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Impact of Poor WASH Infrastructure on Environmental Contamination with Pathogens Known to Cause Neonatal Sepsis

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2016

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Abstract

Impact of Poor WASH Infrastructure on Environmental Contamination with Pathogens Known to Cause Neonatal Sepsis

By Kristen Carr

Poor water and sanitation-hygiene (WASH) in health care facilities (HCF) is associated with adverse neonatal outcomes, but coverage remains poor in low to middle-income countries (LMIC). Sepsis is associated with 520,000 neonatal deaths per year globally, and many cases may be acquired in HCF from hand and environmental contamination. It is hypothesized that a large portion of healthcare-associated infections can be prevented by reducing this contamination through good WASH infrastructure and practice, but little evidence exists to show the impact of WASH on HCF contamination. This study examines if 1) bacteria known to cause hospital-acquired neonatal sepsis in LMIC (*E. coli, S. aureus,* and *K. pneumoniae*) can be detected in environmental samples from maternity and neonatal intensive care wards with limited WASH, and 2) how WASH and environmental conditions change over time. A modified WASH Conditions Assessment "WASHCon" was deployed in multiple wards in two Ethiopian hospitals in the Amhara Region over 32 weeks. WASHCon collects data on hand hygiene, infection prevention and control practices, environmental cleanliness and water availability and quality. Responses were scored "Good (1)", "Moderate (.5)" or "Poor (-1)" and a composite score was created by hospital, ward and time. Assessments included environmental sample collection. Surface swabs, handrinses, drinking water, and medical device water were collected and tested for *E. coli, S. aureus,* and other coliforms and results were matched with scores by hospital, ward, and time. WASH conditions and environmental contamination varied over time. Positive swab and handrinses indicate increased exposure opportunities. A logistic regression model using WASH score, hospital, ward, month and sample type was constructed to predict bacterial contamination. Hospital, ward and sample type were significant (α=0.05). Felege Hiwot had significantly lower odds of contamination compared to Debere Tabor (OR 0.42, $p<0.001$). The Kangaroo-Mother Care ward had significantly higher odds of contamination compared to Post-natal Care (OR 2.89, $p<0.001$). Compared to handrinses, swabs did not have significantly lower odds of contamination (OR 0.76, p=0.29). The WASH score was not a significant predictor of contamination, suggesting multiple factors not measured by WASHCon may be associated with bacterial contamination of these hospital wards (OR 1.01, p=0.48).

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Table of Contents

Introduction

Brief Background on Topic:

Despite the goal to ensure universal basic water and sanitation-hygiene (WASH) for all by 2030 in Sustainable Development Goal (SDG) 6 put forth by the United Nations, little evidence exists on how WASH infrastructure and practice impacts environmental contamination in healthcare facilities (HCF), and further, if this environmental contamination is associated with a greater risk of healthcare-acquired infections, such as sepsis, in neonates receiving care in these facilities.

Achieving universal access to WASH in healthcare facilities is also key to achieving other Sustainable Development Goals to reduce maternal mortality and preventable infant deaths (1, 2).

Literature Review

The Joint Monitoring Programme (JMP) 2017 Report and the Current State of Global WASH

Inadequate water, sanitation, and hygiene (WASH) has been associated with negative health outcomes, particularly among women, children, and other vulnerable populations (3-5). Although much progress has been made in recent years, as of 2015, it was estimated that 844 million people lacked a basic drinking water service, 2.3 billion people lacked a basic sanitation service, and 892 million still practice open defecation (5). In 2019, the United Nations reported that in many countries the rate of change per year would need to be doubled to reach universal basic sanitation access by 2030, showing a large gap still left to close (6). Currently the WHO and UNICEF Joint Monitoring Programme's (JMP) 2017 Report *Progress on Drinking Water, Sanitation and Hygiene* serves as the gold-standard for monitoring the global state of WASH - However, the data are highly aggregated and there is not enough information available to determine global hygiene estimates (5).

JMP 2017 Drinking Water Service Ladder

The JMP defines basic drinking water service as: "Drinking water from an improved source, provided collection time is not more than 30 minutes for a round trip, including queuing" where an improved source is defined as "piped water, boreholes or tube wells, protected dug wells, protected springs, rainwater, and packaged or delivered water" (5).

JMP 2017 Sanitation Service Ladder

The JMP defines basic sanitation service as: "Use of improved facilities that are not shared with other Households" where "improved facilities include flush/pour flush to piped sewer systems, septic tanks or pit latrines; ventilated improved pit latrines, composting toilets or pit latrines with slabs" (5).

JMP 2017 Hygiene Ladder

The JMP defines basic hygiene service as: "Availability of a handwashing facility on premises with soap and water" where "handwashing facilities may be fixed or mobile and

include a sink with tap water, buckets with taps, tippy-taps, and jugs or basins designated for handwashing. Soap includes bar soap, liquid soap, powder detergent, and soapy water but does not include ash, soil, sand or other handwashing agents" (5).

Sustainable Development Goal 6 (SDG 6) and Core Indicators

The United Nation's Sustainable Development Goal 6 (SDG 6), to "Ensure availability and sustainable management of water and sanitation for all", lays out the framework to move towards universal safe, equitable, and sustainable management of water and sanitation by 2030 with a special focus on vulnerable populations (5, 6). SDG 6.2, achieving universal access to WASH in HCF, is declared by the United Nations as critical to the realization of human rights (7). The JMP report, *Core questions and indicators for monitoring WASH in healthcare facilities in the Sustainable Development Goals*, details how improving WASH in health care facilities (HCF) is critical to achieving SDG Goal 6, as well as other health-related SDGs of ending preventable newborn deaths and universal healthcare access (2). It also includes a description of key indicators, service ladders, and questions to use in evaluation (2, 8).

The Global State of WASH in Healthcare Facilities (HCF) and the Global Baseline Report

The definition of healthcare facility (HCF) is not standardized in the field, but in general is a place where individuals will go to seek health care such as a hospital or clinic. Inadequate WASH and environmental conditions in HCFs hamper the ability to provide quality care and services, especially during childbirth, as well as can serve as a deterrent to patients seeking medical care in facilities (2, 9). In 2015, the WHO analyzed the state of WASH in 54 LMICs, using available secondary data, and found that over a 38% of facilities did not have an improved water source within 500 meters, only 19% had improved sanitation, and 35% lacked water and soap needed for handwashing (5, 8). Cronk and Bartram, in the survey of 129,557 HCFs in LMICs from secondary data, found that 59% of HCFs lacked a dependable energy service, which jeopardizes the ability to provide clean and safe care (10). Lack of energy can, for example, cause piped water systems to lose pressure and compromise the water quality (11).

In *WASH in healthcare facilities: Global Baseline Report 2019*, 74% of countries assessed met the JMP 2017 guidelines for basic water service (without consideration of water quality or safety), 21% had no sanitation service, and 16% had no hygiene service (8). WASH service inequalities were often found to vary greatly within a country, by facility type, urban or rural location, and by facility management (8-10). In this report a service ladder was tailored for WASH in HCFs based on the 2017 JMP (5, 8). This service ladder contains definitions of different levels of water, sanitation, hygiene, as well as new ladders for waste management and environmental cleaning.

JMP WASH in HCF 2019 Water Service Ladder

The JMP defines basic water service in HCF as: "Water is available from an improved source on the premises" (8).

JMP WASH in HCF 2019 Sanitation Service Ladder

The JMP defines basic sanitation in HCF as: "Improved sanitation facilities are usable, with at least one toilet dedicated for staff, at least one sex-separated toilet with menstrual hygiene facilities, and at least one toilet accessible for people with limited mobility" (8).

JMP WASH in HCF 2019 Hygiene Service Ladder

The JMP defines basic hygiene in HCF as: "Functional hand hygiene facilities (with water and soap and/or alcohol-based hand rub) are available at points of care, and within five metres of toilets" (8).

JMP WASH in HCF 2019 Waste Management Service Ladder

The JMP defines basic waste management in HCF as: "Waste is safely segregated into at least three bins, and sharps and infectious waste are treated and disposed of safely" (8).

JMP WASH in HCF 2019 Environmental Cleaning Ladder

The JMP defines basic environmental cleaning in HCF as: "Basic protocols for cleaning are available, and staff with cleaning responsibilities have all received training" (8).

WHO Essential Environmental Health Standards in Healthcare

The WHO's report on *Essential environmental health standards in healthcare* was also used in crafting the JMP service levels shown above, and has the most comprehensive guide on developing, implementing, and monitoring environmental standards in HCF (12). It pulls together components of several WASH related reports into a succinct format fit for use in HCF globally. In addition, it contains information on staff infection prevention training, hygiene promotion, and a checklist to be used in assessing facility conditions. An overview of the standards is provided below.

Water Standards

Guidelines for water quality, quantity and management of water systems are laid out in this report. Drinking water should meet the standards described in the WHO *Guidelines for Drinking-water Quality* which includes undetectable levels of *E. coli* and coliforms at the 100 mL sample volume, residual chlorine between 0.5 and 1 mg/L, and turbidity of 1 NTU or below (12, 13). There should be an accessible drinking water point available for staff, patients and other caregivers (12). Water below the quality needed for drinking, such as for cleaning purposes, should be labeled as such (12). The water quantity needed per patient must also be taken into account, with 40-60L per day needed for inpatient services, and upwards of 100L per day for maternity units (12). This water quantity includes water used for drinking, hygiene, and sanitation purposes.

Excreta Disposal (Sanitation and Hygiene)

A sufficient number of toilets is defined in this report as "one per 20 users for inpatient settings; at least four toilets per outpatient setting (one for staff, and for patients: one for females, one for males and one for children)" (12). It is noted that the facilities need to be culturally sensitive and accessible to all groups, have a handwashing facility, and be cleaned twice per day (12).

Healthcare Waste Disposal

Waste should be separated into 4 bins (alternatively the JMP states 3), for sharps, infectious waste, non-infectious waste, and hazardous waste at the point of generation (12). Sharps can be buried in a designated pit, or shipped out to be treated while infectious waste should be steam-sterilized on site if possible (12).

Environmental Cleaning

Mopping with detergent and hot water is recommended daily, which provides the mechanical action necessary in the cleaning process to remove dirt and contaminants (12). Cleaning the room thoroughly with a disinfectant or a detergent is recommended daily, when the room is made dirty, and after each patient and intervention especially for areas with vulnerable patients such as intensive care units (ICUs) and operating suites (12). Cleaning routines/plans may be developed based on the location and services provided therein.

Training and Hygiene Promotion

Heavy emphasis is put on education for infection prevention and control practices, including hand hygiene, for healthcare workers and staff (12). This should not be a one-time learning process, but a continual process, that also includes educating patients on how to properly use the available facilities (12).

Current Limitations of Estimating WASH Conditions in Healthcare Facilities (HCF)

The ability of countries to perform WASH monitoring and surveillance activities is highly constrained, as human resources and available finances are quite limited in these low-resource and income settings (14). In the most recent *Global Analysis and Assessment of Sanitation and Drinking Water (GLAAS) Report*, conducted from a WASH infrastructure and policy perspective, it was found that most countries have national guidelines for drinking and wastewater treatment, but very few of the authorities tasked with oversight were able to fulfill their duties due to constrained financial and human resources (14). Less than 15% of countries surveyed reported that they had the finances to carry out their national WASH plans, and only 12% reported urban drinking water surveillance was

done at the required frequency (14). This highlights the importance that policies supporting WASH, stakeholder engagement, and financing have on WASH conditions in HCF. For these reasons, it is likely that the limited data available on WASH in HCF is an underestimation of true conditions.

Different definitions are frequently used for what constitutes a HCF, and the WASH indicators measured vary across and within countries, making comparisons difficult (9). Data from each country, especially the least developed countries, is often of low quality and highly aggregated which may mask the true conditions present in population subsets. In addition, the true state of WASH services is not assessed holistically with most definitions for improved WASH services only reporting presence/absence at the time of assessment and not collecting information on the functionality or continuity of service (9). The largest standardized tool, the JMP, assesses WASH in this manner. This approach poses a major problem as, for example, the presence of a hand hygiene station or toilet does not mean it is functional. Therefore, the estimations obtained are likely underestimating the true burden of the lack of WASH services. Similarly, it has been noted that the presence of an improved water source does not guarantee the amount of water is adequate to serve patient needs, that it meets water quality standards (as outlined previously), or that it is always available (8, 9).

The focus for HCFs in recent years has primarily been on increasing the capacity to house more patients, due in part to increasing numbers of women choosing to give birth in facilities, but has neglected improving the conditions of the facility itself, which is a critical oversight (1). Data on environmental contamination and cleaning is even more limited, with little data available on WASH infrastructure as it relates to environmental contamination in HCF, and what data is available is not standardized (2, 5, 8, 9). Only four countries have national level data for basic environmental cleaning practices in HCF, and this is arguably the largest gap in assessing WASH standards and the impact of cleaning on environmental conditions (9). It has been noted in several studies and reports that in general there is a lack of knowledge, training and protocols related to environmental cleaning in HCF (2, 8, 15-17).

Environmental Cleaning, Conditions and Contaminants in Healthcare Facilities (HCF)

In many WASH surveillance and monitoring approaches, visible cleanliness is used as an indicator for microbiological safety, but that does not mean the surface is free of contaminants as microbes are invisible to the naked eye (18, 19). The environment in HCF is of particular concern, as ill patients may shed microbes during their stay, contaminating hospital surfaces, air and equipment (20). Pathogens such as *E.coli, Klebsiella spp.*, and *S. aureus,* amongst others, have been demonstrated to survive for months on surfaces, and the global community has not reached a consensus on the proper way to disinfect HCF surfaces (21). Environmental surfaces are those fixed in the patient's environment, and equipment used, such as bed rails, floors, and ultrasound machines (22). In *Best Practices for Environmental Cleaning in Healthcare Facilities in Resource-Limited Settings* the Centers for Disease Control and Prevention (CDC) recommends that cleaning procedures should be risk-based by the area of the hospital, taking into account the type of surface, probability of contamination, risk of exposure (high-touch surfaces pose greater risk), and vulnerability of the patients being served (23). The especially vulnerable include those in the ICU and labor and delivery wards. Recommendations for the ICU include cleaning high-touch surfaces twice a day with detergent, weekly for low-touch surfaces, and terminal cleaning after each patient leaves (23). For labor and delivery wards, it is recommended that high-touch surfaces, floors, and any visibly dirty surface be cleaned after each procedure and at least daily. Terminal cleaning of labor and delivery wards should include cleaning the handwashing stations and using detergents to disinfect the floors (23). Drawing on *Water and Sanitation for Health Facility Improvement (WASHFIT)* indicators, this report identifies necessary WASH elements for a successful environmental cleaning program. These elements include a functioning improved water supply on the premises, a constantly available and sufficient water supply for all uses, functioning stations for hand hygiene at points of care, and greywater drainage systems (23, 24).

Even with proper cleaning, it has been demonstrated that viable pathogens can remain on surfaces and can also persist in biofilms- a highly resistant matrix of polymers and microorganisms-

serving as a potential source of subsequent infection (25, 26). Biofilms in water distribution systems have been noted to contain organisms such as *Pseudomonas aeruginosa*, that contaminate the water and pose a threat to immunocompromised populations (11, 12, 27, 28). Vickery et al. found that after terminally cleaning an ICU with conventional methods, surfaces and equipment remained positive for methicillin-resistant *Staphylococcus aureus* (25). Overall, evidence is mounting that improving environmental cleaning practice provides better prevention and control of outbreaks and has been shown to reduce transmission of healthcare-associated infections (HCAI) in HCF, but the transmission dynamics are not entirely understood (8, 15, 20, 26, 29, 30). However, Dancer et al. showed in a matched study that adding one additional environmental cleaner per ward reduced environmental contamination by 32.5% and was associated with a 26.6% reduction in new *S. aureus* cases (31).

Healthcare-Associated Infections (HCAI) and Transmission Dynamics

The environmental conditions, as well as the specific medical procedures provided, can pose a hazard to patients in HCF. Patients may experience HCAI as a result of contact and subsequent infection with a pathogen encountered during the course of the hospital stay (32). These infectious pathogens can be transferred via healthcare workers' hands, patient contact with the hospital environment, equipment, water, or even from the patient's own flora (19, 33, 34). The link between environmental contamination and HCAI is well-established (19, 23, 29, 35, 36). In addition, HCF are known reservoirs for antimicrobial resistant bacteria, and may even play a part in driving the development of antimicrobial resistance (16, 37-39). Between 5-30% of patients are estimated to contract an HCAI during their stay in a hospital, but data is limited and often unreliable due to little to no HCAI surveillance in LMIC (12, 32). The burden of HCAI is also known to disproportionately affect LMICs (16, 33, 34, 40). For example, Zaidi et al reported that resource-limited countries had up to 20 times higher neonatal infection rates compared to industrialized countries (16). Patients in ICUs are at additional risk, due to longer stays in the hospital, lower immunity, invasive procedures, and other potential comorbidities (32, 34). In their systematic review, Allegranzi et al. found that in neonatal ICUs the incidence of HCAI was 15.2-60 per 1000 patient days (34). Even less is known

about community-acquired HCAI, especially among mothers giving birth at home, or outside of HCF, which is common practice in many LMIC (41).

"High touch" sites, such as bedrails, doorknobs and light switches are known to be especially problematic for HCAI transmission as healthcare workers or patients may pick up microbes from these surfaces and transmit them easily to others (20, 23, 30, 36). It has also been demonstrated that proper cleaning of high touch surfaces decreases the amount of environmental contamination present (42). Conversely, the strain on human resources to provide these services often means these tasks go undone or are done poorly (8, 14, 15, 19). Dancer et al. demonstrated that by simply adding an extra cleaning staff, microbial contamination at hand touch sites was decreased by 35%, and *Staphylococcus aureus* acquisition by 26%, compared to a matched ward (31). In addition to the lack of standards around environmental cleaning, the cleaners themselves are often not acknowledged for their work and are undervalued by institutions (15). The financial burden of HCAI in the long run outweighs the costs involved to improve environmental cleaning practice (32).

It has been estimated that 20-40% of HCAI are from cross-transmission from healthcare workers' hands (30, 33). *Staphylococcus aureus* has been demonstrated as one of the primary pathogens transmitted through this route and is known to be a common colonizer of the skin and mucous membranes (18, 33, 43). In *Guidelines on Hand Hygiene in Healthcare,* the WHO notes that adequate WASH in LMIC HCF cannot be reached without the component of good hand hygiene (33). It has been demonstrated that improved hand hygiene can reduce the incidence of HCAI and decrease neonatal mortality (12, 19, 44-46). The availability of materials needed for hand hygiene, such as soap and sanitizer, has also been associated with greater hand hygiene compliance (47).

Infection Prevention and Control (IPC) Programs

With the known association between environmental contamination and HCAI, environmental cleaning is a critical component of any IPC program (23, 39). In the WHO's *Guidelines on Core Components of Infection Prevention and Control Programs at the National and Acute Healthcare*

Facility Level, it is recommended that each facility should have a team trained on IPC working to prevent HCAI and antimicrobial resistance (AMR) (39). One IPC specialist per 250 hospital beds is recommended, along with laboratory support, adequate training, facility-level surveillance, and monitoring of adherence to guidelines (39). Patient occupancy should not be exceeded, and the facility should not be understaffed, as overcrowded conditions and insufficient staffing contribute to increased risk for infection (32, 33, 39).

