreasing hospital-associated rotavirus infection: a multidisciplinary hand hygiene campaign in a children's hospital.* Pediatr Infect Dis J, 2005. **24**(5): p. 397-403.
76. Sickbert-Bennett, E.E., et al., *Reduction of Healthcare-Associated Infections by Exceeding High Compliance with Hand Hygiene Practices.* Emerg Infect Dis, 2016. **22**(9): p. 1628-30.
77. Cairncross, S., et al., *The public and domestic domains in the transmission of disease.* Trop Med Int Health, 1996. **1**(1): p. 27-34.
78. Boyce, J.M., *Environmental contamination makes an important contribution to hospital infection.* J Hosp Infect, 2007. **65 Suppl 2**: p. 50-4.
79. Bures, S., et al., *Computer keyboards and faucet handles as reservoirs of nosocomial pathogens in the intensive care unit.* Am J Infect Control, 2000. **28**(6): p. 465-71.
80. Dancer, S.J., *How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals.* J Hosp Infect, 2004. **56**(1): p. 10-5.
81. Griffith, C.J., et al., *An evaluation of hospital cleaning regimes and standards.* J Hosp Infect, 2000. **45**(1): p. 19-28.
82. Lewis, T., et al., *A modified ATP benchmark for evaluating the cleaning of some hospital environmental surfaces.* J Hosp Infect, 2008. **69**(2): p. 156-63.
83. Boyce, J.M., et al., *Monitoring the effectiveness of hospital cleaning practices by use of an adenosine triphosphate bioluminescence assay.* Infect Control Hosp Epidemiol, 2009. **30**(7): p. 678-84.
84. Huang, Y.S., et al., *Comparing visual inspection, aerobic colony counts, and adenosine triphosphate bioluminescence assay for evaluating surface cleanliness at a medical center.* Am J Infect Control, 2015. **43**(8): p. 882-6.
85. Layton, M.C., et al., *An outbreak of mupirocin-resistant Staphylococcus aureus on a dermatology ward associated with an environmental reservoir.* Infect Control Hosp Epidemiol, 1993. **14**(7): p. 369-75.
86. Boyce, J.M., et al., *Environmental contamination due to methicillin-resistant Staphylococcus aureus: possible infection control implications.* Infect Control Hosp Epidemiol, 1997. **18**(9): p. 622-7.
87. Barg, N.L., *Environmental contamination with Staphylococcus aureus and outbreaks: the cause or the effect?* Infect Control Hosp Epidemiol, 1993. **14**(7): p. 367-8.
88. Chen, K.H., L.R. Chen, and Y.K. Wang, *Contamination of medical charts: an important source of potential infection in hospitals.* PLoS One, 2014. **9**(2): p. e78512.
89. Hota, B., *Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection?* Clin Infect Dis, 2004. **39**(8): p. 1182-9.

90. Russotto, V., et al., *Bacterial contamination of inanimate surfaces and equipment in the intensive care unit.* J Intensive Care, 2015. **3**: p. 54.
91. Hauri, A.M., G.L. Armstrong, and Y.J. Hutin, *The global burden of disease attributable to contaminated injections given in health care settings.* Int J STD AIDS, 2004. **15**(1): p. 7-16.
92. Whittington, A.M., et al., *Bacterial contamination of stethoscopes on the intensive care unit.* Anaesthesia, 2009. **64**(6): p. 620-4.
93. Frazee, B.W., et al., *Emergency department ultrasonographic probe contamination and experimental model of probe disinfection.* Ann Emerg Med, 2011. **58**(1): p. 56-63.
94. Muradali, D., et al., *Can ultrasound probes and coupling gel be a source of nosocomial infection in patients undergoing sonography? An in vivo and in vitro study.* AJR Am J Roentgenol, 1995. **164**(6): p. 1521-4.
95. Capelletti, R.V. and A.M. Moraes, *Waterborne microorganisms and biofilms related to hospital infections: strategies for prevention and control in healthcare facilities.* J Water Health, 2016. **14**(1): p. 52-67.
96. WHO, *Guidelines for drinking-water quality*. 2011, World Health Organization: Geneva, Switzerland.
97. Stauber, C., et al., *Evaluation of the compartment bag test for the detection of Escherichia coli in water.* J Microbiol Methods, 2014. **99**: p. 66-70.
98. Bain, R., et al., *A summary catalogue of microbial drinking water tests for low and medium resource settings.* Int J Environ Res Public Health, 2012. **9**(5): p. 1609-25.
99. Bhalchandra, R., et al., *Role of water quality assessments in hospital infection control: experience from a new oncology center in eastern India.* Indian J Pathol Microbiol, 2014. **57**(3): p. 435-8.
100. Huttinger, A., et al., *Evaluation of Membrane Ultrafiltration and Residual Chlorination as a Decentralized Water Treatment Strategy for Ten Rural Healthcare Facilities in Rwanda.* Int J Environ Res Public Health, 2015. **12**(10): p. 13602-23.
101. Tammelin, A., et al., *Nasal and hand carriage of Staphylococcus aureus in staff at a Department for Thoracic and Cardiovascular Surgery: endogenous or exogenous source?* Infect Control Hosp Epidemiol, 2003. **24**(9): p. 686-9.
102. Horn, W.A., et al., *Microbial flora on the hands of health care personnel: differences in composition and antibacterial resistance.* Infect Control Hosp Epidemiol, 1988. **9**(5): p. 189-93.
103. Bauer, T.M., et al., *An epidemiological study assessing the relative importance of airborne and direct contact transmission of microorganisms in a medical intensive care unit.* J Hosp Infect, 1990. **15**(4): p. 301-9.
104. Weber, S., et al., *An outbreak of Staphylococcus aureus in a pediatric cardiothoracic surgery unit.* Infect Control Hosp Epidemiol, 2002. **23**(2): p. 77-81.
105. Haley, R.W., et al., *The efficacy of infection surveillance and control programs in preventing nosocomial infections in US hospitals.* Am J Epidemiol, 1985. **121**(2): p. 182-205.
106. Ebbing Lautenbach, K.F.W., Preeti N. Malani, *Practical Healthcare Epidemiology* third ed. 2010: University of Chicago Press Journals. 484.