Environmental Sampling and Monitoring in Healthcare Facilities (HCF)

Environmental sampling may be performed in HCF for surveillance and monitoring purposes, as well as to determine the source of an outbreak or infection. In a systematic review Rawlinson et al. described the methods currently used in the field and highlighted the techniques most suited for certain surfaces and pathogens. Surfaces can be sampled indirectly, such as with a swab, sponge or wipe, and then the microorganisms on the sampling device can be extracted or eluted into a sterile saline solution or transport media (17). Microorganisms can also be captured directly by pressing a contact plate or petri film against a surface (17). Contact plates are preferred when the suspected contamination level is low, whereas swabs are better for surfaces with high levels of contamination because dilutions of the saline can be tested in order to detect quantifiable numbers of bacteria on the growth media (48). Molecular methods, such as polymerase chain reaction (PCR), can be used for sample analysis to gain a bigger picture of the microbial diversity, link environmental and patient strains, as well as detect unculturable species and nucleic acid from dead or non-viable organisms (17).

The sampling method chosen depends on a variety of factors such as the type of surface, suspected level of contamination, and pathogen targeted (17). The most commonly reported method used to sample the HCF environment is swabs, which also outperform contact plates for recovery of gram-negative bacteria (49). However, the swabbing method is difficult to standardize, with different swab angles, pressure and the swab material known to influence results (17, 50, 51). The recovery of the organism also depends on many factors including the method used, transport and processing (17).

Results can be assessed by colony counts, presence absence, and antimicrobial resistance. Very little to no guidance or legislation exists on environmental sampling in HCF, in part because there is limited evidence on the most appropriate sampling methods to use in this context (17). Because there are no standardized procedures in place, results reported in the literature vary widely and make comparisons difficult.

There are few tools or guidelines to monitor and evaluate environmental cleaning in healthcare facilities. Standards exist for surgical wards, but not for other hospital wards (50, 52). Proposed indicator organisms for routine environmental sampling include *E. coli* and *S. aureus* (50, 52). A qualitative aerobic colony count (ACC) of <5 colony forming units (CFU) per cm^2 has been proposed for high touch surfaces, which if exceeded would indicate increased cleaning and a risk assessment are needed (50, 52). The methods described above can be used for monitoring and routine sampling in healthcare facilities (50). In addition PCR, amongst other molecular methods, can be used to provide a faster turnaround of results, but these methods detect both viable and non-viable pathogens and may not always be appropriate (50). The lack of standardization of these methods, as mentioned above, can pose a challenge to interpreting results (50, 52).

WASH and Maternal and Newborn Health

Achieving universal access to WASH in HCF is key to achieving the SDGs to reduce maternal mortality and preventable infant deaths (2, 45, 53-55). Basic WASH practices, such as good hand hygiene, have been shown to positively impact maternal and neonatal health, but coverage is very low in LMIC childbirth settings (1, 45, 53, 54). In the least developed countries (LDC), which represent one in five births globally, an estimated 17 million women each year give birth in facilities with inadequate WASH (8). Globally over 1 million maternal and neonatal deaths are associated with unclean births per year (56). It has been estimated that 40% of neonatal deaths in developing countries are due to infections (16, 57). An estimated 99% of neonatal deaths occur in LMIC, where comprehensive solutions that integrate WASH with maternal and newborn health are most needed (58, 59). Adverse pregnancy outcomes, including maternal mortality, have clearly been associated with

poor WASH conditions in the birthing environment since Semmelweis showed an association between decreased Puerperal Fever with birth attendant handwashing in the mid-19th century (1, 53, 55, 60). A few studies exist linking hand hygiene of birth attendants to neonatal all-cause mortality, but there is generally a lack of concrete evidence of the impact of WASH in HCFs on neonatal mortality and less on neonatal sepsis specifically (45, 46, 61). More attention has been brought to this area recently in the 2016 report *Standards for Improving Quality of Maternal and Newborn Care in Health Facilities,* where the WHO proposed standards and indicators integrating WASH into maternal and newborn health in HCF (59).

Neonatal Sepsis

Sepsis is one of the leading causes of neonatal death, with 520,000 deaths per year globally (45). A large portion of these deaths are estimated to be from HCAIs, and could potentially be reduced by improving WASH practices and environmental conditions believed to drive these HCAIs (15, 45, 57). Several studies and reviews have indicated a link between WASH conditions and lower neonatal sepsis deaths but few causal studies exist (16, 44, 45, 57, 61). It has been estimated in a systematic review that with a clean delivery, 27% of neonatal infections could be reduced (45).

Current Limitations of Estimating the Global Burden of Neonatal Sepsis

During the first month of a child's life, the mortality risk is the highest (62). Of the 2.5 million neonatal deaths in 2018, 15% are attributed to sepsis according to UNICEF's *Levels & Trends in Child Mortality* (62). However the reliability of available estimates of neonatal sepsis should be questioned, as they are open to misclassification and often not collected in a standardized manner across countries (41, 63). Sepsis is most often diagnosed by symptoms only due to the lack of lab capacity to confirm with blood cultures in many LMICs (41). This creates the possibility of misclassification because, the symptoms of sepsis overlap with other severe infections like pneumonia, and also because of the varying definitions of sepsis used by clinicians (41, 58). It is also more difficult to diagnose sepsis by symptoms alone in neonates, as the symptoms may be more subtle and differ in presentation compared to adults (64). In addition, data on important determinants, such as

birthweight and gestational age, are often not collected in facility births in LMICs, leaving unknown the burden of sepsis amongst vulnerable populations such as low birthweight (LBW) and preterm babies (58). LBW and preterm babies are known to have increased risk of contracting sepsis due to under-developed immune systems and additional invasive procedures that their conditions may necessitate (16, 65, 66). In many LMIC, more women give birth at home than in facilities, causing morbidity and mortality from sepsis to be missed, leading to an underestimation of neonatal deaths and sepsis (41, 58).

Pathogens Causing Neonatal Sepsis and Potential Environmental Acquisition

In their systematic review, Zaidi et al. showed that in the first week of life gram-negative rod shaped bacteria make up the majority of hospital-acquired neonatal sepsis (67). For early-onset neonatal sepsis in LMICs *Klebsiella pneumoniae* is associated with 25% of cases, *Escherichia coli* with 15%, and the gram-positive *Staphylococcus aureus* with 18% (67). After the first week, for late-onset sepsis the distribution of etiologically-confirmed cases was *Staphylococcus aureus* (14%), Group-B *Streptococci* (12%), *Streptococcus pneumoniae* (12%), and *Salmonella* species (13%). Late-onset cases are usually considered to be contracted from environmental transmission, and early-onset from flora encountered in the birthing canal, but it has been noted in several reviews and studies that this grouping is not likely to hold true with unhygienic birthing environments and the high levels of environmental contamination often found in LMIC (16, 41, 67). The high proportion of neonatal infections associated with gram-negative rod-shaped bacteria in LMIC hospitals, estimated from 55-61% based on the global region, and *Staphylococcus aureus,* estimated from 8-22%, indicate likely environmental acquisition (16, 67). *Klebsiella*, among other gram-negatives, is known to persist in the hospital environment on multi-use containers, and poorly cleaned equipment and can survive on surfaces for months in biofilms (16, 21). Due to its ability to form biofilms, *Klebsiella* colonizes water distribution systems and can serve as a source of HCAI (13, 66). In an interesting case study, a South African neonatal intensive care unit (NICU) performed a genetic analysis and traced the source of a neonatal *Klebsiella* outbreak to cockroaches in their poorly-kept facility (68). *Escherichia coli,* a fecal

coliform, is commonly used as an indicator for fecal contamination in assessing microbiological water quality, hygiene, and sanitation (13, 37). Therefore, high numbers of sepsis cases associated with *E. coli* have been hypothesized to be linked to unsafe water and poor sanitation and hygiene conditions in a facility (2, 8, 13, 16, 37). *Staphylococcus aureus* is a common skin commensal bacterium, and a common environmental contaminant, and is frequently passed by cross-transmission through the hands of healthcare workers (13, 18, 33, 45, 69, 70). High levels of antimicrobial resistance have been observed in these bacterial populations, especially among *Klebsiella*, and 70% of neonatal sepsis cases in LMIC are estimated to not be treatable by the WHO empiric regimen of antibiotics (16, 32, 38).

Current Limitations in Estimating Impact of Environmental Contamination on Neonatal Sepsis

Little evidence exists to show the impact of environmental contamination in HCF on health outcomes, especially for healthcare-acquired neonatal sepsis in LMIC. The epidemiology of neonatal sepsis, and its transmission pathways, is poorly understood (63, 69). Few studies have been conducted in LMIC, and fewer specifically on neonatal health (15). The strongest area of evidence to date is hand hygiene linked to HCAI, as summarized in the WHO's *Hand Hygiene in Healthcare*, where multiple studies have linked hand hygiene with lower incidence of HCAI (19, 33, 44, 46, 71). A few studies show a link between environmental conditions and clean birthing practices in HCF with improved maternal and neonatal outcomes, but they do not address neonatal sepsis specifically (45, 53). There are several case studies of outbreaks in NICUs where environmental contamination is implicated, but due to the study design causality cannot be proven (31, 72, 73). A review by Blencowe et al., conducted on clean birth practices and neonatal mortality, concluded that there is very little good quality evidence on this topic especially in LMIC where there is poor surveillance and monitoring (18, 45). In addition, as mentioned previously, the little data that does exist is prone to misclassification and not standardized. Further research is needed to causally link WASH infrastructure and practice with environmental contamination in HCF and to link environmental contamination with neonatal sepsis.

Ethiopia in Context

Ethiopian Healthcare: Health Sector Transformation Plan (HSTP) 2015/16-2019/20

Ethiopia is a country in East Africa that is home to over 90 million people from diverse backgrounds and cultures (74). There are 150 national hospitals, over 3,000 health centres, and 16,000 health posts in the country (56). In recent years Ethiopia has made substantial process in improving WASH infrastructure, and in the past decade it met the Millennium Development Goal 7c (MDG 7c) to halve the population without access to improved drinking water (74). The country is prioritizing accessible and safe healthcare and setting ambitious goals for the future in its *Health Sector Transformation Plan* (HSTP) (74). The plan begins by detailing the context of Ethiopia, its past struggles and successes in the health sector, and future goals to bring all Ethiopians access to health services through increased investment in HCF and human resource development (74).

One of the Ethiopia's greatest successes is the launch of the *Health Extension Program* (HEP) where over 38,000 heath care workers have been deployed to bring basic health services to all Ethiopians, especially women and children (74). These workers pass along knowledge and practical skills to families to empower them to improve their health in their specific community context (74). Among the packages offered by the HEP, there is a strong WASH component where families are educated on topics such as hand hygiene and safe disposal of excreta (74). The HSTP also details a national sanitation marketing strategy, working in conjunction with the HEP's educational component, to create a demand for the development of sanitation products (74).

In Ethiopia healthcare utilization is low, and health seeking behavior is noted to differ strongly based on educational status (74). Health facilities often lack specialized services and resources for maternal and child health (74). Many facilities do not have neonatal or NICU units, posing a challenge for the country's goals to increase the number of facility births and decrease the number of neonatal deaths (74). Expansions of health centres are underway to address this gap, but financial resources are limited (74). In addition, human resources are severely constrained with far fewer health professionals than needed to equip facilities and their expanding services (74). In response, the government is investing in education and training to fill this gap (74).

WASH in Healthcare Facilities (HCF)

According to the 2017 JMP, of the 94 countries with national estimates of safely managed drinking water services, Ethiopia ranks one of the lowest for basic drinking water accessibility, availability and contamination (39% national coverage for basic water service) (5). Ethiopia is noted to vary widely in water availability, accessibility and quality especially by regional location, urban vs rural, and socio-economic status (5). In addition, it ranks poorly for sanitation (7% national coverage for basic sanitation service) and hygiene services (1% national coverage for basic hygiene service) (5). In 2017 27% of the country was noted to practice open defecation, although tremendous progress has been made in this area since the estimate of 80% reported in 2000 (5). The *Global Baseline Report* details the WASH conditions in Ethiopian HCFs with a national estimate of 30% basic water coverage, 59% basic sanitation coverage, and 64% basic waste management services (8). There are no estimates available for basic hygiene and environmental cleaning services in Ethiopian HCFs in this report. WASH coverage was noted to differ by urban vs rural, hospital vs non-hospital, and government vs non-government operated facilities. Sanitation coverage had the highest variability based on these factors (5). Urban HCFs had higher national coverage across all categories, with a 63% estimated difference in sanitation coverage when compared to rural HCFs, highlighting the gap in coverage between urban and rural areas (8). Hospitals out-performed non-hospital HCFs across all categories, with the largest difference of 76% in basic sanitation coverage (8). Non-government operated HCFs outperformed government operated HCFs in all estimated categories, with the largest difference of 50% in sanitation coverage (8). This report is based on presence/absence of infrastructure, so these coverage estimates are likely overestimates of the true conditions.

Clean and Safe Health Facilities (CASH) Initiative

The Clean and Safe Health Facilities Initiative (CASH) is a government sponsored program launched in 2014 at the national and regional level (75). The goal is to reduce HCAI, and create a safer environment for patients, through improved infection prevention and cleaning practices (56). The initial target was all hospitals, with subsequent scaling up to include all other health facilities. The

approach relies heavily on a tiered leadership system, with buy-in from the national to community level (75). A national plan, budget, auditing tools and leadership structure were developed (75). Hospitals are supported by the regional health bureaus as they work to establish cleaning campaigns, improve patient satisfaction, and engage their community (56). Local celebrities adopt and advocate for hospitals, and the private sector is involved in improving and managing WASH infrastructure and practice (56). An attitude of "Cleanliness is everybody's responsibility" was fostered among the hospital staff and healthcare workers, changing the culture from the ground up (56, 75). National minimal standards were implemented in the CASH hospitals, and in some facilities renovations and aesthetic improvements were made (75). The healthcare staff and community take great pride in the improvement of their facilities and patient satisfaction (56).

The CASH initiative has been implemented in all 150 national hospitals, and many have been trained in infection prevention and control (75). The initiative is expanding to health facilities outside of hospitals, but because these number in the thousands the financial and resource challenges are high (56, 75). Overall, the initiative has shown that engagement on multiple levels, as well as with key political and community investors, is critical to improving WASH conditions in HCF on both the national and local scale (75). Community and hospital staff buy-in was also key in bringing the issue of WASH in HCF to the forefront, and creating a culture shift towards cleanliness in the community (75).

Estimation of Neonatal Health Outcomes in Healthcare Facilities (HCF)

Sub-Saharan Africa is known to have some of the poorest WASH coverage and maternal and child health outcomes (5, 8, 62). The birth rate in Ethiopia is 4.35 per woman, and 32.75 per 1,000 people (62). In 2018, Ethiopia had a neonatal death rate of 28 per 1000 live births but the proportion of deaths attributable to neonatal sepsis is unknown on a national level (62). In a recent prospective study, Muhe et al. reported a neonatal death rate of 28.8% in 3,852 neonates admitted into NICUs in five Ethiopian hospitals (76). Furthermore they reported a prevalence of neonatal sepsis of 37%, with

26% of neonatal mortality attributed to neonatal sepsis (76). Low birthweight was noted to be inversely associated with mortality (76). However, it is not clear if the deaths attributed to sepsis were culture-confirmed, which could lead to misclassification bias. In a retrospective chart review in Felege Hiwot hospital in northwestern Ethiopia, the all cause neonatal death prevalence was 13.3% and the neonatal sepsis prevalence was 23.9% (77). In a subsequent study at the University of Gondar Hospital to identify the causative agents of neonatal sepsis, gram-positive bacteria were most commonly isolated (78). *Staphylococcus aureus*, coagulase negative *Staphylococci*, and *Klebsiella pneumoniae* were associated with 40.8%, 21.6%, and 15.8% of neonatal sepsis deaths respectively (78).

Little causal evidence exists in Sub-Saharan Africa, and Ethiopia, to link WASH conditions to health outcomes, and to the reported high rates of neonatal sepsis. Further evidence is warranted to determine the nationwide burden of neonatal sepsis in Ethiopia and to determine how WASH and environmental conditions in HCF affect the risks and etiology of neonatal sepsis.

Research Questions and Rationale:

Research Questions:

Do hospital maternity wards and neonatal intensive care units (NICUs) with limited WASH infrastructure and practices have hand contamination and environmental contamination with pathogens known to cause neonatal sepsis (E.g. *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*)?

- Which sites in the wards are most frequently contaminated?
- How do WASH conditions and environmental contamination in maternity wards and NICUs vary over time?
- How are WASH conditions and environmental contamination related?

Brief Description of the Public Health Significance of Proposed Question:

This study will determine if hospital maternity wards and NICUs are contaminated with pathogens known to cause hospital-acquired neonatal sepsis. This study will also address the knowledge gap on how WASH infrastructure and practice in healthcare facilities affects the frequency and levels of microbiological environmental contamination. This study will provide a greater understanding of the risk posed to neonates by the WASH and environmental conditions found in maternity wards and NICUs.

This is the first study to our knowledge to assess how WASH infrastructure and practice affects environmental contamination with pathogens known to cause neonatal sepsis in Ethiopia.

Manuscript

Title, Authors and Abstract

Title: Impact of Poor WASH Infrastructure on Environmental Contamination with Pathogens Known to Cause Neonatal Sepsis

By: Kristen Carr, Kun Zhao, Habib Yakubu, Lamesgin Alamineh, Mulusew Belew, Abebe Gebremariam, Gizachew Yismaw, John Cranmer, Christine L. Moe

Abstract

Poor water and sanitation-hygiene (WASH) in health care facilities (HCF) is associated with adverse neonatal outcomes, but coverage remains poor in low to middle-income countries (LMIC). Sepsis is associated with 520,000 neonatal deaths per year globally, and many cases may be acquired in HCF from hand and environmental contamination. It is hypothesized that a large portion of healthcare-associated infections can be prevented by reducing this contamination through good WASH infrastructure and practice, but little evidence exists to show the impact of WASH on HCF contamination. This study examines if 1) bacteria known to cause hospital-acquired neonatal sepsis in LMIC (*E. coli, S. aureus,* and *K. pneumoniae*) can be detected in environmental samples from maternity and neonatal intensive care wards with limited WASH, and 2) how WASH and environmental conditions change over time. A modified WASH Conditions Assessment "WASHCon" was deployed in multiple wards in two Ethiopian hospitals in the Amhara Region over 32 weeks. WASHCon collects data on hand hygiene, infection prevention and control practices, environmental cleanliness and water availability and quality. Responses were scored "Good (1)", "Moderate (.5)" or "Poor (-1)" and a composite score was created by hospital, ward and time. Assessments included environmental sample collection. Surface swabs, handrinses, drinking water, and medical device water were collected and tested for *E. coli, S. aureus,* and other coliforms and results were matched with scores by hospital, ward, and time. WASH conditions and environmental contamination varied over time. Positive swab and handrinses indicate increased exposure opportunities. A logistic regression model using WASH score, hospital, ward, month and sample type was constructed to predict bacterial contamination. Hospital, ward and sample type were significant (α=0.05). Felege Hiwot had significantly lower odds of contamination compared to Debere Tabor (OR 0.42, $p<0.001$). The Kangaroo-Mother Care ward had significantly higher odds of contamination compared to Post-natal Care (OR 2.89, $p<0.001$). Compared to handrinses, swabs did not have significantly lower odds of contamination (OR 0.76, p=0.29). The WASH score was not a significant predictor of contamination, suggesting multiple factors not measured by WASHCon may be associated with bacterial contamination of these hospital wards (OR 1.01, p=0.48).

Introduction

Good water and sanitation-hygiene (WASH) practice in the birthing environment, such as a clean delivery surface and birth attendant handwashing, have been associated with a reduction in neonatal mortality (1, 45, 46, 55). With a global upward trend of births in healthcare facilities (HCF), ensuring adequate WASH and clean birthing environments is paramount for the protection of maternal and newborn health (1). However, little evidence exists showing the impact of WASH infrastructure and practice on environmental contamination in HCF, and furthermore, the impact of this contamination on neonatal health outcomes (9, 15). Gaining a clear understanding of this relationship will provide evidence to create and improve standards for WASH infrastructure and practice in HCF, and may lead to cleaner, safer environmental conditions and better neonatal health outcomes.