107. Chalmers, C. and M. Straub, *Standard principles for preventing and controlling infection.* Nurs Stand, 2006. **20**(23): p. 57-65; quiz 66.
108. Zafar, A.B., et al., *Effectiveness of infection control program in controlling nosocomial Clostridium difficile.* Am J Infect Control, 1998. **26**(6): p. 588-93.
109. Huang, S.S., R. Datta, and R. Platt, *Risk of acquiring antibiotic-resistant bacteria from prior room occupants.* Arch Intern Med, 2006. **166**(18): p. 1945-51.
110. Jarvis, W.R., *Infection control and changing health-care delivery systems.* Emerg Infect Dis, 2001. **7**(2): p. 170-3.
111. Hugonnet, S., et al., *Nursing resources: a major determinant of nosocomial infection?* Curr Opin Infect Dis, 2004. **17**(4): p. 329-33.
112. Ith, P., et al., *Practices of skilled birth attendants during labour, birth and the immediate postpartum period in Cambodia.* Midwifery, 2013. **29**(4): p. 300-7.
113. Cambodia, R.G.o., *Annual progress report: achieving the Millennium Development Goals*. 2013, Ministry of Planning: Phnom Penh, Cambodia.
114. MoH, M.a.I.I., *Cambodia Demographic and Health Survey 2014*. 2015, National Institute of Statistics: Phnom Penh.
115. Hancart-Petitet, P., et al., *Social and cultural dimensions of hygiene in Cambodian health care facilities.* BMC Public Health, 2011. **11**: p. 83.
116. Hearn, P., et al., *Prospective surveillance of healthcare associated infections in a Cambodian pediatric hospital.* Antimicrob Resist Infect Control, 2017. **6**: p. 16.
117. P.A. Khun, S.S., K. Emary, C. Moore, S. Soeng, C. Ngoun, V. Kumar, N. Day, C. Parry, N. Stoesser, *Surveillance of healthcare-associated infection at Angkor Hospital for Children, Siem Reap, Cambodia.* International Journal of Infectious Diseases, 2012. **16**(1): p. e375.
118. Srun, S., et al., *Surveillance of post-caesarean surgical site infections in a hospital with limited resources, Cambodia.* J Infect Dev Ctries, 2013. **7**(8): p. 579-85.
119. Erasmus, V., et al., *Systematic review of studies on compliance with hand hygiene guidelines in hospital care.* Infect Control Hosp Epidemiol, 2010. **31**(3): p. 283-94.
120. Salmon, S., et al., *Health care workers' hand contamination levels and antibacterial efficacy of different hand hygiene methods used in a Vietnamese hospital.* Am J Infect Control, 2014. **42**(2): p. 178-81.
121. Nogueras, M., et al., *Importance of hand germ contamination in health-care workers as possible carriers of nosocomial infections.* Rev Inst Med Trop Sao Paulo, 2001. **43**(3): p. 149-52.
122. Larson, E.L., et al., *Assessment of two hand hygiene regimens for intensive care unit personnel.* Crit Care Med, 2001. **29**(5): p. 944-51.
123. Monistrol, O., et al., *Hand contamination during routine care in medical wards: the role of hand hygiene compliance.* J Med Microbiol, 2013. **62**(Pt 4): p. 623-9.
124. Snehlata Singh, A.K.S., *Prevalence of bacteria contaminating the hands of healthcare workers during routine patient care: A hospital-based study.* Journal of the Academy of Clinical Microbiologists 2016. **18**(1): p. 60-62.
125. Rhee, V., et al., *Maternal and birth attendant hand washing and neonatal mortality in southern Nepal.* Arch Pediatr Adolesc Med, 2008. **162**(7): p. 603-8.
126. Saboori, S., et al., *Impact of regular soap provision to primary schools on hand washing and E. coli hand contamination among pupils in Nyanza Province, Kenya: a cluster-randomized trial.* Am J Trop Med Hyg, 2013. **89**(4): p. 698-708.

TABLES AND FIGURES

Table 1. *Characterization and WASH conditions of both hospitals.*

	Hospital A	**Hospital B**
Number of inpatient beds	90	15
Number of clinical staff on a typical day	~120	~32
Number of deliveries per month	~900	~60
Number of cesarean sections per month	~200	~20
WATER		
Primary water source	Utility water*	Utility water*
Primary water source treated with chlorine	Yes	Yes
Healthcare facility chlorinates water onsite	Yes	Yes
Access to water	Sinks or piped taps	Piped taps or buckets
Primary drinking water source	Bottled water	Bottled water
SANITATION		
Type of toilets	Flush toilets	Pour squat toilets
Frequency of toilet cleaning	At least once per day	At least once per day
Toilet cleaning procedure	Wet mopping with water and disinfectant or detergent	Wet mopping with water and disinfectant or detergent
HYGIENE		
Handwashing station near toilets	Yes, alcohol-based hand rub or soap are provided	No
Number of sinks	24	6
Number of functional sinks	24	5
Number of functional sinks with soap**	3	1
Number of sinks in patient care areas	19	2
Hand rub in patient care areas	Yes	No
Written guidelines on handwashing	Yes	Yes

**The Tonle Sap River is the water supply for the municipal utility that receives full conventional water treatment (flocculation, coagulation, sedimentation, filtration and chlorination).*
***Sinks with soap were only available in delivery rooms.*

Table 2. *Samples obtained from three major high-touch surfaces* in two hospitals that were positive for each target microorganism (N=74).*

Unit of CFU/swab	Handles (n=30)	Bedside rails (n=15)	Bed covers (n=29)
Median *E. coli* (range)			
Hospital A	9.5 (4-26.5)	14 (8-18.5)	6 (1-41.5)
Hospital B	4.5 (1-8)	25.5 (22.5-39.5)	2.5 (1-6.5)
Median Total coliforms (range)			
Hospital A	13 (2-74)	>200 (0.5->200)	4 (0.5->200)
Hospital B	7.3 (2-13)	36 (1-116.5)	8 (1->200)
Median *S. aureus* (range)			
Hospital A	4 (2-4)	2 (2-4)	2 (1.5-6)
Hospital B	0.5 (0.5-1)	12.5 (3-22)	2 (1->200)

**Handles included door handles and faucet handles. Bed covers included surfaces of delivery beds and surfaces of patient bed covers.*

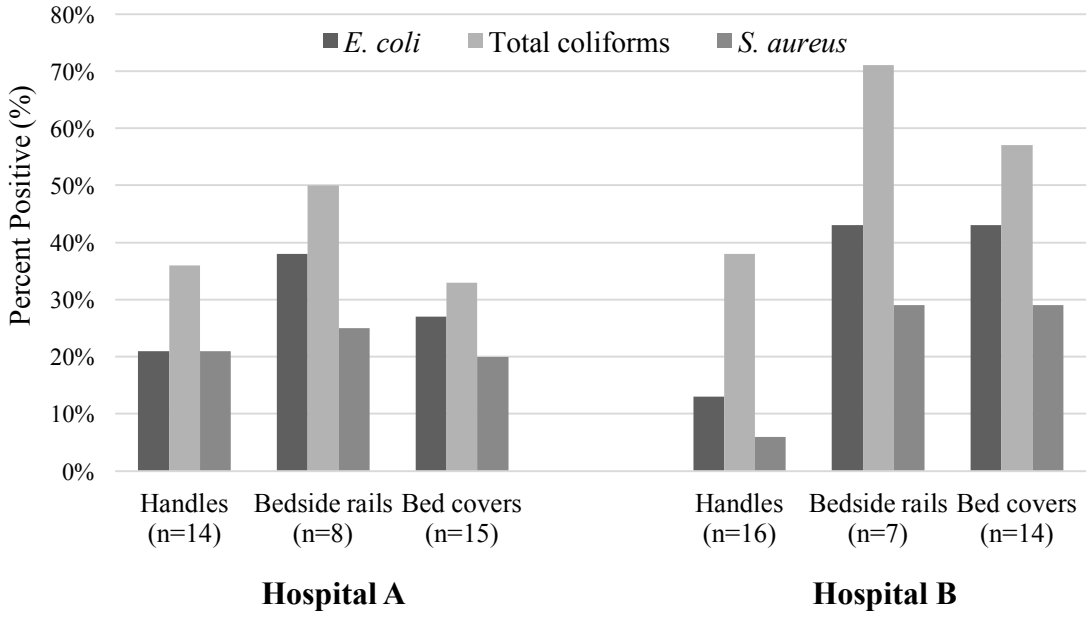
Table 3. *Quality of water samples collected from tap at two hospitals (N=58).*

	Hospital A n (%)	Hospital B n (%)
Number of Samples	29	29
***E. coli* (CFU/100 mL)**		
< 1	29 (100%)	24 (83%)
1-10	0 (0%)	5 (17%)
Total coliforms (CFU/100 mL)		
< 1	28 (97%)	15 (52%)
1-10	1 (3%)	14 (48%)
Number of Samples	8	11
Free chlorine residual (mg/L)		
Mean	0.5	0
Median	0.5	0
Range	0.2-0.8	0-0.1
Total chlorine residual (mg/L)		
Mean	0.6	0.1
Median	0.5	0.1
Range	0.4-0.8	0-0.2

Table 4. *Proportions and odds ratios of healthcare workers' hand rinse samples (N=66) with target microorganisms. 95% CI = 95% confidence interval.*

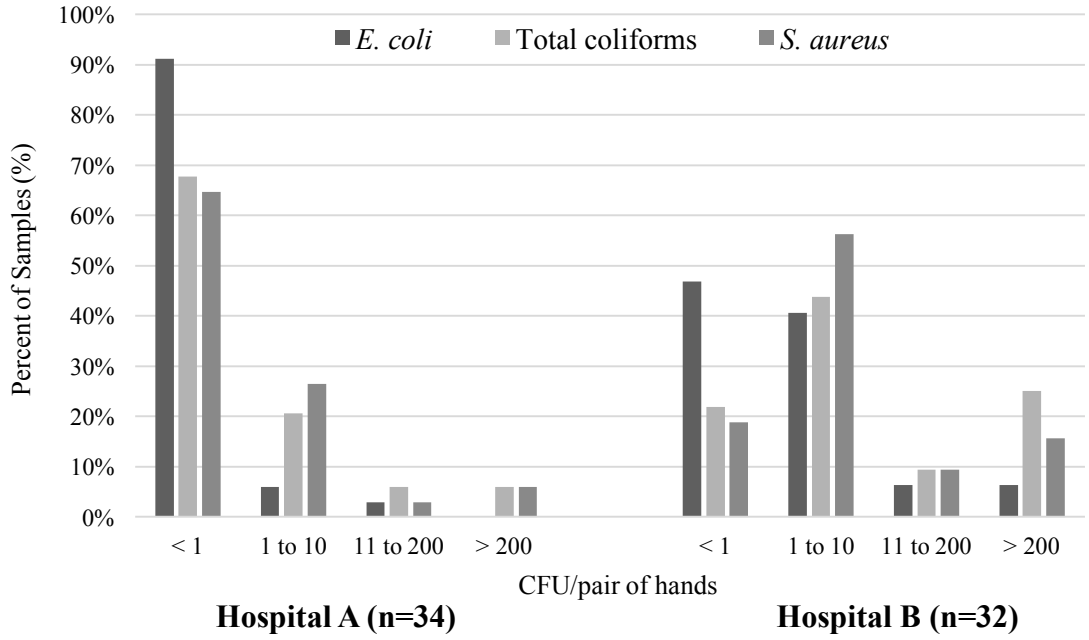
	Presence n (%)	**Odds Ratio (95% CI)**
E. coli **(CFU/pair of hands)**		
Hospital A (n=34)	6 (18%)	Reference
Hospital B (n=32)	18 (56%)	6.00 [1.95, 18.48]
Total coliforms (CFU/pair of hands)		
Hospital A (n=34)	13 (38%)	Reference
Hospital B (n=32)	27 (84%)	8.72 [2.68, 28.35]
S. aureus **(CFU/pair of hands)**		
Hospital A (n=34)	14 (41%)	Reference
Hospital B (n=32)	30 (94%)	21.43 [4.39, 104.60]