Sepsis is associated with 520,000 neonatal deaths globally every year (45). A large portion are attributed to healthcare-associated infections (HCAI) (57). HCAI are developed after contact and infection with a pathogen encountered during the patient's stay in a HCF (32). These pathogens may be transferred via healthcare workers' hands, the facility environment, equipment, and water (19, 33, 34). "High touch" sites, such as bedrails and doorknobs, may be drivers of pathogen transmission, as staff and patients pick up microbes and pass them to others (20, 30). The most common causes of neonatal sepsis in low to middle-income countries (LMIC) include the gram negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*, and the gram-positive *Staphylococcus aureus* (67). Gram-negative bacteria represent a large proportion of environmental contamination, and high proportion of sepsis cases associated with gram-negative bacteria indicate likely environmental acquisition of the sepsis-causing pathogen (16, 67). These bacteria can persist on HCF surfaces for months and may serve as reservoirs for antimicrobial resistance (16, 21, 38). *Klebsiella* is carried in the human gut and is naturally found in the environment (13). It persists on surfaces and multiplies in water distribution systems, posing an extreme hazard to immunocompromised patients (11, 13, 16, 21). It can also be an indicator of fecal contamination (13). *E. coli,* a fecal coliform, is used as an indicator of fecal contamination for microbiological water quality, hygiene, and sanitation (13, 37).

Therefore, sepsis associated with *E. coli* is hypothesized to be linked to poor facility WASH (2, 8, 13, 16, 37). *S. aureus* colonizes skin and mucous membranes and is cross-transmitted by hands serving as a hand hygiene indicator (18, 33, 43).

HCAI disproportionately burden patients in low to middle-income countries (LMIC) with reported neonatal infection rates up to 20 times higher than those in industrialized countries (16, 33, 34, 40). However, WASH coverage in HCFs remains poor in LMIC and jeopardizes the ability to provide safe care (1, 2, 9, 54). In *WASH in Healthcare Facilities: Global Baseline Report 2019*, 26% HCF in LMIC had no basic water service, 21% had no sanitation service, and 16% had no basic hygiene service (5, 8). In addition, the data available on WASH in HCF in assesses for presence/absence of service and visual cleanliness, and not for functionality, continuity of service, or microbiological quality (5, 9). This approach is problematic as the presence of a toilet does not mean it is functional and a visibly clean surface may not be microbiologically clean (20). Currently there are few tools or guidelines to monitor and evaluate environmental conditions and cleaning in HCF, but proposed indicator organisms for routine environmental sampling include *E. coli* and *S. aureus* (50, 52).

Sub-Saharan Africa has poor WASH coverage and maternal child health outcomes (5, 8, 62). Little causal evidence exists in Sub-Saharan Africa, and Ethiopia, to link WASH conditions to health outcomes or to the high rates of neonatal sepsis that have been reported in several studies (61, 62, 64, 76, 77). Ethiopia has a birth rate of 4.35 births per woman and an estimated 30% basic water coverage, 59% basic sanitation coverage, and 64% basic waste management service in HCFs (8). There are currently no estimates for hygiene and environmental cleaning in Ethiopian HCFs (8, 62). However, Ethiopia has made marked improvement in WASH infrastructure in recent years and has prioritized accessible and safe healthcare and reducing neonatal mortality (74). Average mortality rates in Neonatal Intensive Care Units (NICU) were estimated at 28.8% across five Ethiopian hospitals, with 26% of neonatal mortality attributed to sepsis (76).

The Clean and Safe Health Facilities Initiative (CASH) is a government-sponsored program launched in 2014 at the national and regional level to improve WASH in HCFs (75). The goal is to reduce HCAI, such as neonatal sepsis, and create a safer environment for patients through improved infection prevention and cleaning practices (56). National minimal cleaning standards were implemented in the CASH hospitals (75). Hospitals are supported by the regional health bureaus as they work to establish cleaning campaigns, improve patient satisfaction, and engage their community (56).

Debre Tabor is a regional medium-sized hospital in rural northwestern Ethiopia that is recognized as a CASH hospital by the Ethiopian Ministry of Health. The newly built maternity and post-natal care building has around 260 births per month. Debere Tabor had 31 beds in the obstetrics ward in a recent study (79). Felege Hiwot hospital, located in the same region, is a large crowded urban referral hospital that sees around 450 births per month (80). The neonatal intensive care unit had 30 beds, 5 pediatricians and 11 nurses in a recent study (64, 77). In Felege Hiwot, the 2016 all-cause neonatal death prevalence was estimated at 13.3% and the neonatal sepsis prevalence was 23.9% (77).

Research Objectives

In this study the research goal is to determine if hospital maternity wards and neonatal intensive care units (NICU) with limited WASH infrastructure and practice have hand and environmental contamination with pathogens known to cause neonatal sepsis (E.g. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*). This study also examines which wards and sites are most frequently contaminated, and how that contamination changes over time in relation to the WASH conditions. A modified WASH conditions assessment tool “WASHCon” was developed for use in these two facilities and included environmental sample collection and analyses to determine if the observed WASH conditions could accurately predict environmental contamination with pathogens known to cause neonatal sepsis. The resulting data will be used to guide development of evidence-

based recommendations for WASH infrastructure and practices and environmental cleaning of maternity and NICU wards.

This research will also lay the groundwork for examining exposure to environmental contamination in a cohort of neonates followed in both facilities and the community for the development of neonatal sepsis. These neonates were enrolled in the study during the course of the routine WASHCon assessments and sample collection and followed for two weeks. In addition, the environmental samples collected were tested for antimicrobial susceptibility and will be analyzed in future studies. By determining the WASH and environmental conditions in the hospital wards, and how these conditions change over time, the risk posed to the neonates by the environment in the HCF can be understood and recommendations can be made to improve facility conditions and protect the health of neonates.

Methods

This study was approved by the Emory University Institutional Review Board and the ethics review at the Amhara Public Health Institute.

WASHCon Assessment

A modified version of the WASH conditions assessment tool (WASHCon) was used in this study to assess WASH conditions in healthcare facilities. The full WASHCon was developed in a previous study (81). WASHCon was developed based on the latest recommendations from the literature and the JMP indicators for WASH in HCF. The questions in the assessment tool were targeted to assess WASH conditions in healthcare facilities (81). The full WASHCon assessment tool was deployed in the Felege Hiwot and Debere Tabor hospitals in Ethiopia between October 2018 and June 2019 as a baseline assessment. The Baseline assessment included questions to gather general information about the facility, including number of inpatients and outpatients, number of clinical support staff, health services provided, and assessed baseline WASH conditions. The Baseline assessment included interviews with the director and administrators of each facility. The WASHCon Lite assessment was a subset of the Baseline assessment and was repeated every few weeks in the maternity and neonatal intensive care wards of each study hospital for a 32-week period. The shortest time between WASHCon Lite assessments was 8 days, and the longest 62 days. Felege Hiwot had 9 WASHCon Lite assessments and Debere Tabor had 12 assessments. The assessment topics included environmental cleaning and overall cleanliness, hand hygiene facilities and supplies, infection-prevention and control practices and supplies, drinking water availability and quality, and sanitation availability and cleanliness. The WASHCon assessments were deployed on a mobile device by a program associate using the CommCare mobile data collection platform (Dimagi Inc., Cambridge, Massachusetts).

The WASHCon Lite assessment and sample collection were conducted by hospital ward, and each deployment included data collection in the neonatal intensive care unit (NICU), Kangaroo

Mother Care (KMC) ward for low birthweight babies, Labor and Delivery ward, and the post-natal care (PNC) ward. These wards were selected to examine the exposure risks a newborn may experience during his/her journey through the HCF. For example, a baby may be born in the Delivery ward and then be moved into NICU for a complication, and later be transferred to the KMC ward for further development and observation.

Babies in the NICU may have increased risk for infection due to a number of reasons, including underdeveloped immune systems, life-threatening health conditions, longer hospital stays and more invasive procedures. The KMC ward is for mothers to keep their low birthweight babies (<2,000 g) in close proximity, skin to skin, with their mothers (82). Low birthweight babies are known to be at increased risk of sepsis and death (16, 58). Babies in the Post-natal Care ward are generally otherwise healthy, but still can be infected through exposure to contaminated hands, surfaces and water. The Delivery ward can pose a hazard to the neonate if the delivery surface or instruments (e.g. knife for cutting the umbilical cord) are not clean.

Sampling Methods

Structured observations were conducted in each hospital to guide sample collection. Environmental samples including handrinse, drinking water, surface swabs, and water from medical devices were collected by hospital ward to test for contamination by *Escherichia coli*, *Staphylococcus aureus*, and other coliforms. The samples were collected as close as possible to the WASHCon Lite assessments, usually on the same day or within a few days. The sample collection and contamination data were collected and managed using REDCap, a secure, web-based platform to support data capture, hosted at Emory University (REDCap, Nashville, Tennessee).

Sample Collection

Handrinse samples were collected from physicians, nurses, caregivers, midwives and mothers. Whirl-pak® bags with 100 mL sterile water were used to collect hand rinse samples,

where the individual submersed both hands, one following the other, in the bag. Drinking water samples (100 mL) were collected from the point of use in sterile containers. Water from medical devices, such as oxygen concentrators and C-pap machines, was collected as a 1 mL volume in the NICU only. Swabs were taken from surfaces identified as high-touch sites in the structured observations, as well as from other surfaces in the immediate environment of the infant such as bed sheets and medical equipment.

Sample Testing

The 100 mL handrinse and drinking water samples were tested for *E. coli* using the membrane filtration technique and a quantitative concentration (colony forming units (CFU) per pair of hands, or per 100 mL) was obtained using m-ColiBlue24® Media and filters from VWR (Hach|VWR, USEPA Method #10029) (83). In this method *E. coli* colonies appear dark blue on the media and other coliforms appear red. For membrane filtration tests ,only *E. coli* was recorded. Samples were incubated at 35˚C for 24 hours. Too numerous to count (TNTC) results were set to the maximum detectable count for this method of 200 CFU.

The handrinse samples were also tested by 1 mL volume for *E. coli, S. aureus*, and other coliforms using CompactDry™ XSA and EC plates (Hardy Diagnostics) (84, 85). Water from medical devices was tested by 1 mL volume with the compact dry plates for the same contaminants. Swab samples were also tested for the presence of *E. coli, S. aureus*, and other coliforms using the compact dry plates, but quantitative data was not obtained due to the lack of available standardization methods in the field for swab sample collection in HCF (52). Samples were incubated at 35-37°C for 24 hours. For the EC plates blue colonies were recorded as *E. coli* and pink colonies as other coliforms. For the XSA plates pink colonies were recorded as *S. aureus*. It is important to note that in this study *Klebsiella* was detected as a part of the larger group of coliforms detected on the EC plates. “Other coliforms” in this thesis refers to coliforms other than *E. coli* that were detected.

Data Cleaning and Analysis

The WASHCon Lite assessment and environmental sample data were cleaned using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina). A scoring system was developed to summarize the results of the WASHCon Lite assessment across the categories present in the assessment of hand hygiene, infection prevention and control, environmental cleanliness, drinking water availability and quality, sanitation availability and cleanliness. Questions were assigned to each category, or multiple categories when relevant. Question responses were evaluated by the research team and assigned as "Good", "Moderate", or "Poor" in accordance with recommendations in the WASH in HCF literature. "Good", "Moderate", and "Poor" responses were assigned 1, 0.5, and -1 values respectively. Each WASHCon Lite deployment was run through the scoring model in SAS to create an additive "WASH score" for the hospital ward on the date of assessment deployment. The full scoring model and WASHCon Lite assessment can be found in the appendices.

Each environmental sample was matched by hospital, ward, and date to the closest WASHCon Lite assessment and assigned the respective WASH score. If the number of days was the same between two assessments, environmental samples collected before 12pm were matched to the prior assessment. The dates of the matched WASHCon Lite assessments and environmental samples can be found in the appendices. In one instance two WASHCon assessments were deployed in Felege Hiwot on the same day. The assessment closest in time to the collected environmental samples was kept, and the other was removed from the analysis. A pilot test of the WASHCon Lite assessment in Felege Hiwot was also removed from the final database for analysis. Questions in the WASHCon Lite assessment that focused on a single ward, such as the NICU, were not included in the scoring model due to missing data in the other wards.

A variable was created to indicate if each sample was positive for any of the three target bacteria tested. Bacterial-positive samples were coded as 1 and negative as 0. This variable was compared by hospital, ward, and assessment period with the matched WASH score for analysis. This

variable was used to look for a visual association between the WASH score and the proportion of samples without bacterial contamination in the hospital ward and time. A correlation plot was produced by plotting the WASH score versus the percent of bacterial-negative samples by ward over time. Six environmental samples with missing data were removed from this analysis.

A logistic regression model was constructed in SAS 9.4 using the WASH score, hospital, ward, sample type and month to predict risk (log odds ratio) of contamination with *E. coli, S. aureus,* and/or other coliforms (where event =1). The covariates and interaction terms used in the model were chosen based on preliminary results from the WASHCon Baseline and WASHCon Lite assessments and covariates of noted importance in the literature. Results were considered significant at α=0.05. The categorical variables hospital (coded 1-2), and ward (coded 1-4), and sample type (coded 1-4) and their interactions with WASH score, were included in the full model. The continuous variable month, coded by calendar month (1-12), was also included in the model.

The full logistic regression model was run in SAS and compared with forward and backward selection models. The final model was constructed by removing insignificant

covariates. The final model was constructed by removing insignificant covariates. The WASH score predictor was not eligible for removal from the final model in this analysis.

Full Model:

$$\ln(Odds\ of\ Any\ Contamination) = \alpha + \beta_1\, WASH\ Score + \beta_2 Hospital + \beta_3\, Ward + \beta_4\, Sample\ Type + \beta_5\, Month + \beta_6\, WASH\ Score * Hospital + \beta_7 WASH\ Score * Ward + \beta_8\, WASH\ Score * Sample\ Type + \beta_9\, WASH\ Score * Month + \varepsilon$$

Tables and figures were made in Microsoft Excel Version 1808 (Microsoft Corporation, Redmond, Washington), JMP (SAS Institute Inc., Cary, North Carolina) and SAS 9.4.

Results

WASHCon Baseline Hospital Demographics and Characteristics

Both of the hospitals in the study provided health care services to large populations, including maternity wards with 300-500 births per month (Table 1). However, there were some important differences. The large urban referral hospital, Felege Hiwot, had 480 inpatient beds, and the regional and rural medium-sized hospital, Debere Tabor, had 170 inpatient beds (Table 1). Felege Hiwot provides services to approximately 25,000 outpatients and 2,333 inpatients per month, but only sees patients 20 days out of the month. In contrast, Debere Tabor is open the entire month and provides services to approximately 34,578 outpatients and 1,600 inpatients. Approximately 1,300 surgical procedures and 500 births were reported in Felege Hiwot per month, while approximately 300 surgeries and 300 births were reported to take place per month in Debere Tabor.

The workforce at Felege Hiwot Hospital includes 1,000 clinical staff, 300 of which are doctors, in addition to 350 non-clinical staff, of which 110 are cleaning staff. Debere Tabor has 250 clinical staff, of which 36 are doctors, with 165 non-clinical staff, of which 39 are cleaning staff. Both hospitals responded that they do not treat water on-site with chlorine, but that their main water source is chlorinated by the municipal water authority. Both hospitals also reported that they provide soap to clinicians and staff for handwashing, but not to patients.

Both Felege Hiwot and Debere Tabor reported that they had WASH guidelines for the facility and indicated that money is allocated in the budget for WASH infrastructure. Staff are trained on infection prevention and control on an annual basis in both facilities. Floors, surfaces, and toilets are disinfected at least daily and the number of cleaning staff was reported to be sufficient by the management of both facilities. Felege Hiwot indicated there are not enough toilets to meet the needs of the facility.

WASHCon Lite Assessment

Water

The results used to calculate the WASH score from the WASHCon Lite assessment can be seen in Table 2, where the results are displayed by hospital and ward. Piped water was available at both hospitals, but not always in each ward of the hospital. The functionality of the piped water was lowest in the PNC ward in Felege Hiwot (57%), and the Delivery ward in Debere Tabor (42%). The cause of the loss of functionality was not recorded in this study. Water availability was notably higher in Debere Tabor across all wards compared to Felege Hiwot, where water availability reached as low as 1 in 7 assessments (14%) in the Felege Hiwot PNC ward. Treated water was not observed to be available at any time period or hospital ward in Felege Hiwot but was nearly always available (83% and above) in Debere Tabor. Felege Hiwot hospital wards were observed to rarely store water (14-29%), except for the PNC ward that had stored water 4 of 7 assessments (57%), and what was stored was not treated. Debere Tabor had high rates of water storage observed (83% and above), and it was most often treated water (75% and above).

Hand Hygiene

Hand hygiene stations at points of care were observed for clinicians and staff for most of the assessments in Felege Hiwot (71% and above), except for in the PNC ward where only 2 of the 7 assessments (29%) observed hand hygiene stations. Availability of both water and soap (together) at these clinician and staff hand hygiene stations was high (86%) in the NICU, but rare or absent in the other three wards in Felege Hiwot (29% and below). Debere Tabor also had hand hygiene stations for clinicians and staff observed for all or most (67% and above) of the assessments in each ward. Water and soap availability was highest in the KMC ward (75%), less often (58%) in NICU, and rarely (25% and below) in the Delivery and PNC wards. For patients and caregivers, hand hygiene stations were observed in Felege Hiwot for most assessments (86%) in the NICU and KMC wards, and about half (57% and

43%) of the assessments in the Delivery and PNC wards. In Felege Hiwot, the availability of water and soap at the hand hygiene stations for patients and caregivers was rarely observed or absent (14% or below) across all wards. In Debere Tabor hand hygiene stations for patients and caregivers were observed for nearly all assessments in the NICU and KMC (83%) wards, and over half of the assessments in the Delivery and PNC wards (50 and 58%). None of the ward hand hygiene stations for patients and caregivers in Debere Tabor were observed to have both water and soap available for handwashing.

Hand hygiene promotion materials were visible in the NICU and Delivery wards in Felege Hiwot in 3 and 2 of the 7 assessments, respectively, but were never observed in the KMC and PNC wards. In Debere Tabor, hand hygiene promotion materials were visible in nearly all assessments (92% and above) in each ward.

Infection Prevention and Control

Gloves and disinfectant were available at most observations (71% and above) in the wards in Felege Hiwot, except for the KMC ward where gloves were available in 4 and disinfectant was available in 3, of 7 assessments, respectively. In Debere Tabor both gloves and disinfectant were nearly always available (92% or above). In the Delivery wards of Felege Hiwot and Debere Tabor, clean delivery surfaces were observed in for 71% and 41% assessments respectively. Both hospitals were observed to have a controlled access point for entry into the NICU, but at Felege Hiwot it was only enforced in 5 of 7 assessments. Neither hospital required handwashing to pass the controlled access point into the NICU. Personal Protective Equipment (PPE) was required past the control access point in 6 of 7 assessments in Felege Hiwot, and 7 of 12 assessments in Debere Tabor.

Environmental Cleanliness

For visible cleanliness of the hospital environment, Felege Hiwot was consistently cleaner than Debere Tabor. The wards were observed as visibly clean from dust and soil in Felege Hiwot in nearly all observations (86% and above) across all wards. In Debere Tabor the NICU and KMC wards were observed to be visibly clean in most assessments (83% and above), but in contrast the Delivery and PNC wards were observed to be rarely visibly clean (17% and below). Debere Tabor often had uncleaned spills and unclean floors in the Delivery and PNC wards. In Felege Hiwot and Debere Tabor the staff toilets were visibly clean in all or most (71% and above) observations across all wards. However, the patient toilets were observed to be visibly clean less often reaching as low as 2 out of 7 assessments (29%) in the Felege Hiwot NICU. In Debere Tabor, the patient toilet that was observed as visibly clean the most often (75%) was in the NICU, but patient toilets were reported as visibly clean as low as 1 out of 12 observations in the Delivery ward.