Figure 1. *Frequency of detection of target microorganisms on three major surfaces* in the two hospitals (N=74).*



**Handles included door handles and faucet handles. Bed covers included surfaces of delivery beds and surfaces of patient bed covers.*

Figure 2. *Distribution of target microorganisms on the hands of healthcare workers in the two hospitals (N=66).*



APPENDICES

Appendix A. WASH Conditions Assessment Tool

SECTION 1 – Interview with the Director		
1	**Which of the following services or departments are available at this healthcare facility?** Read all options aloud. Check all that apply.	☐ Adult Inpatient Care ☐ Adult Outpatient Care ☐ Antenatal Care ☐ Dentistry ☐ Emergency Department ☐ Environmental Services ☐ Eye Clinic ☐ HIV/VCT/ARV Clinic ☐ Housing for Staff ☐ Intensive Care Unit ☐ Kitchen ☐ Labor and Delivery ☐ Laboratory ☐ Major surgery ☐ Morgue ☐ Minor surgery ☐ Nutrition Services ☐ Pediatric Inpatient Care ☐ Pediatric Outpatient Care ☐ Pharmacy ☐ TB Services ☐ Other: ☐ None of the above ☐ Don't know
ELECTRICITY: Now I am going to ask you some questions about electricity.		
2	**What sources of electricity are available at the healthcare facility?** Read all options aloud. Check all that apply. If "No power source" is selected, skip to Q5.	☐ Utility power ☐ Solar power ☐ Generator ☐ No power source ☐ Other: ☐ Don't know

3	**If there is more than one source of electricity, which is the main source used by the healthcare facility?**	☐ Utility power ☐ Solar power ☐ Generator ☐ Other: ☐ Don't know
4	**How many days last month was the electricity from the main source interrupted for more than 2 hours at a time?** Read all options aloud.	☐ Everyday ☐ Most days but not every day ☐ Several times ☐ Once ☐ Never ☐ Don't know
WATER SUPPLY: Now I am going to ask you questions about water supply.		
5	**Please tell me which of the following sources of water are available to the healthcare facility:** (Read all options aloud. Check all that apply.)	☐ Piped supply from outside the facility ☐ Tube well ☐ Borehole ☐ Protected dug well ☐ Rain Water ☐ Unprotected dug well ☐ Surface water ☐ Tanker truck ☐ Other ☐ Don't know ☐ No water source
6	**What is the main source of water?** Note: This question refers to the source of water for general purposes, including drinking, washing, and cleaning. In case of water being available at multiple points, record the response closest to the outpatient area.	☐ Piped supply from outside the facility ☐ Tube well ☐ Borehole ☐ Protected dug well ☐ Rain Water ☐ Unprotected dug well ☐ Surface water ☐ Tanker truck ☐ Other ☐ Don't know ☐ No water source
7	**Where is the main water source for the facility?**	☐ On premises ☐ Off premises, within 500m ☐ Off premises, further than 500m ☐ No water source ☐ Don't know
8	**What is the round trip travel time to collect water off premises?** (in minutes)	_____ minutes
9	**Who collects the water off premises?** Read all options aloud.	☐ Patients ☐ Staff ☐ Both patients and staff ☐ Other ☐ Don't know
10	**Are there times when [the main water source] is unavailable?** If NO, skip to Q14.	☐ Yes ☐ No ☐ Don't know

11	**If yes, why?** Read all options aloud. Check all that apply	☐ Power outage ☐ Water rationing/shortage ☐ Equipment malfunction (i.e. broken pump) ☐ Season (dry or wet) ☐ Pipe breakage ☐ Problems at the provider ☐ Other: ☐ Don't know
12	**How often is the main water supply unavailable?** Read all options aloud.	☐ For part of the day, rarely ☐ For part of the day, frequently ☐ For part of the year (seasonal problem), frequently ☐ For part of the year (seasonal problem), rarely ☐ Don't know
13	**If water is not available from the main source, is water available from an alternative source at this time? If yes, what is the source?**	☐ *Yes*, and the alternative source is *improved* (ex. Piped supply from outside the facility, Tube well, Borehole, Protected dug well, Protected spring, Rain Water) ☐ *Yes*, and the alternative source is *unimproved*. (ex. Bottled water, Unprotected dug well, Surface water, Tanker truck) ☐ No alternative source available ☐ Have alternative source but is unavailable ☐ Don't know
14	**Does the healthcare facility ever ration water?** (i.e. is water use intentionally limited or used sparingly) If NO, skip to Q16	☐ Yes ☐ No ☐ Don't know
15	**If yes, why?** Select all that apply.	☐ Cost of water ☐ Concerned water will run out ☐ Other: ☐ Don't know
16a	**Which users have access to water?** Select all that apply.	☐ Patients/caregivers ☐ Staff ☐ Community members ☐ Don't know
16	**Is water accessible to all users at all times?** (i.e. water can be accessed any time of day by anyone at the HCF) Note: this questions has to do with equity of access, not with water outages	☐ Yes ☐ No, patients/caregivers do not have access at all times ☐ No, staff do not have access at all times ☐ No, both do not have access at all times ☐ Don't know

17	**Are there tastes, odors or colors that discourage consumption or use of the drinking-water?**	☐ Yes ☐ Sometimes ☐ No ☐ Don't know
18	**How is water accessed within the healthcare facility?** Read all options. Select all that apply.	☐ Sinks or piped taps ☐ Uncovered buckets/barrels ☐ Covered buckets/barrels ☐ Covered buckets with taps on bottom ☐ Uncovered buckets with taps on bottom ☐ Other ☐ Don't know
19	**If buckets/barrels selected, how is water removed from buckets/barrels for use on the wards?** Read all options. Select all that apply.	☐ Cup/Ladle ☐ Tap ☐ Pour ☐ Other ☐ Don't know
20	**Does this healthcare facility expect that pregnant women will bring their own water when they come to deliver?**	☐ Yes ☐ Sometimes ☐ No ☐ Don't know
WATER TREATMENT: Now I am going to ask you some questions about water treatment.		
21	**Is water from the main water source chlorinated (treated with chlorine)?** Read all options aloud. If NO, skip to Q23	☐ Yes ☐ No ☐ Don't know
22	**If yes, does the healthcare facility chlorinate the water?** (as opposed to the water being chlorinated by the water utility)	☐ Yes ☐ No ☐ Don't know
23	**Does the healthcare facility purchase or produce drinking-quality water for patients?** Note: this includes bottled water If NO, skip to Q26	☐ Yes ☐ No ☐ Don't know
24	**If yes, how does the healthcare facility provide treated drinking water?** Read all options, select all that apply.	☐ Chlorination of drinking water onsite ☐ Filtration of drinking water onsite ☐ Boiling of drinking water onsite ☐ UV treatment of drinking water onsite ☐ Bottled (or sachet) water available for purchase ☐ Water is treated before reaching the healthcare facility (i.e. by a utility treatment plant) ☐ Other:

		☐ Don't know
I'm now going to ask you questions about water treatment for various medical purposes. *Note: Only those services which you selected in Q1 will show up here*.		
25	**How is water treated for the following medical purposes?** Read all purposes aloud. Check all that apply and circle the type of treatment. Note: Select "Not Applicable" if the medical purposes does not occur at this facility	☐ Surgical procedures No Treatment, Chlorination, Filtration, Boiling, Distillation, Purchase, UV, Other, Don't know, Not Applicable ☐ Childbirth/labor and delivery No Treatment, Chlorination, Filtration, Boiling, Distillation, Purchase, UV, Other, Don't know, Not Applicable ☐ Wound and burn care No Treatment, Chlorination, Filtration, Boiling, Distillation, Purchase, UV, Other, Don't know, Not Applicable ☐ Sterilization/cleaning equipment No Treatment, Chlorination, Filtration, Boiling, Distillation, Purchase, UV, Other, Don't know, Not Applicable ☐ Use in medical devices (CPAP, incubator, etc.) No Treatment, Chlorination, Filtration, Boiling, Distillation, Purchase, UV, Other, Don't know, Not Applicable ☐ Dentistry No Treatment, Chlorination, Filtration, Boiling Distillation, Purchase, UV, Other, Don't know, Not Applicable ☐ Mixing medication No Treatment, Chlorination, Filtration, Boiling, Distillation, Purchase, UV, Other, Don't know, Not Applicable ☐ Laboratory No Treatment, Chlorination, Filtration, Boiling, Distillation, Purchase, UV, Other, Don't know, Not Applicable ☐ Other: No Treatment, Chlorination, Filtration, Boiling, Distillation, Purchase, UV, Other, Don't know, Not Applicable ☐ Don't know
HYGIENE: Now I am going to ask you some questions about hygiene.		
26	**Does the healthcare facility provide soap for handwashing for staff?**	☐ Yes ☐ Sometimes ☐ No ☐ Don't know
27	**Does the healthcare facility provide soap for handwashing for patients and caregivers?**	☐ Yes ☐ Sometimes ☐ No ☐ Don't know

28	**Are bathing facilities available to patients?**	☐ Yes ☐ No ☐ No inpatient services ☐ Don't know
29	**Are beds, mattresses, pillows and/or mats cleaned between patients?** i.e. bed rails and mattresses are cleaned, linens are laundered or changed Read all options aloud.	☐ Yes, always ☐ Yes, sometimes ☐ Bedding is not provided. Patients bring bedding from home. ☐ No, beds, mattresses and pillows are rarely or never cleaned between patients. ☐ No inpatient services at this facility
30	**Is there an infection prevention and control committee either at the health facility itself or one that the facility is a member of?** (i.e. designated staff in charge of infection prevention and control or committees tasked with monitoring or improving infection prevention and control.)	☐ Yes, and they have met within the past 6 months ☐ Yes, but they have ***not*** met within the past 6 months ☐ No ☐ Don't know
31	**Are there written guidelines pertaining to water, sanitation, and hygiene for the healthcare facility?** Note: Guidelines can be printed or digital. Select all that apply.	☐ Yes, water ☐ Yes, sanitation ☐ Yes, hygiene ☐ No
32	**What functional sterilization equipment is available at the healthcare facility today?** Read all responses. Select all that apply.	☐ Autoclave (pressure & wet heat) ☐ Dry heat sterilizer ☐ Boiler or steamer (no pressure, electric or not) ☐ Other: ☐ No functional sterilization equipment available. ☐ Don't know
SANITATION: Now I am going to ask you some questions about sanitation.		
33	**Are toilet facilities available on the healthcare facility premises?**	☐ Yes ☐ No ☐ Don't know
33a	**Are toilet facilities sufficient to meet the facility's needs and in use?**	☐ Yes, sufficient and in use ☐ Not sufficient ☐ Not in use ☐ Neither sufficient nor in use ☐ Don't know