To safely segregate waste, WHO guidelines recommend at least three separate and labeled bins (sharps, infectious and non-infectious waste). Felege Hiwot had safely segregated waste in 5 of 7 observations in the Delivery ward but much less frequently (43% and below) in the other wards. Debere Tabor had safely segregated waste in 25% and 17% of the NICU and KMC ward assessments respectively, but in the other two wards the waste was never segregated safely.

Environmental and Handrinse Samples

There was a total of 202 environmental samples collected in Felege Hiwot and 240 collected in Debere Tabor during the study period. The sample sizes are similar between hospitals (Table 3).

Swab Samples from Surfaces

In Felege Hiwot a total of 106 swab samples was collected from surfaces across the four hospital wards during the study period (Table 3). *E. coli* detection was relatively

infrequent (4-12%), with the highest frequency of detection of 12% in the Delivery ward (Table 4). The frequency of detection of *S. aureus* was also low (0-6%) across all wards. The frequency of detection of other coliforms was slightly higher at 12% in all wards but the PNC ward, where the frequency of detection was 6%. In Debere Tabor a total of 123 swab samples was collected across the four hospital wards (Table 3). The frequency of detection of bacteria was notably higher in Debere Tabor (Table 4). The frequency of detection of *E. coli* ranged from 5% to 45% across the wards, with the lowest (5%) frequency in the Delivery ward and highest (45%) in the KMC ward. *S. aureus* had a low (5%) frequency of detection in the Delivery and PNC wards, but moderate (18% and 25%) in the NICU and KMC wards. Frequency of detection of other coliforms stands out with 75% in the KMC ward, 52% in the NICU and 30% in the PNC ward. No other coliforms were detected in the Delivery ward.

When grouping the three categories of bacteria together to determine if any bacterial contamination was present, the Delivery ward showed the highest frequency of detection of the target bacteria (33%) in Felege Hiwot, with the lowest (6%) in the PNC ward. For Debere Tabor, the KMC ward had the highest frequency of detection with 85% of swabs testing positive and the Delivery ward the lowest (10%). The NICU in Debere Tabor also showed a high frequency of detection at 58%.

The surface samples were grouped by the type of surface and the proximity to patients. In Felege Hiwot, surface fomites in direct contact with neonates, such as bedsheets and bedrails, were more often contaminated with *E. coli, S. aureus*, and especially other coliforms, than fomites the patient would contact indirectly such as doorknobs (Table 5). The frequency of other coliform detection differed by 29% between direct patient contact fomites and other fomites. The same trend was observed in Debere Tabor hospital where the sample fomites in direct contact with patients had higher frequency of detection of bacteria than other fomites (Appendix Figure 2). The largest difference in frequency of detection was 32% for *E. coli*.

Drinking Water Samples

E. coli was not detected in any of the 25 drinking water samples collected in Felege Hiwot (Table 4, Table 6). *E. coli* was detected in six of the 32 drinking water samples collected in Debere Tabor. A moderate frequency of detection (40%) was found in the PNC ward with a lower frequency in the other three wards (20% and below).

For the quantitative results, the concentration of *E. coli* (colony forming units (CFU) per 100 mL), in the Debere Tabor PNC ward had the highest observed *E. coli* concentration at 9 CFU per 100 mL (median 5.5) followed by the Delivery ward at 7 CFU per 100 mL (median 7), NICU at 2 CFU per 100 mL (median 2) and the KMC ward at 1 CFU per 100 mL (median 1) (Table 6). No *E. coli* was detected in Felege Hiwot drinking water samples.

Water Samples from Medical Devices

In the NICU wards, water samples were collected from medical devices such as water reservoirs attached to oxygen tank tubing or within oxygen concentrators. Twenty samples were collected in Felege Hiwot and 27 were collected in Debere Tabor (Table 3). In Felege Hiwot, the presence of *E. coli* and *S. aureus* was not detected but other coliforms were detected in 5% of samples (Table 4). In Debere Tabor *E. coli* and *S. aureus* were also not detected, but other coliforms were detected in 30% of samples collected.

Handrinse Samples

Overall, a total of 108 handrinse samples was collected from both hospitals, and *E. coli* was detected in 15.7%, *S. aureus* was detected in 19.5 %, and other coliforms were detected in 26% of samples (Table 4). In Felege Hiwot 50 handrinse samples were collected from individuals across the four wards and included samples from clinical staff and mothers (Table 3). Twice as many handrinse samples were collected from individuals in the NICU compared to the other wards. *E. coli* was not detected in any handrinses from the Felege

Hiwot Delivery ward, and the other three wards had relatively low frequencies of (15% or less) of *E. coli* positive handrinses (Table 4). *S. aureus* was detected in 60% of handrinse samples in the Felege Hiwot PNC ward, 33% in Delivery ward handrinse samples, and 10% or less in the other wards. Other coliforms were detected in 30% and 40% of handrinse samples collected in the NICU and KMC wards, and less frequently (11% or less) in the Delivery and PNC wards. In Debere Tabor, the three target bacteria were detected at higher frequencies in handrinse samples. *E. coli* was detected in 24% and 30% of the handrinse samples collected from the NICU and KMC wards, respectively, and in 11% or less from the handrinses collected in the Delivery and PNC wards. In the KMC ward, *S. aureus* was detected in 50% of the handrinse samples, but in 10% or less in the other 3 wards. Other coliforms were detected in 70% of the handrinse samples collected in the KMC ward, in 31% of the samples in the NICU, and in 11% or less of the handrinse samples in the other two wards.

When grouping the three categories of target bacteria together to determine if any bacterial contamination was present, Felege Hiwot had the highest frequency of hand contamination in the PNC ward (70%), followed by the KMC ward (50%), the NICU (40%) and Delivery wards (41%). Debere Tabor had the highest frequency of detection of bacterial contamination on hands in the KMC ward at 80%. The KMC ward was followed by the NICU, with the frequency of detection of bacteria at 41%, and the hand rinses from other two wards had detection rates below 20%.

The overall concentrations of *E. coli* measured by the membrane filtration in the handrinse samples ranged from 1 CFU to 132 CFU per pair of hands across the two hospitals (Table 6). In Felege Hiwot, *E. coli* positive handrinse samples were detected by membrane filtration in the NICU and KMC wards. The *E. coli* concentrations ranged from 2 to 94 CFU per pair of hands in the NICU, and 4 CFU per pair of hands in the KMC ward. In Debere Tabor, all wards except for the Delivery ward had *E. coli* positive handrinse samples detected

by membrane filtration. The *E. coli* concentrations in the handrinses from the NICU ranged from 1 to 132 CFU per pair of hands, in the KMC ward from 1 to 12 CFU, and in the PNC ward 5 CFU per pair of hands.

There were 19 handrinse samples collected from mothers, and *E. coli* was detected in 37% of these samples. The majority of the detected *E. coli* contaminated handrinse samples were from females (89%) (Table 7). *E. coli* positive handrinse samples were also obtained from nurses, caregivers, midwives and doctors.

WASH Score Results

Table 8 and the boxplots in Figures 1 and 2 show the distribution of the WASH scores by hospital and ward based on the scoring model for the WASHCon Lite assessment results. The spread of the WASH scores in Felege Hiwot was 28.5 (Max 25, Min -3.5) and for Debere Tabor 32.5 (Max 31.5, Min -1), with higher WASH scores indicating better WASH conditions and infection prevention and control practices (Figure 1). The distribution of the WASH scores by ward in Felege Hiwot show that the Delivery ward had the greatest variability in scores over time, and the NICU had the smallest variability (Figure 2a). In Debere Tabor, the PNC and Delivery wards had the greatest variability in WASH scores over time and the NICU and KMC wards showed less variation (Figure 2.b). Figure 3 shows how the WASH scores in each hospital and ward varied over the assessment time points. For example, the Felege Hiwot NICU started with a score of over 20 and through the course of assessments the score dropped to a minimum of 5 and then rose again to around 15 by the end of the study period (Figure 3a). In the Delivery and PNC wards in Debere Tabor the scores varied up and down during the study period (Figure 3.g, h.).

The temporal relationship between the WASH scores and the proportion of environmental samples (all sample types combined) that were negative for all the target bacterial indicators was examined. The percent of the environmental samples that were negative for any of the three target bacteria, varied over the study period in each ward of the hospital (Figure 3). Sample sizes per

assessment period in the wards ranged from 2 to 27 samples. The percentage of bacterial-negative samples from some wards remain relatively stable over time, such as in the Felege Hiwot NICU. Whereas the percentage of bacterial-negative samples from other wards, such as in the Felege Hiwot PNC ward and Debere Tabor KMC wards varied considerably over the study period (Figure 3.d, f). Based on a visual assessment, there does not appear to be an association between the WASH score and percentage of bacterial-negative environmental samples over time.

The correlation between the WASH score and the percentage of bacterial-negative environmental samples over time by hospital and ward was examined (Figure 4). There was a significant positive correlation between these two measures for the NICU in Felege Hiwot (Figure 4.a), and for the NICU and Delivery wards in Debere Tabor (Figure 4.e, g). No significant correlation was observed between these two measures for the KMC and Delivery wards in Felege Hiwot (Figure 4.b, c), and PNC ward in Debere Tabor (Figure 4.h) Significant negative correlations were observed between the WASH score and the percentage of environmental samples that were bacterial-negative in the Felege Hiwot PNC ward (Figure 4.d), and Debere Tabor KMC ward (Figure 4.f).

Logistic Regression Model

Model diagnostics showed a rough linearity between the predictors and log odds, and the residual distribution showed no significant violations of model assumptions. There was no strong evidence to suggest collinearity between predictors in the model. It was assumed that testing results from environmental samples are independent of one another, with samples collected across different areas and timepoints.

Full Model:

$$\ln(Odds\ of\ Any\ Contamination) = \alpha + \beta_1\ WASH\ Score + \beta_2 Hospital + \beta_3\ Ward + \beta_4\ Sample\ Type + \beta_5\ Month + \beta_6\ WASH\ Score * Hospital + \beta_7 WASH\ Score * Ward + \beta_8\ WASH\ Score * Sample\ Type + \beta_9\ WASH\ Score * Month + \varepsilon$$

Both forward and backward selection reached the same conclusion and removed WASH score, month, and all interaction terms from the model as insignificant (α=0.05). The WASH score is the predictor variable and for the purposes of this analysis was ineligible for removal.

It was observed in preliminary data analysis that the WASH score and contamination levels varied by hospital and ward, and interaction may happen between the WASH score and these variables. However no significant interaction was observed by hospital or ward. Since water availability in the facilities was reported to vary by time of year in the WASHCon Baseline assessment and is known to vary by season in the literature, interaction could be expected between month and the WASH score (86). This interaction between WASH score and time (in months) was in fact not significant and month was dropped from the model. The results of the full model, backward selection and the proposed final model are shown in Table 9. The likelihood ratio test was highly significant at p <0.0001, indicating the final model is a better fit for the data than the full model.

Proposed Final Model:

$$\ln(Odds\ of\ Any\ Contamination) = \alpha + \beta_1\ WASH\ Score + \beta_2 Hospital + \beta_3\ Ward + \beta_4\ Sample\ Type + \varepsilon$$

In the proposed final model, the WASH score was not significant at α=0.05 (p=0.48) (Table 9). A one unit increase in WASH score was not significant in predicting odds of contamination (OR 1.01, p=0.48, 95% CI 0.98, 1.05) (Table 10). The hospital was significant in predicting contamination in the proposed final model (p<0.0001). The estimated odds ratio for Felege Hiwot versus Debere Tabor hospital is 0.42 (p=<0.001, 95% CI 0.26, 0.70) (Table 10). These results indicate samples

collected from Felege Hiwot are less likely to be contaminated than samples collected in Debere Tabor. The hospital ward was also significant in predicting contamination in the model ($p<0.001$). The estimated odds ratio is 0.87 for the NICU ward versus the PNC ward (reference) ($p=0.71$, 95% CI 0.42, 1.82). The estimated odds ratio of the KMC ward versus the PNC ward is 2.89 and for the Delivery ward versus the PNC ward is 0.39 ($p<0.009$, 95% CI 1.29, 6.49; $p<0.03$, 95% CI 0.17, 0.92). These results indicate that samples collected from the KMC ward are more likely to be contaminated than samples collected in the PNC ward, and that samples in the Delivery ward are less likely to be contaminated than samples collected in the PNC ward.

The sample type was also significant in the model ($p<0.0001$). The estimated odds ratio of swabs versus handrinse (reference) is 0.76 ($p<0.29$, 95% CI 0.46, 1.23). The estimated odds ratio of drinking water samples versus handrinse is 0.11, and for water from device samples is 0.30 ($p<0.0001$, 95% CI 0.039, 0.31; $p=0.007$, 95% CI 0.13, 0.72). According to these results, swabs from surfaces and handrinses are more likely to be contaminated than drinking water and water from medical devices.

Discussion

This study sought to determine if maternity wards and neonatal intensive care units (NICUs) in two hospitals in Amhara, Ethiopia with limited WASH infrastructure and practices have hand and environmental contamination with pathogens known to cause neonatal sepsis (e.g. *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*). High frequencies of contaminated handrinse samples were found in each hospital and ward, with a notably high frequency of detection of other coliforms found in samples from both clinical staff and mothers. In the light of the observed low availability of water and soap for clinicians in some wards, and even less availability for patients, this finding of frequent hand contamination is likely linked to both lack of hand hygiene materials and low compliance with handwashing recommendations. Surface swabs were also frequently contaminated with target bacteria. Surfaces in closer proximity to patients were found to be contaminated at a higher frequency compared to other surfaces and equipment in the hospital. This indicates inadequate environmental cleaning, and in conjunction with barriers to, and lack of, adequate hand hygiene suggests that neonates may be at risk of exposure to etiologic agents of sepsis from surfaces and from the hands of staff and their mothers.

WASHCon Baseline Hospital Demographics and Characteristics

When selecting the hospitals for this study, Felege Hiwot was observed to be a much busier, larger, and more crowded hospital than Debere Tabor. However, the information on the number of patients who receive services each month indicated that Debere Tabor hospital sees nearly 10,000 more outpatients than Felege Hiwot (Table 1). This difference in outpatient load is less pronounced when considering Debere Tabor is open every day of the month compared to Felege Hiwot which provides outpatient services only 20 days per month (Table 1). The number of beds per ward was not collected in our study, but in another recent study Felege Hiwot had 30 beds in the NICU (64). No estimate was available for the number of Debere Tabor NICU beds, but it had 30 beds in the obstetric ward in a recent study (79). Over four times the surgeries are performed at Felege Hiwot, and 200 more births take place, compared to Debere Tabor. For facilities with a similar patient capacity there

are far more clinical staff (1,000), doctors (300), and cleaning staff (110) in Felege Hiwot compared to Debere Tabor (250, 36, 39).

WASHCon Lite Assessment

Felege Hiwot had far more dedicated cleaning staff per patient than Debere Tabor. A similar patient load is seen at each hospital per day but Felege Hiwot had 3x the dedicated cleaning staff (Table 1). This could explain the poorer environmental conditions observed in Debere Tabor over the study period, such as more frequently observed dirty floors, uncleaned spills and lower visible cleanliness. Previous studies have reported that under-staffing environmental cleaners was associated with poorer environmental conditions and a greater incidence of HCAI (15, 29, 31, 33, 87). It has also has been shown that lack of training of cleaning staff, lack of recognition of cleaning staff, understaffing, and low moral contribute to the failure to meet environmental cleanliness standards (14, 15, 56). Further investigation is needed to explore which of these reasons may be driving the problem in Debere Tabor. Since Debere Tabor is a CASH hospital, there are national minimal standards that should be met in the facility. Staff should be aware of healthy WASH practice and have a high morale and pride around keeping the facility clean (56, 75). Failure in environmental cleaning and WASH practices in the Debere Tabor facility may indicate the program was not implemented properly, staff need refresher training, or other underlying issues.

The stark contrast between the availability of clinician/staff hand hygiene stations and toilets versus the patient hand hygiene stations and toilets in each facility reveals a great deal about the prioritization of time and resources in both facilities (Table 2). Staff toilets were nearly always visibly clean in both facilities, but patient toilets were frequently observed to be dirty. Soap was reported to only be provided to staff and not to patients. The possibility of exposure to environmental fecal contamination on hands when using the unclean facilities, and then subsequent infection or transmission may be a serious risk. Adequate WASH and environmental cleaning were prioritized for

the facilities used by clinicians/staff, while patient facilities were neglected. Adequate care is not given to waste management either, as few wards had safely managed waste.

Environmental Contamination

In Felege Hiwot, in general the frequency of contaminated surface samples was low (Table 4). The predominant contamination with other coliforms (12% in all wards but PNC) may indicate issues with environmental cleaning compliance and or improper cleaning. *Klebsiella* is a coliform with prolonged survival on surfaces in biofilms that may not be completely removed when cleaning (13, 25). This poses a risk as cross-contamination can occur via this pathway to patients.

In Debere Tabor, coliforms were the most frequently detected bacteria on surface swab samples. This is expected for surfaces with contamination (16, 67). A high frequency of detection (above 50%) of coliforms and a moderate frequency of *E. coli* contamination were detected (20-45%) in all wards, except the Delivery ward, and again this may indicate inadequate environmental cleaning and potential fecal contamination (Table 4). However, disinfectant was nearly always observed in each ward, and in the baseline assessment, Debere Tabor management reported that surfaces, floors and toilets were disinfected daily. This may indicate that routine cleaning was either done poorly or was not actually being performed by the staff at the required frequency. The Centers for Disease Control and Prevention (CDC) recommends that high-touch surfaces be cleaned at twice daily and that floors are to be disinfected twice per day and after each patient in intensive care units (23). Debere Tabor was observed to be visibly clean less often across every ward when compared to Felege Hiwot and uncleaned spills were a common occurrence in the Delivery and PNC wards. The higher frequency of contamination observed is in line with what is expected with the lower level of visible cleanliness in Debere Tabor.

The more frequent detection of any contamination with the target bacteria (85%) on swab samples collected in the Debere Tabor KMC ward is striking, considering the Delivery ward had only 10% positive samples. This data indicates the cleaning practices, available materials and cleaning staff

may be different in the KMC ward. There is also the possibility that the staff may be trained differently, or the perceived need for cleanliness is not as high in the KMC ward compared to the Delivery ward where births take place. There is a greater body of literature around hygiene in the birthplace environment, showing the potential risks of an unclean birthing environment, while research on Kangaroo Mother Care is not as robust (60).

The more frequent contamination on swab samples from surfaces closest in proximity to the neonate, compared to other surfaces and equipment, sheds light on patient risk. The surfaces in close proximity to the patients, and that they are in contact with for prolonged periods, like blankets and bedrails, are not safe (Table 5). *E. coli* and other coliforms were frequently detected on these surfaces, again reflecting that inadequate environmental cleaning is likely responsible. Overall, these results indicate that pathogens associated with neonatal sepsis persist in the environment and have the potential to be transmitted to neonates through contact with contaminated surfaces. Special attention should be paid to these surfaces and items, as high-touch surfaces should be cleaned twice a day with terminal room cleaning, and bedding should be washed after each patient (23). Compliance with cleaning recommendations and protocols in these hospitals should be further evaluated. These results are especially concerning for NICUs who have immunocompromised patients who are at greater risk.