34	**How is human waste (feces) from toilets disposed of?** Read all responses. Select all that apply.	☐ Sewerage system ☐ Septic Tank ☐ Underground holding pit ☐ No toilet ☐ Discharged into drain or immediate environment
WASTE MANAGEMENT: Now I am going to ask you some questions about waste management.		
35	**Are fenced and protected areas available for the storage of waste awaiting disposal or removal?**	☐ Yes ☐ Sometimes ☐ No ☐ Don't know
45	**Is there a functional incinerator? If yes, is fuel available for it?** Read all options aloud.	☐ Yes, and fuel is available today. ☐ Yes, but no fuel is available today. ☐ No ☐ Don't know
36	**Is infectious waste separated from other waste?** Give example such as: blood soaked clothes, body parts removed during surgery, catheters, vomit, etc. If NO, skip to Q39	☐ Yes ☐ Sometimes ☐ No ☐ This kind of waste is not generated. ☐ Don't know
37	**If yes or sometimes, how do you treat infectious waste most of the time?**	☐ Autoclave ☐ Chemical disinfection with hypochlorite (*ex: chlorine, bleach, etc.)* ☐ Other: ☐ Not treated ☐ Don't know
38	**If yes or sometimes, how do you dispose infectious waste most of the time?**	☐ Incinerate (two chamber, 850-1000 C) ☐ Incinerate (brick incinerator) ☐ Bury in a lined, *protected* pit ☐ Open burning ☐ Open dumping ☐ Collect for medical waste disposal ☐ Other: ☐ Don't know
39	**Is sharps waste separated from other waste?** Give example, such as disposable needles. If NO, skip to Q42.	☐ Yes ☐ Sometimes ☐ No ☐ This kind of waste is not generated ☐ Don't know

40	**If yes or sometimes, how do you treat sharps waste most of the time?**	☐ Autoclave ☐ Chemical disinfection with hypochlorite (*ex: chlorine, bleach, etc.)* ☐ Other: ☐ Not treated ☐ Don't know
41	**If yes or sometimes, how do you dispose of sharps waste most of the time?**	☐ Incinerate (two chamber, 850-1000 C) ☐ Incinerate (brick incinerator) ☐ Bury in a lined, *protected* pit ☐ Open burning ☐ Open dumping ☐ Collect for medical waste disposal ☐ Other: ☐ Don't know
42	**How do you dispose of non-infectious general waste most of the time?** Read each bolded category aloud and the probe for more specific location.	☐ Incinerate (two chamber, 850-100 C) ☐ Incinerate (brick incinerator) ☐ Bury in a lined, *protected,* pit ☐ Open burning ☐ Open dumping ☐ Other ☐ Don't know
43	**Are placentas separated from other waste?** If NO, survey completed.	☐ Yes ☐ Sometimes ☐ No ☐ Women bring placentas home ☐ This kind of waste is not generated ☐ Don't know
44	**If yes or sometimes, how does the heath facility dispose of placentas?**	☐ Incinerate (two chamber, 850-1000 C) ☐ Incinerate (brick incinerator) ☐ Bury in a lined, *protected* pit ☐ Open burning ☐ Open dumping ☐ Women bring placentas home ☐ Collect for medical waste disposal ☐ Other: ☐ Not applicable ☐ Don't know

SECTION 2 – ADMINISTRATIVE DATA		
1	**Does this healthcare facility have outpatient services?** If NO, skip to Q4	☐ Yes ☐ No
2	**How many outpatients were seen last month?**	
3	**How many days per month are outpatients seen?**	
4	**Does this healthcare facility have inpatient services?** If NO, skip to Q8.	☐ Yes ☐ No
5	**How many inpatients were seen last month?**	
6	**On an average day, how many inpatients are at the healthcare facility?**	
7	**How many inpatient beds are available?**	
8	**How many deliveries were there in the past month?**	
9	**Of these, how many cesarean sections were performed in the last month?**	
10	**Are surgical procedures performed at this healthcare facility?** If no, skip to Q12	☐ Yes ☐ No
11	**If yes, how many surgical procedures are performed each month?** (if unknown, ask about how many procedures are performed per day, then extrapolate to per month)	
12	**How many clinical staff are employed at the healthcare facility?** (i.e. doctors, midwives, nurses, etc.)	
13	**Of the clinical staff, how many are medical doctors?**	
14	**How many non-clinical staff are employed at the healthcare facility?** (i.e. administrative staff, janitorial staff, etc.)	
15	**On average, how much water is used daily (in liters)?** Note: information may be found on water bill or best estimate from reliable source	

Cleaning Routines: Please find a participant who is knowledgeable about cleaning routines in the healthcare facility and ask them the following questions.		
16	**How are floors in patient areas typically cleaned?** Read all options aloud.	☐ Wet mopping with water and disinfectant or detergent ☐ Wet mopping with water only ☐ Sweeping only ☐ Don't know
17	**How frequently are floors in patient areas typically cleaned?**	☐ At least once per day ☐ Less than once per day
18	**How are toilet areas typically cleaned?** Read all options aloud.	☐ Wet mopping with water and disinfectant or detergent ☐ Wet mopping with water only ☐ Sweeping only ☐ Don't know
19	**How frequently are the toilets typically cleaned?**	☐ At least once per day ☐ Less than once per day

Water Supply: Observe the main source of water.		
20	**Is water available from the main source at the time of the survey?**	☐ Yes ☐ No, but water is available from an alternative source. ☐ No, water is not available from any source.