Drinking Water Quality

Both hospitals reported in the WASHCon Baseline assessment that their main water source was chlorinated by the municipal authority. No *E. coli* was detected in Felege Hiwot drinking water samples, whereas drinking water samples collected from each ward in Debere Tabor showed *E. coli* contamination at some time during the study period. *E. coli* was detected in 40% of the drinking water samples in the Debere Tabor PNC ward (Table 4). In the WASHCon Lite assessment, Debere Tabor had functional piped water a greater percentage of the time compared to Felege Hiwot, and treated water was nearly always available when in Felege Hiwot it was never available. The *E. coli* positive samples in Debere Tabor suggest that the drinking water may not have been adequately treated at the

municipal water utility, or it became contaminated between treatment and use in the healthcare facility, or that water may not have been stored safely in the hospital. *E. coli* is an indicator of fecal contamination and may point to some structural problems in the facility pipes, allowing fecal matter to contaminate the water supply, or inadequate hygiene when handling stored water (11). *E. coli* contamination occurs where there is unsafe water, and poor sanitation and hygiene conditions in a facility (2, 8, 13, 16, 37). These findings suggest unsafe water may contribute to the risk of neonatal sepsis associate with *E. coli* in Debere Tabor. For all the wards in Debere Tabor, especially the PNC ward with the highest frequency of water contamination, the water collection, treatment, and storage practices should be evaluated.

The frequent detection of *E. coli* in the drinking water at Debere Tabor indicates failures to meet the WHO guidelines for drinking water quality (Table 6) (13). The magnitude of contamination, while not safe for human consumption, was not egregious in its contamination with the highest concentration detected at 9 CFU per 100 mL (Table 6). It should also be noted that while Felege Hiwot did not have any *E. coli* contamination detected in the 25 drinking water samples tested, it should not be concluded that drinking water contamination never occurred in this hospital.

It is important to note that it is possible that "treated water" was interpreted differently by each hospital. Both hospitals reported water from the main water source (municipal water utility) was chlorinated, and both hospitals reported water was not treated on-site. Under these circumstances a misinterpretation of "treated water" is possible. Treated water could have been thought to mean the initial chlorination at the municipal authority (off-site), or further treatment at the facility (on-site).

Water Samples from Medical Devices

Contaminated water in medical devices has been associated with sepsis in neonates in previous reports of hospital outbreaks (16, 88-90) . In this study, other coliforms were detected in only one of 20 samples (5%) of water samples from medical devices in Felege Hiwot, and in 8 of 27 samples (40%) in Debere Tabor (Table 4.). This is similar to the results of contamination that was

detected for drinking water samples from the two hospitals. The frequency of contamination in these water samples from Debere Tabor may indicate that the water reservoirs in the equipment were not cleaned adequately or often enough, and/or that the water used in these devices was not changed frequently enough or was of poor quality initially. Because the drinking water samples from Debere Tabor were frequently found to be contaminated, if this water is also used in medical devices, it is not surprising that these water samples were also frequently contaminated. Of additional concern is that coliforms were detected in these small volume (1 mL) samples- indicating high concentrations of contamination. Gram-negative rods associated with neonatal sepsis are thought to be acquired from environmental sources and can survive on surfaces for months (16, 67). The ability of *K. pneumoniae* to form biofilms and colonize water distribution systems provides further support for the possibility that contaminated water and biofilms were in the reservoirs of medical devices (13). However, the piped water in this study was not tested specifically for *K. pneumoniae.* But due to similar properties of *E. coli* and *K. pneumoniae,* it is not unreasonable to suspect that other coliforms such as *K. pneumoniae* may have also been present in the piped water system at Debere Tabor. These results are alarming because they indicate high levels of contamination in the NICU among neonates with immature immune systems who require intensive care. Exposure to pathogens in water used in medical devices is a potential risk factor for neonatal sepsis in Debere Tabor.

Hand Contamination

The results of the handrinse samples indicate there was generally less hand contamination among individuals in Felege Hiwot compared to individuals in Debere Tabor (Table 4). However, a moderate frequency of detection (30%) of other coliforms was observed in the NICU and KMC wards, and *S. aureus* was detected in 33% and 60% of handrinse samples from individuals in the Delivery and PNC wards, respectively, in Felege Hiwot. The WASHCon Lite data indicated that hand hygiene stations for both clinicians/staff and patients/caregivers in the Felege Hiwot PNC ward were frequently out of water and soap. The PNC ward also showed the highest frequency of *S. aureus* handrinse contamination compared to the other wards. The presence of both water and soap at Felege

Hiwot hand hygiene stations was common in the NICUs for clinicians/staff, but not in the other wards, and was rarely or never available in the patient/caregiver hand hygiene stations. Hand hygiene promotion materials were reported to be visible sometimes in the Felege Hiwot NICU and the Delivery wards, and never in the KMC and PNC wards. The wards that had water and soap available, at any assessment time point, the NICU and Delivery wards, were also the two wards that had hand hygiene promotion materials visible at any assessment time point. This suggests there may be a greater knowledge of good hand hygiene and the necessary materials in these wards in Felege Hiwot compared to the KMC and PNC wards. Perhaps because no medical or surgical procedures take place in the KMC or PNC wards, as mentioned before, there was less of a perceived need for hand hygiene behaviors such as handwashing. However direct contact with neonates occurred in all wards in this study, and contaminated hands are a known risk factor for sepsis transmission, which can be especially dangerous for low birthweight infants that are in the KMC ward (29, 33, 58, 65).

Hand hygiene promotion materials were reported to be nearly always visible in each ward in Debere Tabor, but a greater frequency of contaminated hands was detected in this hospital. For example, in the KMC ward *E. coli*, *S. aureus*, and other coliforms were observed in 30%, 50%, and 70% of handrinse samples respectively (Table 2, 4). *S. aureus* is a common colonizer of skin and can serve as an indicator of hand hygiene (18, 33). The frequent detection of *S. aureus* contamination observed in the handrinse samples from individuals in the KMC ward may indicate poor hand hygiene practices. The staff and mothers in Debere Tabor were likely aware of appropriate hand hygiene behavior, but either did not practice hand hygiene, did not practice an adequate level of hand hygiene, or did not have the supplies for the promoted hand hygiene behaviors. Hand hygiene stations for clinicians/staff were frequently reported in Debere Tabor but were available less often for patients/caregivers. Water and soap for the clinician/staff hand hygiene station was reported to not always be available in each ward, and never present in the patient/caregiver hand hygiene stations. Hand hygiene stations were not always available across the wards, indicating that the functionality of

these stations may be intermittent and not be dependable. This may explain the high frequency of hand contamination in Debere Tabor.

When the three categories of bacteria were combined to look at any contamination with the target bacteria in handrinse samples, the PNC ward had the highest frequency of samples with contamination in Felege Hiwot (70%) and the KMC ward had the highest frequency of contaminated handrinse samples in Debere Tabor (80%). In Felege Hiwot, the PNC ward had the lowest availability of hand hygiene stations for both clinicians and patients. However, in Debere Tabor the PNC ward does not follow this same trend, and functional hand hygiene stations were present at most assessment timepoints. A higher percentage of samples were contaminated with *E. coli* across three of the four study wards in Debere Tabor compared to Felege Hiwot.

In addition, the high frequency of surface swabs that were positive for the target bacteria in Debere Tabor suggest that hands may have become contaminated from contact with contaminated environmental surfaces. This leaves open the possibility of subsequent cross-transmission of pathogens associated with neonatal sepsis when hand hygiene is not adequate. Staff and patients could also transmit fecal bacteria, such as *E. coli*, if they were not able to wash their hands with soap and water after using the toilet facilities.

The WASHCon Baseline results indicated that soap was not provided to patients/caregivers in either hospital. This makes it challenging to practice good hand hygiene and identifies this as a key gap in infection prevention and control in both facilities. The presence of hand hygiene promotion materials without providing staff and patients the needed supplies to practice good hand hygiene is counter intuitive. Having a functional hand hygiene facility at points of care is recognized as an essential element of an environmental cleaning program by the CDC, and essential for meeting the basic hygiene definition in the JMP *Global Baseline Report* (8, 23). Lack of needed materials for handwashing, such as water and soap, has also been shown to affect hand hygiene compliance (47). It was reported that financing for WASH infrastructure was included in both of the hospitals' budget,

but it is not known if financial reasons were the barrier to providing necessary hand hygiene materials. In addition, there may be a financial constraint on hiring more cleaning staff. The financial situation of each hospital should be reviewed in depth, gaps in funding identified, and a plan made to increase financial resources available where needed.

In both hospitals, the results from the handrinses from both clinical staff, mothers, and caregivers show there were facility-wide challenges with maintaining adequate hand hygiene (Table 7). In addition, handwashing was not required in either facility before entering the NICU. Taken together these findings provide strong evidence for the transmission of pathogens associated with neonatal sepsis through contaminated hands. However, the different distributions of *E. coli, S. aureus,* and other coliforms across each hospital and ward suggest that different transmission dynamics were occurring.

WASH Score Model

The WASH scores for Debere Tabor hospital was on average higher than the WASH scores for Felege Hiwot (Figure 1). However, Debere Tabor had less frequent reports of visual cleanliness according to the WASHCon Lite assessment. In general, Debere Tabor had higher scores than Felege Hiwot for water access, as well as infection prevention and control, so it is possible these domains are responsible for the higher overall scores.

A higher WASH score was correlated with less frequent detection of contamination in environmental samples from the NICU for both hospitals (Figure 4.a, e). However, in the other wards, the WASH score correlation with the percent bacterial-negative samples was inconclusive or negatively correlated with the percent of bacterial-negative samples. It is also of importance to remember that the correlation plots do not adjust for potential confounders, and outliers were not removed due to an already small sample size in some wards. Overall, there was temporal variability in the WASH scores, and the proportion of environmental samples that were negative for the target bacteria, in some of the hospital wards over the study period. This makes it difficult to draw

conclusions about the value of the WASH score (based on observation and reported practices) as an accurate indicator of microbial contamination and risk of healthcare-associated infections in these wards.

Logistic Regression Model

In the proposed final logistic model, the WASH score was not significant in predicting hand and environmental contamination. The preliminary data, including the correlation plots, provided an early indication that this may be the case. In the correlation plots, which show subsets of the data by ward, the WASH score was correlated with percent negative samples in the NICU wards and Debere Tabor Delivery. The other correlation plots showed no significant correlation or negative correlation. However, it must be kept in mind the correlation plots do not adjust for potential interactions or confounders. When considering these mixed results, it is not surprising the WASH score was not significant in the model. In addition, there were not significant interactions with the WASH score, which is expected if WASH score is not significant in the model.

The WASH conditions and environmental contamination did not vary significantly over time (by month) in the proposed final model (Figure 3, Table 9). The significance of the hospital in predicting contamination is consistent with what should be expected based on the frequencies of contamination detected in the environmental samples. This contrast between the two facilities, with the odds ratio of 0.42 for Felege Hiwot versus Debere Tabor, shows there are facility effects and conditions that impact contamination. Hospital ward also affected the likelihood of environmental contamination, where the odds of contamination, compared to the PNC ward, was significantly lower in the Delivery ward and significantly higher in the KMC ward. Taking a more detailed look at the ward conditions, and differing practices between these wards, in future research could elucidate this relationship further.

The type of sample was significant in the model, which is consistent with the fact that handrinse and swab samples showed more frequent contamination in preliminary analysis compared

to the other sample types of drinking water and water from devices. This observation could be applied to adjust the WASH score model in further analysis, where greater weight could be given to the hand hygiene and environmental cleanliness questions. These results reinforce the WASHCon Lite assessment results showing the ward environments were often not visibly clean and the need for greater attention towards hand hygiene and environmental cleaning in the health care facilities.

The results of this model suggest that the WASH scoring system needs further adjustment to accurately reflect the odds of contamination or that there are other factors affecting the probability of environmental contamination that need to be added to the model. It is also possible that the WASH score is significant in the model, but our study does not have the statistical power to detect it due to small samples sizes.

Adjusting the WASH scoring model, such as applying different question and category weights as mentioned above, could improve the scoring system. In the current WASH scoring model, questions are used in multiple categories which may introduce some redundancy into the model. For example, a WASHCon question may be used in both hand hygiene and infection prevention and control categories. Properly weighting these questions could impact the WASH score significance in the model.

Future calibrations to the scoring system and model could also include creating separate WASH scoring models for the categories included in the WASH scoring system: hand hygiene, infection prevention and control, environmental contamination and drinking water. Having a WASH score for each of these elements, instead of a composite WASH score, may allow a better understanding of what exactly may cause contamination in the facilities and wards. This model could also be modified to predict contamination in different sample types, such as a model for surface swabs only. Since the type of sample was significant for predicting contamination in the final model, this would allow for an estimate of the relationship between the WASH score and a specific type of

contamination, such as hand contamination. To do this type of analysis, a greater number of the specific type of sample would need to be collected.

Creating a model exclusively for each ward could also improve the scoring system, as the WASHCon Lite questions that were specific to one ward (and not used in this analysis) could be incorporated. Because of potential effect modification by ward (Figure 4), a separate model should be considered for each ward of the hospital. Different wards may have different environmental cleaning practices, personnel, and WASH infrastructure due to the procedures and populations in each ward, and by tailoring a different model to each ward it is possible that the WASH score may serve as a significant predictor for risk of exposure to bacterial contamination in the ward environment (23).

The logistic model should also be adjusted for the baseline WASH score and environmental contamination levels. The WASHCon Baseline assessment is far more extensive than the WASHCon Lite, and an additional scoring system would need to be developed to add the Baseline score as a predictor variable in the logistic model.

The odds of contamination obtained from the proposed final model could serve as a risk metric for hospital and ward. An increased odds of contamination may indicate a greater risk to the neonate. It is possible that future studies linking the neonatal cohort data to the environmental data may provide support for the use of the model as a risk metric. Further research is warranted.

Strengths and Limitations

To our knowledge, this is the first study to investigate the relationship between hand and environmental contamination with WASH infrastructure and practice in health care facilities over time. Strengths of this study include its wholistic approach to assessing WASH infrastructure and practice in healthcare facilities and the longitudinal nature of the study. The WASHCon Assessment covers many aspects of WASH in healthcare facilities including hand hygiene, infection prevention and control, environmental contamination, sanitation and waste management, and drinking water

quality and access. The assessment provides an overview of WASH infrastructure and practices across each ward, and how WASH conditions varied over time through multiple assessments over the study period. The large number of environmental samples that were collected over the study period show how the frequency of bacterial contamination varied in each ward environment. By examining the WASH scores and the bacterial contamination results from the time-matched environmental samples, this study explored the association between WASH conditions and environmental contamination over time. It is especially valuable that this study was able to match data on bacterial contamination in the environment with the visual assessment data, as visibly clean surfaces may still be contaminated with bacteria (12).

In Debere Tabor, the environmental samples were most often taken on the same day or within a few days of the WASHCon Lite assessment. However, in Felege Hiwot the average time between the assessment and sample collection times was longer. The longer the time between the assessment and sample collection, the more the WASH conditions have the opportunity to change and introduce bias in the results. Overall spatial, and temporal heterogeneity in the data made it challenging to accurately estimate risk of exposure for neonate patients to bacterial contamination by ward, hospital, and time.

The coding system used for the environmental samples was often disorganized. For example, different numerical codes were used for the same hospital ward across different sample types. When reconciling the data, this increases the likelihood that errors were made. In addition, there was not a concrete system to record sample IDs, which then required a lot of back-end work to reconcile, which may present when matching the samples to antibiotic susceptibility results, and the environmental exposure data to the clinical data from the neonatal cohort.

The WASH scoring model developed for this study was a pilot. One challenge of this scoring model is that it combined diverse data on water quality, water availability, hand hygiene, infection prevention and control, sanitation, and environmental cleaning. To develop the composite score, each

response was categorized. Literature and industry standards were used to inform these choices, but there is a degree of subjectivity remaining. Each category in the scoring model was weighted equally, but it could be that one category, such as hand hygiene, may have a greater impact on bacterial contamination compared to another category.

By calculating an aggregate score, and combining results from multiple sample types, there is a loss of detail that is needed to provide information about specific problems or gaps in WASH and infection prevention and control measures. A facility could have excellent hand hygiene and poor environmental cleanliness, which could effectively cancel each other out in an aggregate score. In addition, using a measure of "any contamination" instead of information on the detection of specific bacteria can miss clues about where the contamination is coming from. The different target bacteria in this study were selected because they provide different information about sources and risks of contamination. Also, several questions in the assessment are based on "visible cleanliness" which is somewhat subjective as it is based on an enumerator's own idea of what "visibly clean" looks like.

Large variability in the bacterial results from the environmental samples, such as in the Felege Hiwot PNC ward, may affect the ability of the WASH scoring model to predict accurately. In addition, outliers influence analysis of the correlation between environmental contamination and the WASH score, and sample sizes were often small which makes estimates more prone to influence by outliers. The small number of specific types of samples, and temporal variability in proportion of samples with bacterial detection, made it difficult to accurately determine the frequency and magnitude of environmental contamination. When the bacterial results from all sample types were aggregated into a measure of "any contamination" for a specific time point, hospital, and ward, one type of environmental sample may have more weight than other types of samples simply because there were more of certain types of samples for a specific place and time. A larger sample size for each type of sample, as well as more samples for each sample collection time point, should be taken in future work.

Finally, *Klebsiella pneumoniae* is an important etiologic agent of neonatal sepsis, but for this study we were not able to find a bacterial media that specifically identified *Klebsiella* colonies without confirmatory tests. In this study, *Klebsiella* contamination in the environmental samples was only captured as part of the group of non-*E. coli* coliforms that were detected on the EC Compact Dry Plates.

This study only assessed the quantity of contamination for *E. coli* in the handrinse and drinking water membrane filtration samples. This is because standardized methods do not exist in the field for swab sample collection in HCF. Because of this, we could only assess swab samples for the presence/absence of specific target bacteria and not quantify the concentration of surface contamination. This did not allow us to examine if one type of surface had a higher magnitude of contamination compared to others. Future research should include methods to elute the bacterial targets from the swab and measure concentration with a quantitative assay. In addition, the maximum colony count for membrane filtration plates for *E. coli* was set to 200 CFU, in order to be able to count the colonies, which could bias the results down if the true contamination is a large quantity.

An advantage of using a logistic regression model in the study is that it gives a measure of association between the predictor and the outcome and the direction of that association. However logistic regression does require that the log odds ratio of the response variable is normally distributed, and a normal distribution of the error term. Regression also allowed for the consideration of potentially confounding variables and interactions, and to examine the relationship between the WASH conditions and the percentage of bacterial-negative samples over time. The logistic model has some limitations, such as not all variables can be included in the model (fully parameterized) and thus only a few were selected based on the knowledge from the literature and preliminary data.

Conclusions and Recommendations

This study shows that hospital maternity wards and NICUs with limited WASH infrastructure and infection prevention and control practices have hand and environmental contamination with bacteria associated with neonatal sepsis. The transmission pathways of these pathogens through contaminated hands, surfaces, drinking water, and water from medical devices were all demonstrated to be possible based on bacterial detection in handrinse and environmental samples. The NICU and KMC wards were observed to have the most frequently contaminated samples in Debere Tabor. Samples collected in Felege Hiwot had similar levels of contamination across the four wards, with the exception of more frequent other coliform contaminated handrinse samples in the NICU and KMC wards and handrinse samples that were positive for *S. aureus* from individuals in the Delivery and PNC wards.

It is possible that for the KMC and PNC wards, because no medical procedures take place in that area, that inadequate WASH and poor environmental conditions are not perceived as high of a risk as wards where medical procedures are performed. However, pathogen transmission is still possible through cross-contamination between hands and surfaces. Low birthweight babies are known to be at an increased risk of death from sepsis, and since the KMC is for low birthweight babies it is important that good WASH and environmental conditions are maintained (16). Educational interventions for patients and staff about the importance of good WASH and infection prevention and control practice, such as hand hygiene, should be implemented in the KMC and PNC wards in Debere Tabor.

The frequency of environmental contamination detected, especially in Debere Tabor, shows that environmental cleaning is either not done well or not done at the required frequency in the facilities. High-touch surfaces and surfaces in close proximity to patients were especially likely to be contaminated, and cleaning should be focused on these areas. The number of cleaning staff should be high enough to meet these needs for all parts of the facility. An inspection of plumbing, water points,

and water storage and treatment should be performed to determine the source of drinking water contamination in Debere Tabor. It may also prove useful to provide resources to these facilities on the long-term cost-savings and improved patient outcomes that result from improving WASH infrastructure and practice.

In this study, the WASH conditions and hand and environmental contamination varied over time. However, in the final proposed logistic model, time (by month) was not significant in predicting contamination with the target bacteria. The WASH score was correlated with the frequency of any environmental contamination in the NICU ward. However, for the other wards, the correlation did not exist or was negative. Effect modification of the relationship between the WASH score and the percentage of environmental samples with no detection of the target bacterial indicators was observed by ward. The logistic model did not find the WASH score as a significant predictor of contamination, showing the WASH scoring system is in need of further development and fine tuning. Further calibration of the scoring model may improve the ability of the WASH score to predict environmental contamination. Increasing the number of environmental samples collected and analyzed in each assessment period may help provide a more robust estimate of bacterial contamination that is less prone to being skewed by outliers and more representative of facility conditions.

The facility, hospital ward, and sample type were significant in the logistic regression model to predict any contamination with the target bacteria (Table 10). The estimated odds of any contamination was 0.42 for Felege Hiwot versus Debere Tabor. The estimated odds of contamination in the KMC ward versus the PNC ward was 2.89. Therefore, facility and ward level factors should be considered when considering what may impact the frequency of contamination of environmental samples. When compared to handrinse samples, surface swabs, drinking water and water from device samples had a lower estimated odds of contamination (Table 10). This may indicate that hand hygiene should be given special focus in facility WASH infrastructure and practice in HCF.

Both hospitals should improve maintenance and increase the necessary hygiene supplies in facilities, as soap was not provided for patients, patient hand hygiene stations were frequently reported to be non-functional, and toilet facilities were cleaned less often compared to staff facilities. The JMP and CDC state that providing hand hygiene stations with soap at the point of care is key to achieving basic hygiene and is a foundational element of infection prevention and control. Appropriate environmental cleaning is also essential for infection prevention and control, and Debere Tabor should increase the number of cleaning staff to meet the cleaning demands of the facility which was more often observed in the WASHCon Lite assessment to have poor environmental conditions. Appropriate training and cleaning protocols should also be provided. The CASH program should also be evaluated in this facility, to see if the programs standards are still being followed and if the goals were met.

Summary, Public Health Implications and Future Directions

This study showed that there was hand and environmental contamination with bacteria known to cause neonatal sepsis (*E. coli*, *S. aureus*, and *K. pneumoniae*) in the maternal and neonatal ICUs of facilities with limited WASH infrastructure and practice. The public health implication of this finding is that it is possible for neonates to be exposed to sepsis-causing organisms and acquire an infection in the facility through transmission by hands, the environment, drinking water, and/or medical equipment. Neonates in the intensive care unit are most vulnerable to infection with these pathogens, as they are immunocompromised, have longer hospital stays, and often may be subjected to invasive procedures. Neonates in the Kangaroo Mother Care ward are also very vulnerable, as these babies are low birthweight and are at increased risk of death if they become ill with sepsis.

Even though both facilities claimed to disinfect surfaces, toilets, and floors daily, visible contamination was observed, and bacterial contamination was detected in environmental samples, especially in the Debere Tabor hospital. This indicates either a lack of training among the staff on how to clean properly, and/or that the cleaning frequency is either not being met or is not sufficient to control contamination. The results of this study indicate surfaces in close proximity to neonates were more often contaminated with bacteria associated with neonatal sepsis than other more distal surfaces. The public health implication of this finding is that cross-contamination and transmission of infectious agents can, and is likely, to happen from these surfaces, because these surfaces are closer in proximity and have prolonged and more frequent contact with the patients. Taken together, the findings of bacterial contamination on surfaces and hands of healthcare workers, patients, mothers and caregivers shows how easily transmission may occur. This is further compounded by the facilities not always providing adequate hand hygiene facilities and materials for patients.

The WASH conditions and environmental contamination were found to change over time in the two facilities in our study, and this is likely true for other facilities as well. However, time was not a significant predictor in the proposed final logistic regression model. Further work is needed to

determine how to use simple data on WASH conditions in a facility to predict risk of bacterial contamination in the HCF.

Future directions include creating separate domain-specific WASH score models from the assessment data from each domain, e.g. hand hygiene, infection prevention and control, environmental contamination, and drinking water, and examining the relationship between these scores and the bacterial results from the different types of environmental samples. This would provide a greater understanding of how changes in each of these WASH infrastructure and practice domains affects the likelihood of microbial contamination.

Finally, future analyses can examine whether health outcome data from the neonatal cohort, and etiology and antibiotic susceptibility data from sepsis cases, can be linked to the results of bacterial detection in the environment and antibiotic resistance in environmental isolates to determine if exposure to bacterial contamination in the environment was associated with a incidence of neonatal sepsis and antibiotic resistance.

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Tables and Figures

Table 1. WASHCon Baseline Hospital Demographics

	Felege Hiwot	Debere Tabor
Patient Population		
Avg. Outpatients (per month)	25,000	34,758
Days Outpatients Seen (per month)	20	30
Avg. Inpatients (per month)	2,333	1,600
Avg. Inpatients (per day)	77	53
Inpatient Beds	480	170
Surgical Procedures (per month)	1,300	300
Baby Deliveries (per month)	500	300
Workforce		
Clinical Staff	1,000	250
Doctors	300	36
Non-Clinical Staff	350	165
Cleaning Staff	110	39
WASH Infrastructure		
Facility Has WASH Guidelines	No	Yes
Budget Includes WASH Infrastructure	Yes	Yes
Water		
Type of Water Available	Piped	Piped Protected well
Water Always Available	No	No
Main Water Source Chlorinated	Yes	Yes
Hand Hygiene		
Provide Soap to Staff	Yes	Yes
Provide Soap to Patients	No	No
Infection Prevention and Control		
Separation of Infectious Waste	Yes	Yes
Staff Trained on IPC* Every Year	Yes	Yes
Environmental Cleanliness		
Floors, Surfaces, Toilets Cleaned with Disinfectant Daily	Yes	Yes
Adequate Cleaners and Maintenance Staff	Yes	Yes
Facilities		
Toilets Available on Premises	Yes	Yes
Sufficient Toilet to Meet Facility's Needs	No	Yes
How is Human Waste Disposed	Septic Tank	Septic Tank

*Infection prevention and control

Table 2. WASHCon Lite Assessment* by Hospital and Ward

	Felege Hiwot (N=7)				Debere Tabor (N=12)			
Ward	**NICU ^**	**KMC†**	**Delivery**	**PNC§**	**NICU ^**	**KMC†**	**Delivery**	**PNC§**
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Water								
Piped water	7 (100)	7 (100)	7 (100)	4 (57)	12 (100)	12 (100)	12 (100)	11 (92)
Functional Piped Water	7 (100)	6 (86)	7 (100)	3 (43)	12 (100)	12 (100)	5 (42)	8 (67)
Water Available	4 (57)	5 (71)	5 (71)	1 (14)	12 (100)	12 (100)	11 (92)	11 (92)
Treated Water Available	0 (0)	0 (0)	0 (0)	0 (0)	12 (100)	12 (100)	11 (92)	11 (92)
Ward Water Storage	2 (29)	1 (14)	2 (29)	4 (57)	10 (83)	11 (92)	11 (92)	11 (92)
Ward Water Storage (Treated)	0 (0)	0 (0)	0 (0)	0 (0)	12 (100)	12 (100)	9 (75)	11 (92)
Hand Hygiene								
Hand Hygiene Station (Clinicians/Staff)	6 (86)	5 (71)	6 (86)	2 (29)	12 (100)	12 (100)	8 (67)	9 (75)
Water and Soap Available	6 (86)	1 (14)	2 (29)	0 (0)	7 (58)	9 (75)	3 (25)	2 (17)
Hand Hygiene Station (Patients/Caregivers)	6 (86)	6 (86)	4 (57)	3 (43)	10 (83)	10 (83)	6 (50)	7 (58)
Water and Soap Available	1 (14)	0 (0)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hand Hygiene Promotion Materials Visible	3 (43)	0 (0)	2 (29)	0 (0)	12 (100)	12 (100)	11 (92)	12 (100)
Infection Prevention and Control (IPC)								
Gloves Available	7 (100)	4 (57)	7 (100)	6 (86)	11 (92)	12 (100)	12 (100)	12 (100)
Disinfectant Available	6 (86)	3 (43)	7 (100)	5 (71)	12 (100)	12 (100)	11 (92)	11 (92)
Clean Delivery Surface	-	-	5 (71)	-	-	-	5 (42)	-
Control Access Point for Ward Entry	7 (100)	-	-	-	12 (100)	-	-	-
Control Access Point Enforced	5 (71)	-	-	-	12 (100)	-	-	-
Personal Protective Equipment Required	6 (86)	-	-	-	7 (58)	-	-	-
Hand Washing Required	0 (0)	-	-	-	0 (0)	-	-	-
Fresh Gloves upon Entry	1 (14)	-	-	-	0 (0)	-	-	-
Hand Re-contamination upon Entry	0 (0)	-	-	-	0 (0)	-	-	-
Environmental Cleanliness								
Safely Segregated Waste (3 labeled bins)	3 (43)	2 (29)	5 (71)	2 (29)	3 (25)	2 (17)	0 (0)	0 (0)
Ward Visibly Clean (Dust and Soil)	6 (86)	7 (100)	7 (100)	7 (100)	10 (83)	11 (92)	2 (17)	1 (8)
Ward Spill Free (No spills of bodily fluids)	6 (86)	6 (86)	7 (100)	6 (86)	12 (100)	12 (100)	6 (50)	4 (33)
Ward Floors Visibly Clean	7 (100)	7 (100)	7 (100)	7 (100)	8 (67)	12 (100)	2 (17)	1 (8)
Staff Toilet	7 (100)	7 (100)	7 (100)	6 (86)	12 (100)	12 (100)	12 (100)	12 (100)
Staff Toilet Visibly Clean	7 (100)	5 (71)	6 (86)	6 (100)	10 (83)	9 (75)	11 (92)	11 (92)
Patient Toilet	7 (100)	7 (100)	7 (100)	7 (100)	12 (100)	12 (100)	12 (100)	12 (100)
Patient Toilet Visibly Clean	2 (29)	3 (43)	4 (57)	5 (71)	9 (75)	8 (67)	1 (8)	2 (17)

*Repeated assessments by ward over time

^ Neonatal ICU, † Kangaroo Mother Care, § Post-natal Care

Table 3. Sample Distribution by Hospital and Ward

	Felege Hiwot				Debere Tabor			
	Swab	Drinking Water	Water from Device	Handrinse	Swab	Drinking Water	Water from Device	Handrinse
Neonatal ICU	50	8	20	20	63	14	27	29
Kangaroo Mother Care	19	3	-	10	20	5	-	10
Delivery	19	10	-	10	20	8	-	9
Post-natal Care	18	5	-	10	20	5	-	10
Total	106	26	20	50	123	32	27	58
Hospital Total				202				240
Grand Total								442

*Repeated samples by ward over time

Table 4. Frequency* of Bacterial Contamination by Hospital and Ward

	Felege Hiwot								Debere Tabor							
	E. coli^		*S. aureus*		Other coliforms‡		Any Contamination		*E. coli*^		*S. aureus*		Other coliforms‡		Any Contamination	
	N Total	% Pos.	N Total	% Pos.	N Total	% Pos.	N Total	% Pos.	N Total	% Pos.	N Total	% Pos.	N Total	% Pos.	N Total	% Pos.
Neonatal ICU																
Swab	50	4	50	6	50	12	50	16	63	22	63	17	63	52	63	58
Handrinse	20	15	20	10	20	30	20	40	29	24	29	10	29	31	29	41
Water from Device	20	0	20	0	20	5	20	5	27	0	27	0	27	30	27	30
Drinking Water	8	0	-	-	-	-	8	0	14	7	-	-	-	-	14	14
Kangaroo Mother Care																
Swab	50	4	50	6	50	12	50	16	20	45	20	25	20	75	20	85
Handrinse	10	10	10	10	10	40	10	50	10	30	10	50	10	70	10	80
Drinking Water	8	0	-	-	-	-	8	0	5	20	-	-	-	-	5	20
Delivery																
Swab	17	12	17	0	17	12	17	33	20	5	20	5	20	0	20	10
Handrinse	10	0	9	33	9	11	10	41	9	11	9	0	9	11	9	11
Drinking Water	9	0	-	-	-	-	9	0	8	13	-	-	-	-	8	13
Post-natal Care																
Swab	16	6	16	6	16	6	16	13	20	20	20	5	20	30	20	45
Handrinse	10	10	10	60	10	0	10	70	10	10	10	10	10	0	10	20
Drinking Water	5	0	-	-	-	-	5	0	5	40	-	-	-	-	5	40

*Repeated assessments by ward over time

^Both membrane filtration and compact dry plate tests are included for *E. coli* handrinse samples, all other tests are compact dry plate only

‡Coliforms other than *E. coli*

† Limit of detection is <1 CFU per 1 mL for Compact Dry Plate Test

§ Limit of detection is <1 CFU per pair of hands for Membrane Filtration Test

Table 5. Hospital Swab Contamination by Patient Proximity

Felege Hiwot							
		E. coli		***S. aureus***		**Other Coliforms‡**	
Surface	N Total	N Positive	% Positive	N Positive	% Positive	N Positive	% Positive
Direct Patient Contact^	15	2	13	2	13	6	40
Other§	100	4	4	3	3	11	11

Debere Tabor							
		E. coli		***S. aureus***		**Other Coliforms‡**	
Surface	N Total	N Positive	% Positive	N Positive	% Positive	N Positive	% Positive
Direct Patient Contact^	44	18	41	6	14	25	57
Other§	94	8	9	8	9	30	32

*Repeated assessments by ward over time
‡Coliforms other than *E. coli*
† Limit of detection is <1 CFU per 1 mL for Compact Dry Plate Test
^Bedsheet, Blanket, Bed rail
§ Oxygen cylinder, Cabinet, Door/Door handle, CPAP, IV tube, Machine, Chair, Sink Faucet, Radiant Warmer, Floor, Ambubag, Fetal monitor, Oxygen nasal tube

Table 6. *E. coli* Concentrations in Positive Handrinse and Drinking Water Samples by Hospital and Ward*

	Felege Hiwot		Debere Tabor	
	Handrinse	Drinking Water	Handrinse	Drinking Water
	CFU (per pair of hands)[§]	CFU (per 100 mL)[†]	CFU (per pair of hands)[§]	CFU (per 100 mL)[†]
Cumulative	**N=37**	**N=25**	**N=47**	**N=32**
N Positive	4	-	11	6
Median	25	-	5	2
Range (Max, Min)	92 (94,2)	-	131 (132, 1)	8 (9,1)
Neonatal ICU	**N=15**	**N=8**	**N=22**	**N=14**
N Positive	3	-	5	1
Median	46	-	15	2
Range (Max, Min)	92 (94, 2)	-	131 (132, 1)	0 (2, 2)
Kangaroo Mother Care	**N=8**	**N=3**	**N=9**	**N=5**
N Positive	1	-	4	1
Median	4	-	5.5	1
Range (Max, Min)	0 (4,4)	-	11 (12,1)	0 (1,1)
Delivery	**N=7**	**N=9**	**N=7**	**N=8**
N Positive	-	-	-	1
Median	-	-	-	7
Range (Max, Min)	-	-	-	0 (7, 7)
Post-natal Care	**N=7**	**N=5**	**N=9**	**N=5**
N Positive	-	-	1	2
Median	-	-	5	5.5
Range (Max, Min)	-	-	0 (5, 5)	7 (9, 2)

*Repeated assessments by ward over time

^Membrane Filtration method was used for quantitative colony forming unit counts

§ Limit of detection is <1 CFU per pair of hands

† Limit of detection is <1 CFU per 100 mL

Table 7. *E. coli*^ Contaminated Hand Demographics by Hospital

	Felege Hiwot (n=5)	Debere Tabor (n=13)
	%	%
Male Sex	20	8
Female Sex	80	92
Doctor	20	8
Nurse	20	38
Midwife	20	8
Mother	40	38
Caregiver	-	8

*Repeated assessments by ward over time

^Both membrane filtration and compact dry plate tests are included

§ Limit of detection is <1 CFU per pair of hands for Membrane Filtration

† Limit of detection is <1 CFU per 1 mL for Compact Dry Plate

Table 8. WASH Score Distribution by Hospital and Ward

	Aggregated Hospitals	Felege Hiwot					Debere Tabor				
		All	NICU^	KMC†	Delivery	PNC§	All	NICU^	KMC†	Delivery	PNC§
Max	31.5	25.0	21.5	17.5	25.0	15.5	31.5	31.5	31.5	22.5	23.5
Q3	23.5	20.0	20.0	15.0	25.0	3.0	27.5	28.5	29.0	21.0	21.5
Median	19.5	18.5	19.0	11.5	19.5	0.5	21.0	26.5	27.5	16.0	20.0
Q1	15.0	11.5	18.5	10.0	15.0	-0.5	18.5	19.5	23.0	1.0	10.5
Min	-3.5	-3.5	5.0	-3.5	7.0	-1.0	-1.0	17.5	1.0	1.0	-1.0
Range	35.0	28.5	16.5	21.0	18.0	16.0	32.5	14.0	30.5	21.5	24.5

^ Neonatal ICU

† Kangaroo Mother Care

§ Post-natal Care

Table 9. Logistic Regression Model to Predict Any Contamination‡

	Full Model			Backward Selection			Final Model§		
	Parameter Estimate	95% CI	p value	Parameter Estimate	95% CI	p value	Parameter Estimate	95% CI	p value
Intercept	0.48	0.14, 1.64	0.24	0.29	0.20, 0.42	<0.0001	0.24	0.13, 0.46	<0.0001
WASH Score	0.96	0.90, 1.03	0.24	-	-	-	1.01	0.98, 1.05	0.48
Hospital (1)^	0.99	0.23, 1.84	0.97	0.62	0.50, 0.78	<0.0001	0.65	0.51, 0.83	0.0007
Ward (1)^	2.02	0.67, 6.10	0.21	0.93	0.66, 1.32	0.7	0.87	0.59, 1.29	0.5
Ward (2) ^	1.42	0.50, 4.01	0.51	2.97	1.88, 4.69	<0.0001	2.90	1.82, 4.60	<0.0001
Ward (3)^	0.46	0.13, 1.60	0.22	0.39	0.23, 0.65	0.0004	0.39	0.23, 0.67	0.0005
Sample Type (1)^	0.86	0.29, 2.57	0.79	1.91	1.29, 2.80	0.001	1.92	1.30, 2.82	0.0009
Sample Type (2)^	0.28	0.041, 1.91	0.19	0.28	0.13, 0.59	0.0009	0.28	0.13, 0.59	0.0008
Sample Type (3)^	1.2	0.11, 13.39	0.88	0.76	0.40, 1.43	0.39	0.75	0.40, 1.43	0.39
Month	0.97	0.87, 1.10	0.66	-	-	-	-	-	-
WASH Score * Hospital (1)	0.97	0.94, 1.00	0.088	-	-	-	-	-	-
WASH Score * Ward (1)	0.96	0.91, 1.01	0.13	-	-	-	-	-	-
WASH Score * Ward (2)	1.04	0.98, 1.10	0.17	-	-	-	-	-	-
WASH Score * Ward (3)	1.00	0.93, 1.07	0.95	-	-	-	-	-	-
WASH Score * Sample Type (1)	1.04	0.99, 1.10	0.099	-	-	-	-	-	-
WASH Score * Sample Type (2)	1	0.91, 0.92	0.95	-	-	-	-	-	-
WASH Score * Sample Type (3)	0.98	0.88, 1.09	0.75	-	-	-	-	-	-
WASH Score * Month	1.00	0.99, 1.01	0.31	-	-	-	-	-	-
AIC			503.3			498.84			498.84
Global Null Hypothesis LRT (β=0)			-			<0.0001			<0.0001