SECTION 3 – WARD OBSERVATION CHECKLIST

1	**Which ward are you observing?**	☐ Maternity Ward/Labor & Delivery ☐ Surgery Ward ☐ Pediatric Ward ☐ Inpatient Ward ☐ Outpatient Ward ☐ Kitchen ☐ Other:
2	**Observe if the following resources/supplies used for infection control are available today in the ward:** Select all that apply. Note: This question not applicable to kitchen observation.	☐ Access to water (piped, bucket with tap, pour pitcher) ☐ Disposable latex gloves ☐ Environmental disinfectant (chlorine, ethanol, alcohol) ☐ None ☐ Didn't observe
3	**Is waste safely segregated into at least three labeled bins, including sharps waste, infectious waste and non-infectious general waste?** Note: The bins should be clearly labeled, no more than 75% full, and each bin should not contain waste other than that corresponding to their label.	☐ Yes ☐ Bins are present but do not meet all requirements ☐ No ☐ Didn't observe
4	**Is there at least one functioning handwashing station at point of care?**	☐ Yes, water and soap are present ☐ Yes, alcohol-based sanitizer is present ☐ Yes, both water and soap AND sanitizer are present ☐ No, water is present but no soap or sanitizer ☐ No, water and soap/sanitizer are not present ☐ Didn't observe
5	**Is the ward visibly clean and free from dust and soil?**	☐ Yes ☐ No ☐ Didn't observe
6	**Are there uncleaned spills from bodily fluids (blood, urine, feces, vomit, etc.)?**	☐ Yes ☐ No ☐ Didn't observe

SECTION 4 – TOILET FACILITY OBSERVATIONS	
Is the facility locked from the outside? Note: This question indicates ALL toilets on toilet block	☐ Yes – and key was produced ☐ Yes – and key was not produced ☐ No
What areas/wards does this toilet block primarily serve? (Select all services for which this toilet block is considered the primary toilet block)	☐ Outpatients ☐ Inpatients - adult ☐ Inpatients - pediatrics ☐ Labor & Delivery/Maternity ☐ Administrative Services (and other non-patient care services) ☐ Other: ☐ Didn't observe
Who uses this toilet block?	☐ Staff ☐ Patients ☐ Both ☐ Both, separated ☐ Didn't observe
What gender has access to this toilet block?	☐ Male ☐ Female ☐ Both ☐ Both, separated ☐ Didn't observe
How many toilets are locked and no key could be accessed?	
What type of toilet(s) can be found in this block? Select all that apply.	☐ Pit latrine without slab ☐ Bucket latrine ☐ Flush ☐ Pour-Flush ☐ Ventilated Improved Pit (VIP) Latrine ☐ Pit latrine with slab ☐ Other improved: ☐ Other unimproved: ☐ Didn't observe
How many usable toilets can be found in this toilet block?	
How many usable toilets have doors? (a barrier that provides privacy to the user)	☐ All ☐ Some ☐ None

	☐ Didn't observe
How many usable improved toilets are available to patients?	
How many usable improved toilets are designated for staff?	
How many non-usable toilets can be found in this toilet block?	
Is there functional lighting in the toilet block for use during the night?	☐ Yes, functional lighting ☐ No, non-functional lighting ☐ No lights ☐ No overnight use of this area ☐ Didn't observe
How many usable toilets don't have flies?	☐ All (none have flies) ☐ Some ☐ None (all have flies) ☐ Didn't observe
Is there an unpleasant smell (of urine or feces) on the block?	☐ Yes ☐ No ☐ Didn't observe
How clean is the toilet block?	☐ Clean (No presence of dirt (trash, other) OR urine/feces/blood) ☐ Presence of dirt (trash, other) OR urine/feces/blood ☐ Presence of dirt (trash, other) AND urine/feces/blood ☐ Didn't observe
Is there at least one functioning handwashing station near this toilet block? Near = within 5 meters of toilet block If no, skip to Q18	☐ Yes, water and soap are present ☐ Yes, alcohol-based sanitizer is present ☐ Yes, both water and soap AND sanitizer are present ☐ No, water is present but no soap or sanitizer ☐ No, water and soap/sanitizer are not present ☐ Didn't observe

Is there a functioning disability accessible handwashing station near this toilet block? Note: A handwashing station is considered to be accessible if it meets the following conditions: • can be accessed without stairs or steps • is not too high to be accessed in a wheelchair • easy to turn on or tip container of water	☐ Yes, water and soap are present ☐ Yes, alcohol-based sanitizer is present ☐ Yes, both water and soap AND sanitizer are present ☐ No, water is present but no soap or sanitizer ☐ No, water and soap/sanitizer are not present ☐ Didn't observe
Is there at least one usable improved toilet designated for women and girls, which provides facilities to manage menstrual hygiene needs? Note: To meet these needs, a female-only toilet must have a bin with a lid on it within the cubicle and water and soap available in a private space for washing.	☐ Yes ☐ No ☐ Didn't observe
Is there at least one usable improved toilet that meets the needs of people with reduced mobility? Note: A toilet is considered accessible if it can be accessed without steps or stairs, has handrails for support, has a door that is at least 80cm wide and has a door handle and seat are within reach of people using wheelchairs or crutches/sticks.	☐ Yes ☐ No ☐ Didn't observe

Appendix B. Environmental Sampling Protocol

Preparation

1) Bring letter of approval, identity card/ passport, and badge (if available).
2) Wear head nets, hospital gowns, gloves, and facial masks (if available).

Sample Collection

1) Label swabs and Whirl-Pak bags using a *universal labeling* scheme.
2) Sterilize gloves by spraying with 70% ethanol and rubbing hands together. Air dry gloves completely.
3) Record the name of person, name of hospital, sampling type (i.e. swabbing area, device or equipment type, hand rinses type, etc.), sample ID, plate number, volume (dilution), number and color of colonies, date and time of sampling, processing, and reading in the *data collection form*.