‡ Contamination with *E. coli, S. aureus,* and/or other coliforms

^ Class variables: effect coding, class variables are compared with last level
Levels for class variables: Hospital (1=Felege Hiwot, 2=Debere Tabor), Ward (1=Neonatal ICU, 2=Kangaroo Mother Care, 3=Delivery, 4=Post-natal care), Sample Type (1=Swab, 2=Drinking Water, 3=Water from Device, 4=Handrinse)

§ Retained predictor wash score (not significant)

Table 10. Odds Ratios* of Model Parameters from Proposed Final Model

	Parameter Estimate	95% CI	p value
WASH Score	1.01	0.98, 1.05	0.48
Hospital (1)^	0.42	0.26, 0.70	<0.001
Ward (1)^	0.87	0.42, 1.82	0.71
Ward (2) ^	2.89	1.29, 6.46	0.009
Ward (3)^	0.39	0.17, 0.92	0.03
Sample Type (1)^	0.76	0.47, 1.26	0.29
Sample Type (2)^	0.11	0.039, 0.31	<0.0001
Sample Type (3)^	0.30	0.13, 0.72	0.007

*Estimated Odds Ratios

‡ Contamination with *E. coli, S. aureus,* and/or *o*ther coliforms

^ Class variables: effect coding, class variables are compared with last level

Levels for class variables: Hospital (1=Felege Hiwot, 2=Debere Tabor), Ward (1=Neonatal ICU, 2=Kangaroo Mother Care, 3=Delivery, 4=Post-natal care), Sample Type (1=Swab, 2=Drinking Water, 3=Water from Device, 4=Handrinse)

Figure 1. WASH Score by Hospital

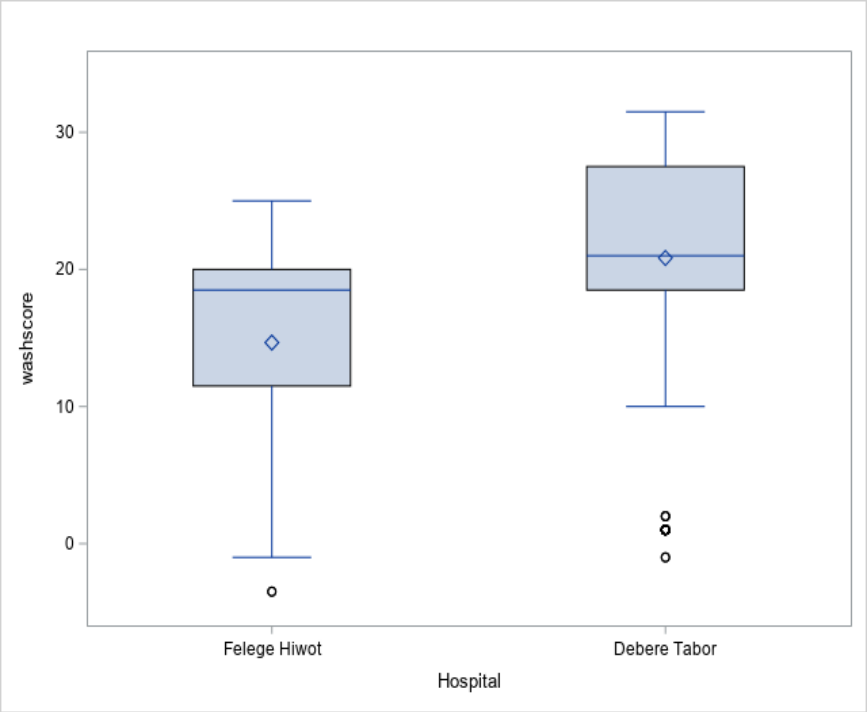
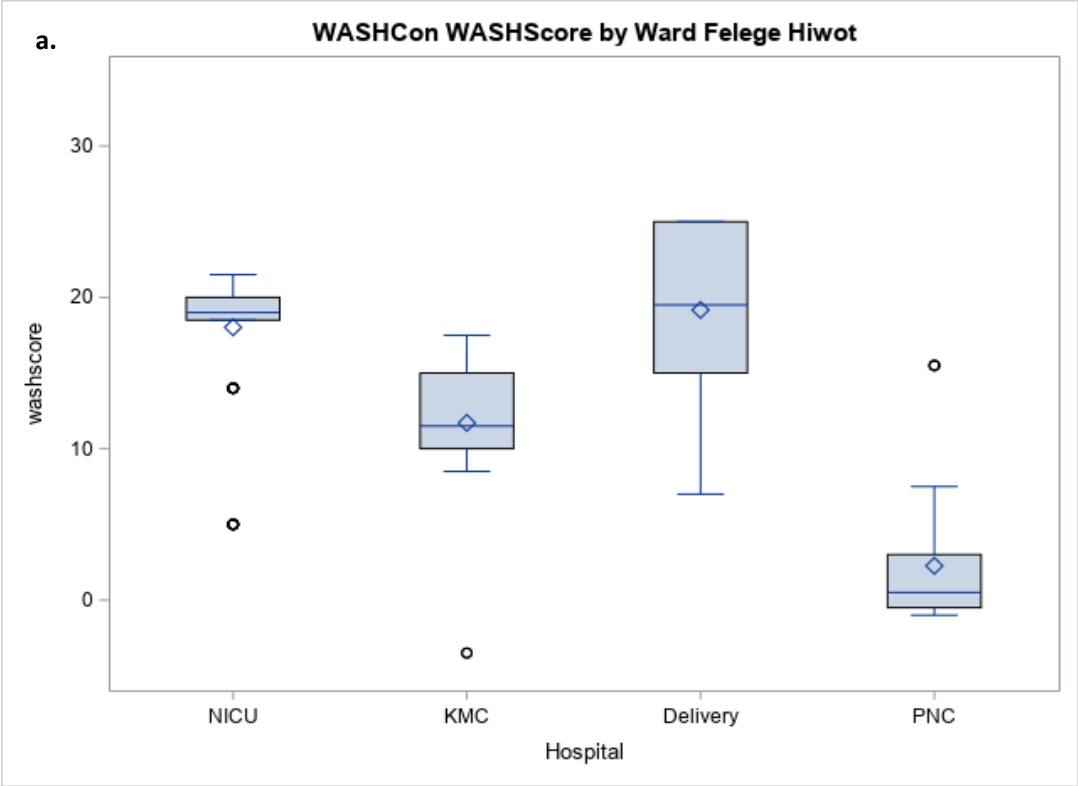


Figure 2. WASHCon Lite WASH Score by Hospital Ward



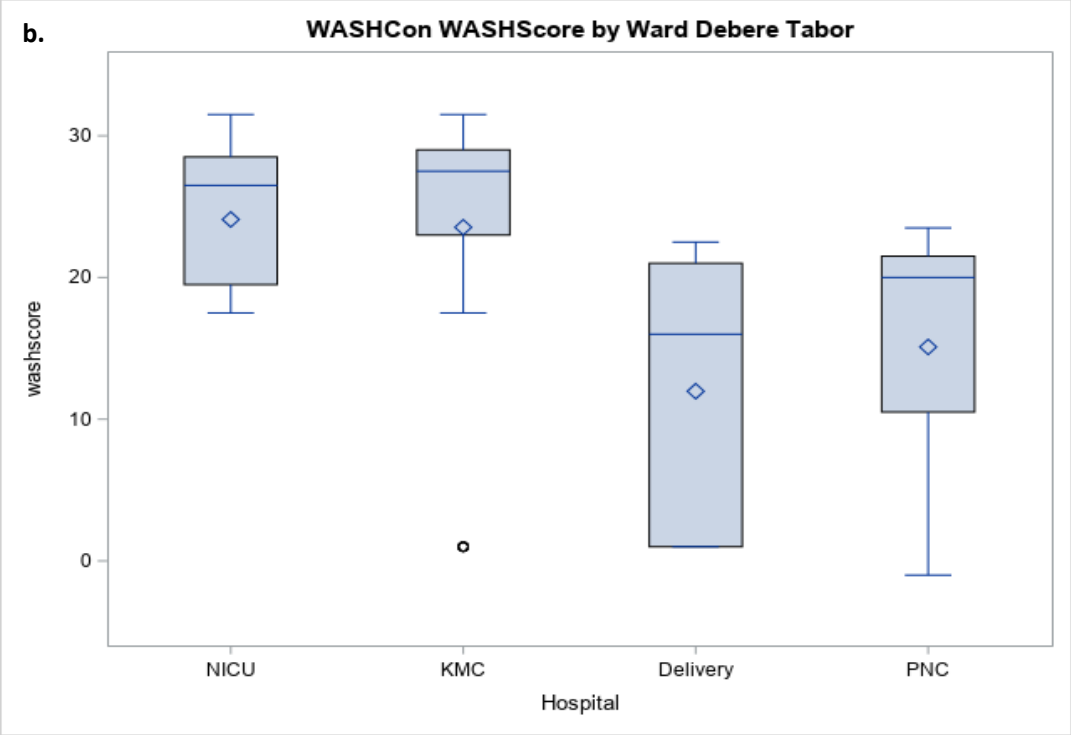


Figure 3. WASH Score and Percent Bacterial-negative Samples over Time by Ward

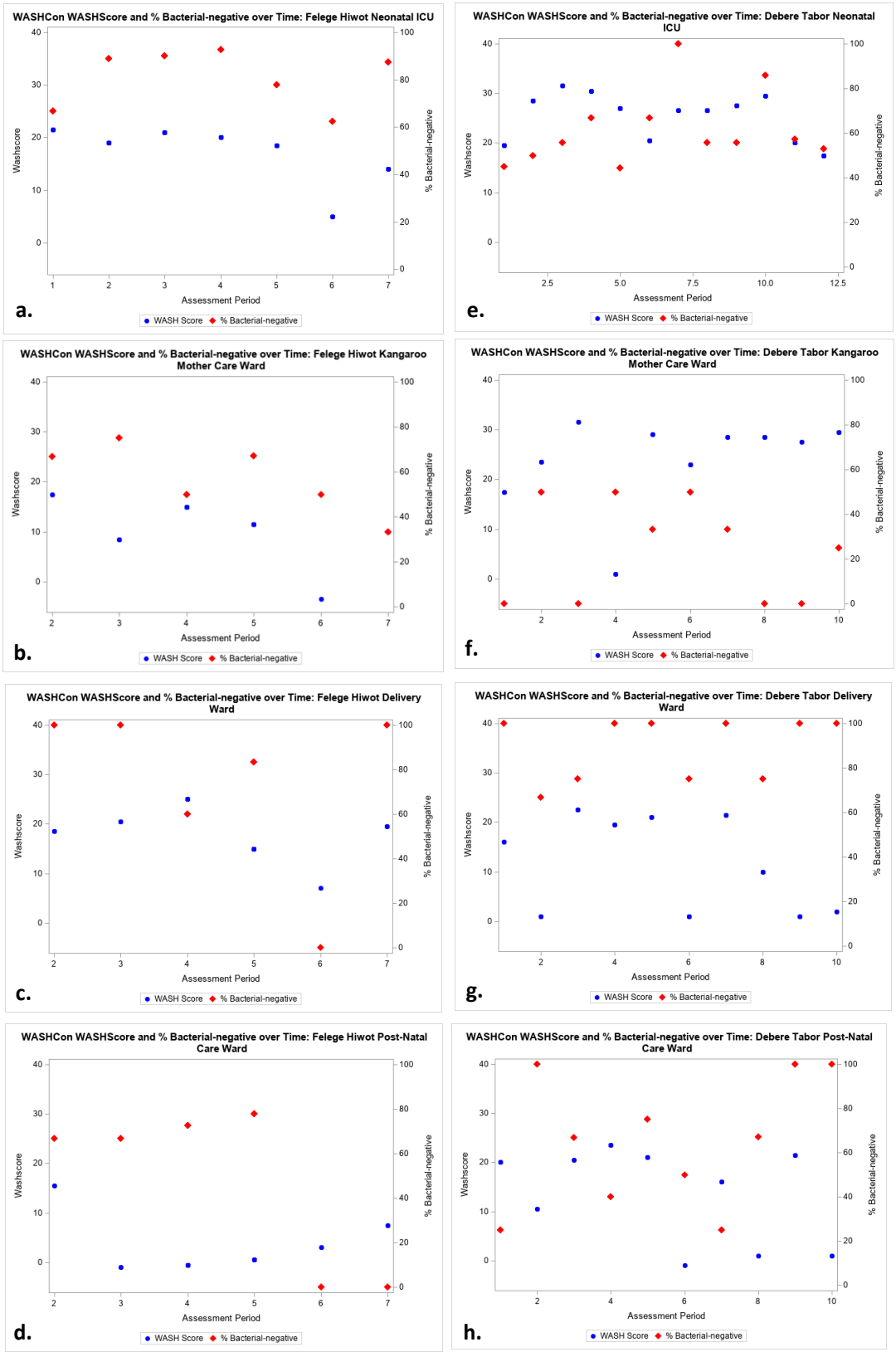
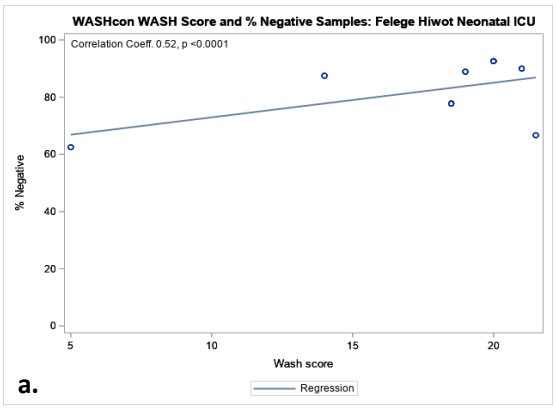
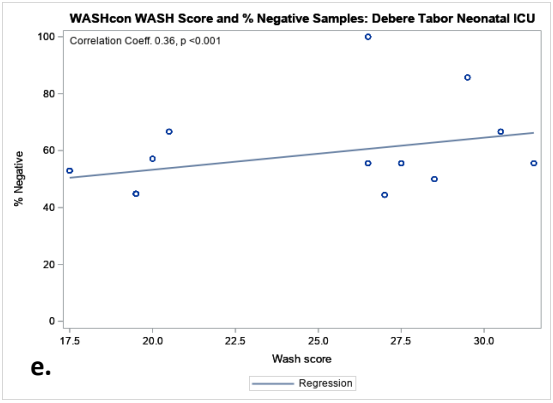
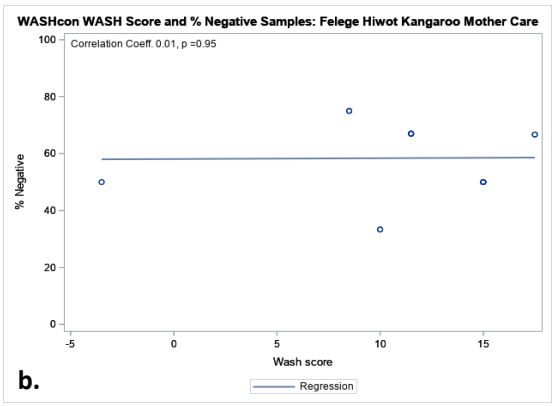
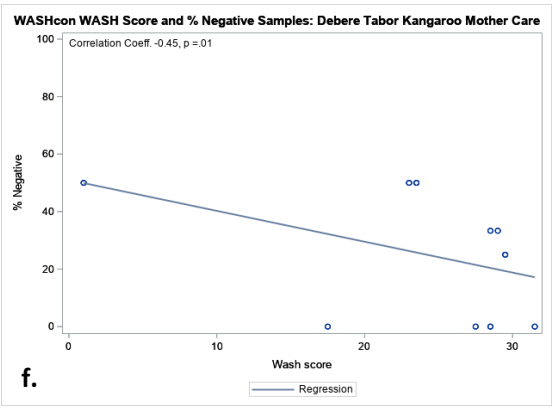


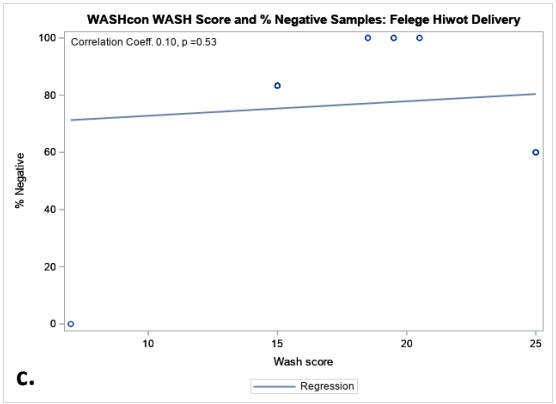
Figure 4. WASH Score and Percent Negative Samples Correlation Plots by Ward

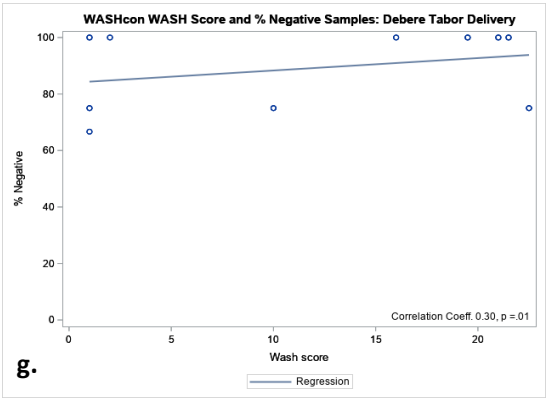


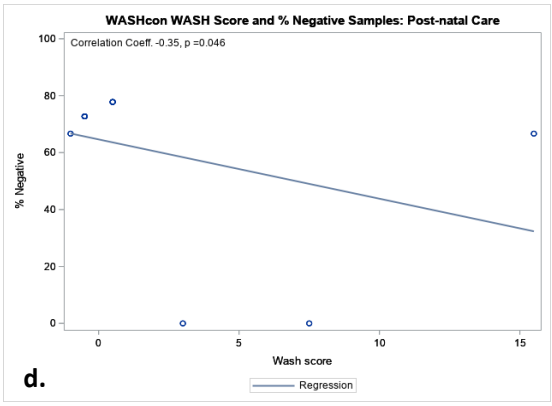


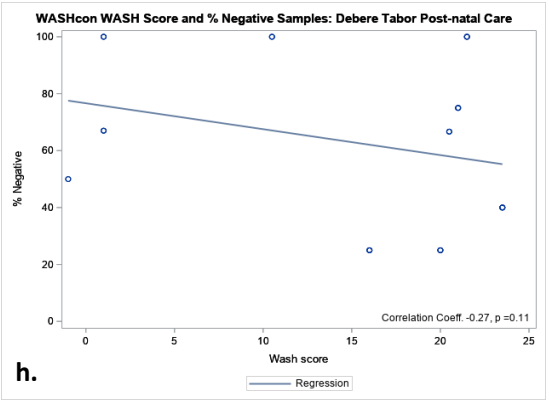












Appendix

Appendix A. WASH Conditions "WASHCon" Lite Assessment Tool

1.	Which ward are you observing?	☐ Delivery Room ☐ Post-natal Care ☐ NICU ☐ KMC ☐ Other
2.	Specify Other *Free Response*	
3.	Is water piped into this ward?	☐ Yes ☐ No
4.	What type of water is currently available in this ward?	☐ Treated water ☐ Untreated water ☐ Treated and untreated water ☐ No water available ☐ Didn't Observe
5.	Is water piped into this ward, functional?	☐ Yes ☐ No
6.	Is water available during the visit?	☐ Yes ☐ No
7.	How is water accessed in the ward? *Select all that apply*	☐ Pipe taps ☐ Uncovered buckets/barrels ☐ Covered buckets/barrels ☐ Uncovered buckets with taps on bottom ☐ Covered buckets with taps on bottom ☐ Jerrycans ☐ Other ☐ Didn't observe
8.	Is water stored in the health facility	☐ Yes ☐ No
9.	Specify other (7) *Free response*	☐
10.	Is water stored in the ward?	☐ Yes ☐ No
11.	How is water stored in the ward?	☐ Storage Tank ☐ Covered container ☐ Uncovered container ☐ Jerrycan ☐ Other ☐ Didn't observe
12.	What type of (stored) water is currently available in the ward?	☐ Treated water ☐ Untreated water ☐ Jerrycan ☐ Other ☐ Didn't observe