Surface Swabs

1) Grasp the cap of the swab and twist off the lid, and swab an area of at least 5 cm^2 (to 10 cm^2).
2) If the target surface is not flat, i.e. small object such as door handle, then swab the entire surface.
3) Start at the bottom right corner of the object and press one side of the swab firmly to the surface. Hold the swab at a slight angle to the object so that the swab surface is in maximal contact with the object but the gloves do not rub against the object (~30° angle).
4) Move the swab from right to left in horizontal lines, overlapping the upper row over the bottom row. At the midway point, turn the swab over and use the opposite side to finish swabbing the upper half of the object.
5) Insert the tip back into the tube and screw the cap on tightly.
6) Shake the tube and invert to mix for 1min.
7) Put the swab into the cooler.

Tap water

1) Open the Whirl-Pak bag and turn the tap on, without touching the mouth or inside of the bag, fill the bag carefully through the central opening to slightly above the 500mL mark. Catch the first flow of water and do not touch the mouth of tap.
2) Fold the Whirl-Pak bag carefully and twist the wire tabs together.
3) Make sure that the bag is completely closed and not leaking. Turn it upside down to check.
4) Put the Whirl-Pak bag into the cooler.

Hand rinses

1) Collect a hand rinse sample from only one person at a time. Ask for consent from the participant by reading the text below explaining purposes of the study and reasons for collecting hand rinse samples.

My name is ___ and I am a Master of Public Health student from Emory University in the United States. We are conducting a research study about environmental contaminations in maternal and neonatal wards in Cambodia. Your hand rinse samples will help us understand how much and what type of contamination people have on their hands. This would allow us to make recommendations to the government on healthcare infection prevention and control policies that will ultimately benefit the health of Cambodian citizens.

The hand rinse sample will be collected by putting both of your hands into a bag filled with sterile water, and no harm will be done to you. The process shall take about 5 minutes. Your participation is completely voluntary and no personal information, such as name, address, or other identifying information will be collected. There is no compensation but your voluntary participation would be very helpful.

2) If the participant consents to the sampling, ask he/ she to remove any devices (like a watch or wristband).
3) Open the Whirl-Pak bag and the bottle containing sterile water. Pour 500 mL of the sterile water carefully into the central opening of the Whirl-Pak bag.
4) Ask the participant to insert his/ her right hand into the bag until the water covers the hand up to the wrist.
5) Grasp the bag around the participant's wrist to secure it and gently massage the fingers and the palm of the hand from the outside of the bag for 30 seconds. Make sure sterile water does not to overflow.
6) Ask the participant to remove his/ her right hand and insert the left hand into the bag. Repeat step 5.
7) Fold the Whirl-Pak bag carefully and twist the wire tabs together.
8) Make sure that the bag is completely closed and not leaking. Turn it upside down to check.
9) Put the Whirl-Pak bag into the cooler.

Equipment rinses

1) Open the Whirl-Pak bag and the bottle containing sterile water. Pour 500 mL of the sterile water carefully into the central opening of the Whirl-Pak bag.
2) Slowly insert the equipment into the bag until it is completely submerged into the water.
3) Grasp the equipment to secure it and gently massage it from the outside of the Whirl-Pak bag for 1 minute. Make sure sterile water does not to overflow.

4) Remove the equipment. Dry the equipment with a clean paper towel before handling back to the owner.
5) Fold the Whirl-Pak bag carefully and twist the wire tabs together.
6) Make sure that the bag is completely closed and not leaking. Turn it upside down to check.
7) Put the Whirl-Pak bag into the cooler.

Sample Processing

All of the samples will be sealed and stored in a cooler and took back to the lab for processing on the same day of collection (ideally within 6hrs). Compact Dry plates, combined with membrane filtration method will be used to identify and isolate target microorganisms. Microorganisms will be counted in colony forming unit (CFU/mL). Remove the set of four trays from the foil pouch and separate each individual tray by gently bending along the connecting edge until each tray snaps free. Remove the lid of the tray using two fingers to hold down one end of the lid and the thumb to lift the opposite end.

Compact Dry swabs

1) Label the tray with appropriate information, including the sample dilution factor.
2) Pipette 1ml of sample directly to the center of the dry sheet. Once dispensed, the sample will automatically diffuse across the surface, manual spreading is discouraged.
3) Invert the plate and incubate, upside down with the medium on top, at 35 ~ 37°C for 24 hours.
4) Count colonies illuminated from the backside of the tray to calculate CFU/ml. If the colony count is high, use the 1cm*1cm molded grid on the back of the tray/ quarter reading to assist in counting.

Compact Dry plates with Membrane filtration

1) Label the tray with appropriate information, including the sample dilution factor.
2) Pipette 1 mL of sterile water directly to the center of the dry sheet.
3) Pick up a membrane filter using a sterilized forceps.
4) Remove the funnel of the sterilized filtering device and place the membrane filter.
5) Set the funnel, pour sample in the funnel, and filter the sample water by turning on the vacuum.
6) After the filtering sample, wash the inner surface of funnel with sterile water then turn off the vacuum.
7) Use a sterilized forceps to hold the edge of membrane filter, and place it gently on the Compact Dry plate while avoiding air bubbles.
8) Invert the plate and incubate, upside down with the medium on top, at 35 ~ 37°C for 24 hours.

9) Count colonies illuminated from the backside of the tray to calculate CFU/ml. On EC plates, blue colonies are *E. coli* and pink to purple colonies are other coliforms. On X-SA plates, blue colonies are *S. aureus*.
10) If the colony count is high, use the 1cm*1cm molded grid on the back of the tray/ quarter reading to assist in counting.