		☐ No water available
13.	Is there a functional hand hygiene facility at the point of care for healthcare providers?	☐ Yes ☐ No
14.	Observe and select available hand hygiene materials. *Select all that apply*	☐ Water only ☐ Soap only ☐ Hand sanitizer only ☐ Water and soap ☐ Water and sanitizer ☐ Soap and sanitizer ☐ Water, soap and sanitizer ☐ No supplies available ☐ Didn't observe
15.	Is there a functional hand hygiene facility accessible to patients/caregivers?	☐ Yes ☐ No
16.	Observe and select available hand hygiene materials. *Select all that apply*	☐ Water only ☐ Soap only ☐ Hand sanitizer only ☐ Water and soap ☐ Water and sanitizer ☐ Soap and sanitizer ☐ Water, soap and sanitizer ☐ No supplies available ☐ Didn't observe
17.	Observe if the following supplies are available today in the ward. *Select all that apply*	☐ Disposable latex gloves ☐ Environmental disinfectant (chlorine, ethanol, alcohol) ☐ Hand sanitizer ☐ Soap/detergent ☐ Mop and bucket ☐ Broom ☐ No supplies available ☐ Didn't observe
18.	Observe if the following supplies are available today in the delivery room. *Select all that apply*	☐ Disposable latex gloves ☐ Environmental disinfectant (chlorine, ethanol, alcohol) ☐ Hand sanitizer ☐ Soap/detergent ☐ Mop and bucket ☐ Broom ☐ Clean blade for cord cutting ☐ Clean cord for tying ☐ Clean towels to wrap baby and mother ☐ Clean delivery surface ☐ Clean diaper ☐ Didn't observe

19.	Is waste safely segregated into at least 3 labeled bins, including sharps waste, infectious waste, and non-infectious waste?	☐ Yes ☐ Yes, but does not meet all requirements ☐ No
20.	Is the ward visibly clean and free from dust and soil?	☐ Yes ☐ No
21.	Are there uncleaned spills from bodily fluids (blood, urine, feces, vomit etc.)?	☐ Yes ☐ No
22.	Are the floors clean?	☐ Yes ☐ No
23.	Is environmental disinfectant used in the ward?	☐ Yes, always ☐ Yes, sometimes ☐ Don't know ☐ No
24.	Are there hand hygiene promotion materials clearly visible and at key places in the ward?	☐ Yes ☐ No ☐ Didn't observe
25.	Is there a control access point into the NICU that is monitored by staff at the time of the visit (PPE)?	☐ Yes ☐ No ☐ Didn't observe
26.	Is controlled access being enforced?	☐ Yes ☐ No ☐ Didn't observe
27.	Do you observe non-family, non-clinical staff beyond the control access point? (Ex. maintenance staff)	☐ Yes ☐ No ☐ Didn't observe
28.	Are you required to wear a mask, shoe covers and fresh gown?	☐ Yes ☐ No
29.	Is the PPE separate for staff and caregivers?	☐ Yes ☐ No ☐ Didn't observe
30.	Does the PPE appear to be clean?	☐ Yes ☐ No ☐ Didn't observe
31.	Are you required to wash your hands before passing through the control access point?	☐ Yes ☐ No ☐ Didn't observe
32.	What materials are used for handwashing? *Select all that apply*	☐ Water ☐ Soap ☐ Hand sanitizer ☐ No supplies available ☐ Didn't observe
33.	Do staff put on fresh gloves before entering the NICU?	☐ Yes ☐ No ☐ Didn't observe
34.	Do staff re-contaminate their hands before entering the NICU?	☐ Yes ☐ No ☐ Didn't observe

35.	Where does the PPE (gloves, mask, shoe covers, gown) go after it is used?	☐ Laundry ☐ Garbage ☐ Reused ☐ Other ☐ Didn't observe
36.	Observe the staff toilet for this ward. Is it visibly clean, with no presence of feces, blood or bodily fluids?	☐ Yes ☐ No ☐ No staff toilet for the ward ☐ Didn't observe
37.	Observe the patient toilet for this ward. Is it visibly clean, with no presence of feces, blood or bodily fluids?	☐ Yes ☐ No ☐ No staff toilet for the ward Didn't observe
38.	Specify other *Free response*	
39.	Provide any comments about the WASH conditions or infection control practices of the staff today in this ward. *Free response*	

Appendix B. Table 1. Felege Hiwot WASHCon Lite Assessment Dates and Matched Environmental Samples

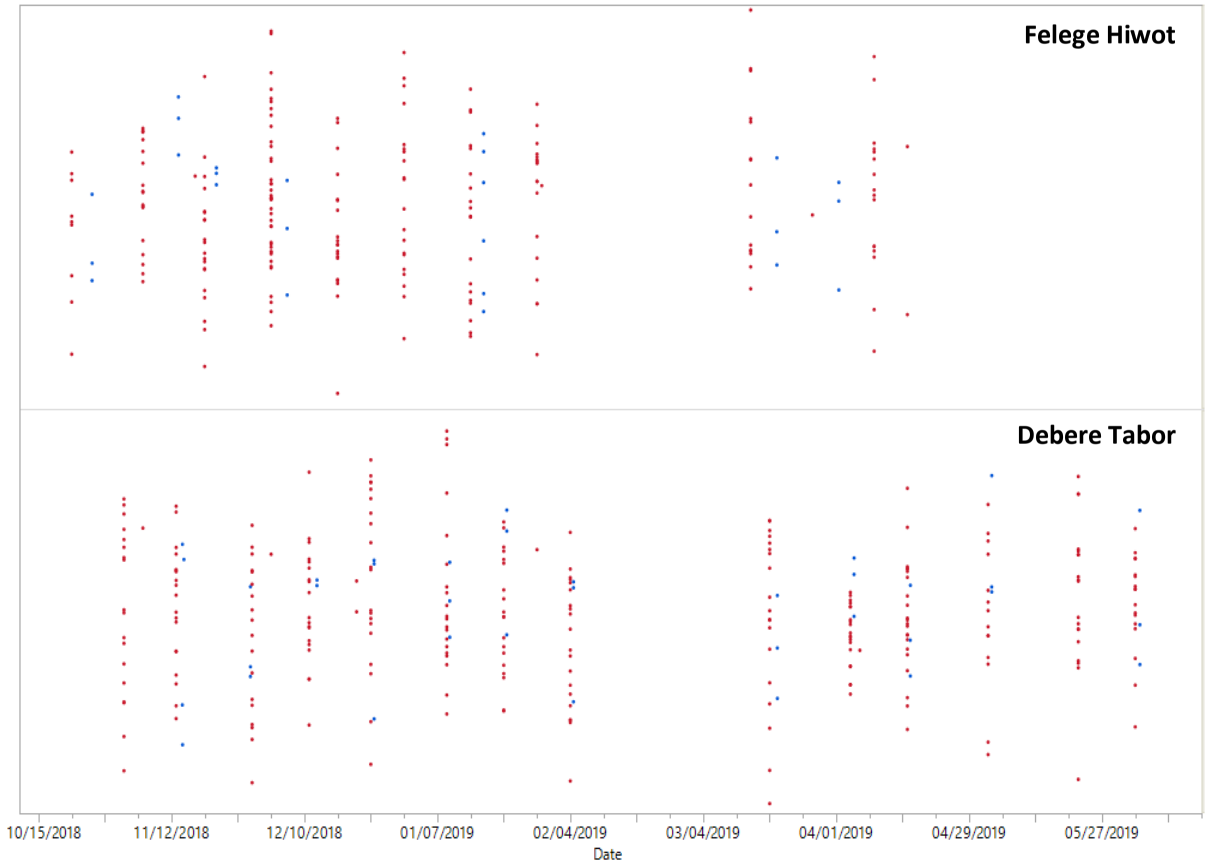
Felege Hiwot

Assessment	Assessment Date	Environmental Samples	Max Days Between
1	10/26/2018	10/22/2018	4
2	11/13/2018	11/6/2018	7
3	11/21/2018	11/17/2018 - 11/19/2018	4
4	12/6/2018	12/3/2018-12/17/2018	11
5	1/16/2019	12/31/2018-1/29/2019	16
6	3/19/2019	3/14/2019	5
7	4/1/2019	3/27/2019 - 4/16/2019	15

Debere Tabor

Assessment	Assessment Date	Environmental Samples	Max Days Between
1	11/14/2018	11/2/2018 - 11/13/2018	12
2	11/28/2018	11/29/2018 - 12/3/2018	5
3	12/12/2018	12/11/2018	1
4	12/24/2018	12/21/2018 - 12/24/2018	3
5	1/9/2019	1/9/2019	0
6	1/21/2019	1/21/2019 - 1/28/2019	7
7	2/4/2019	2/4/2019	0
8	3/19/2019	3/18/2019	1
9	4/4/2019	4/4/2019	0
10	4/16/2019	4/16/2019	0
11	5/3/2019	5/3/2019	0
12	6/3/2019	5/22/2019 - 6/3/2019	12

Appendix C. Distribution of WASHCon and Environmental Samples by Date and Facility

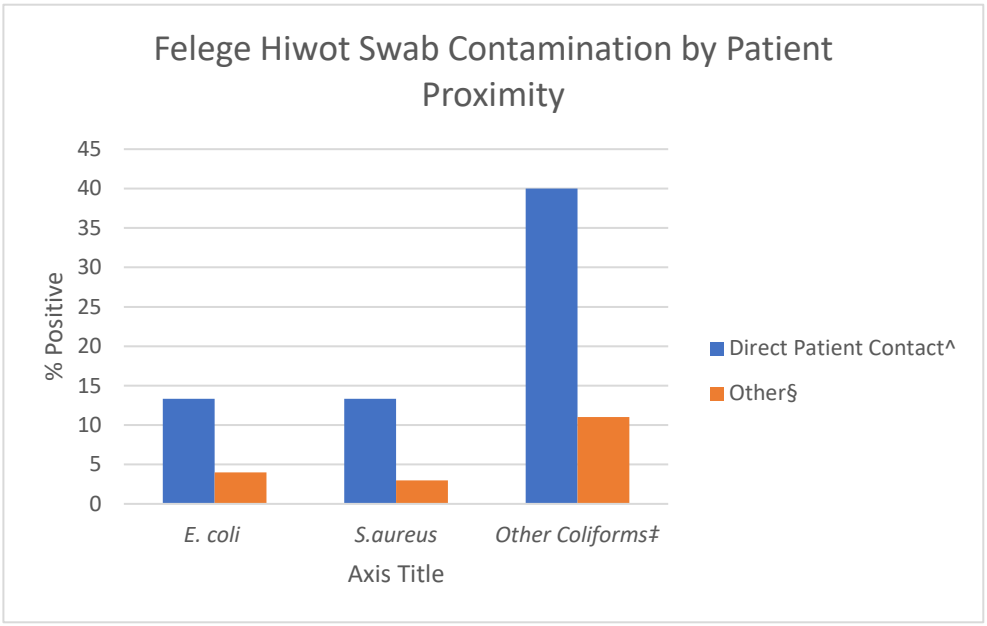


Key:

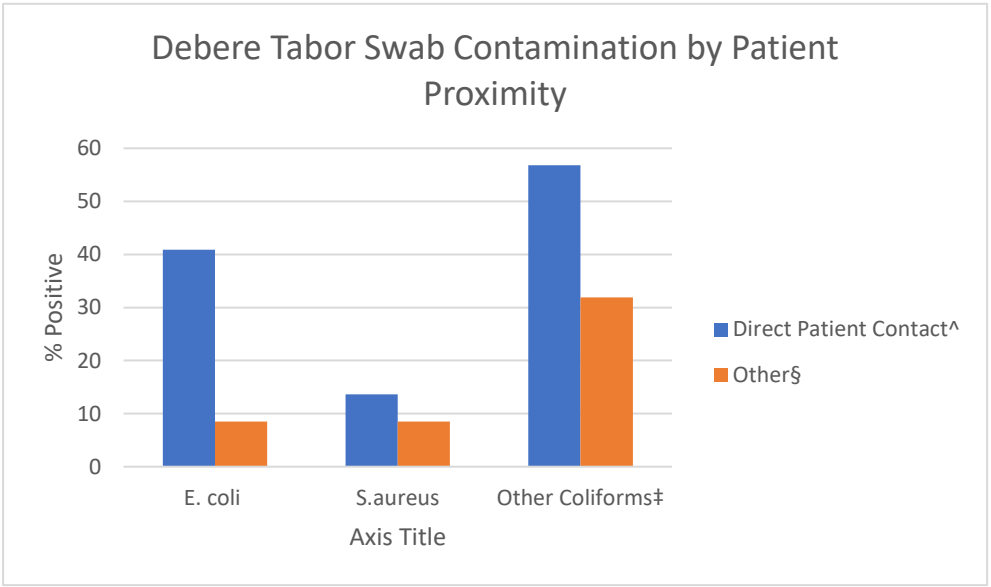
- Environmental Samples
- WASHCon Lite Assessments

Appendix D. Swab Contamination by Patient Proximity

a.



b.



‡Coliforms other than *E. coli*

^Bedsheet, Blanket, Bed rail

§ Oxygen cylinder, Cabinet, Door/Door handle, CPAP, IV tube, Machine, Chair, Sink Faucet, Radiant Warmer, Floor, Ambubag, Fetal monitor, Oxygen nasal tube

Appendix E. WASH Scoring Model

a. Hand Hygiene						
Did not observe coded as missing						
* NICU Only						
**Delivery Only						
used in multiple categories						
		Responses				
Question # (WASHCon Lite)	**Question**	**GOOD**		**MODERATE**		**POOR**
13	Is there a functional hand hygiene facility at POC for health care providers?	Yes				No
14	Observe and select the available hand hygiene materials (Providers)	Water and soap		Water and sanitizer		Soap only
		Water, soap and sanitizer		Water only		No supplies available
				Hand sanitizer only		
				Soap and sanitizer		
15	Is there a functional hand hygiene facility accessible to patients/caregivers?	Yes				No
16	Observe and select the available hand hygiene materials (Patients)	Water and soap		Water and sanitizer		Soap only
		Water, soap and sanitizer		Water only		No supplies available
				Hand sanitizer only		
				Soap and sanitizer		
24	Are there hand hygiene promotion materials clearly visible and understandable at key places within the ward?	Yes				No
*31	Are you required to wash your hands before passing through the control access point? (NICU)	Yes				No
*32	What materials are used for handwashing (control access point)	Water		Water only		No supplies available

b. Hand Hygiene (LONG)

Long format (1 Yes / 0 No) for coding purposes. Responses broken down by question in cases of non-binary answers.

Did not observe coded as missing

* NICU Only

**Delivery Only

used in multiple categories

Question # (WASHCon Lite)	Question	Responses GOOD	MODERATE	POOR
13	Is there a functional hand hygiene facility at POC for health care providers?	Yes		No
14.1	Observe and select the available hand hygiene materials (Providers): **Water and soap**	Yes		
14.2	Observe and select the available hand hygiene materials (Providers): **Water soap and sanitizer**	Yes		
14.3	Observe and select the available hand hygiene materials (Providers): **Water and sanitizer**		Yes	
14.4	Observe and select the available hand hygiene materials (Providers): **Water only**		Yes	
14.5	Observe and select the available hand hygiene materials (Providers): **Hand sanitizer only**		Yes	
14.6	Observe and select the available hand hygiene materials (Providers): **Soap and sanitizer**		Yes	
14.7	Observe and select the available hand hygiene materials (Providers): **Soap only**			Yes
14.8	Observe and select the available hand hygiene materials (Providers): **No supplies available**			Yes
15	Is there a functional hand hygiene facility accessible to patients/caregivers?	Yes		No
16.1	Observe and select the available hand hygiene materials (Patients): **Water and soap**	Yes		
16.2	Observe and select the available hand hygiene materials (Patients): **Water soap and sanitizer**	Yes		
16.3	Observe and select the available hand hygiene materials (Patients): **Water and sanitizer**		Yes	
16.4	Observe and select the available hand hygiene materials (Patients): **Water only**		Yes	
16.5	Observe and select the available hand hygiene materials (Patients): **Hand sanitizer only**		Yes	
16.6	Observe and select the available hand hygiene materials (Patients): **Soap and sanitizer**		Yes	
16.7	Observe and select the available hand hygiene materials (Patients): **Soap only**			Yes
16.8	Observe and select the available hand hygiene materials (Patients): **No supplies available**			Yes
24	Are there hand hygiene promotion materials clearly visible and understandable at key places within the ward?	Yes		No
*31	Are you required to wash your hands before passing through the control access point? (NICU)	Yes		No
*32.1	What materials are used for handwashing (control access point): **Water**	Yes		
*32.2	What materials are used for handwashing (control access point): **Soap**	Yes		
*32.3	What materials are used for handwashing (control access point): **Hand Sanitizer**	Yes		
*32.4	What materials are used for handwashing (control access point): **Water only**		Yes	
*32.5	What materials are used for handwashing (control access point): **No supplies available**			Yes

c. Infection Prevention and Control (IPC)						
Did not observe coded as missing						
* NICU Only						
**Delivery Only						
used in multiple categories						
		Responses				
Question # (WASHCon Lite)	**Question**	**GOOD**		**MODERATE**		**POOR**
17	Observe if the following resources/supplies used for infection control are available today in the ward	Disposable Latex Gloves				No supplies available
		Environmental Disinfectant				
		Soap/detergent				
		Hand sanitizer				
**18	Observe if the following resources/supplies used for infection control are available today in the delivery room	Soap/detergent				
		gloves				
		Hand sanitizer				
		Environmental Disinfectant				
		Clean blade				
		Clean cord				
		Clean towels				
		Clean delivery surface				
		Clean diaper				
19	Is waste safely segregated into at least three labeled bins, including sharps, infectious waste and non-infectious?	Yes, and met all requirements		Yes but did not meet all requirements		No
*28	Are you required to wear a mask, shoe covers, and a gown?	Yes				No
30	Does the PPE appear to be clean?	Yes				No
*31	Are you required to wash your hands before passing through the control access point? (NICU)	Yes				No
*32	What materials are used for handwashing (control access point)	Water		Water only		No supplies available
		Soap				
		Hand sanitizer				
*33	Do staff put on fresh gloves before etering the NICU?	Yes				No
*34	Do staff re-contaminate hands before entering the NICU?	Yes				No

d. Infection Prevention and Control (IPC) (LONG)

Long format (1 Yes / 0 No) for coding purposes. Responses broken down by question in cases of non-binary answers.

Did not observe coded as missing

* NICU Only

**Delivery Only

used in multiple categories

Question # (WASHCon Lite)	Question	Responses GOOD	MODERATE	POOR
17.1	Observe if the following resources/supplies used for infection control are available today in the ward: **Disposable Latex Gloves**	Yes		
17.2	Observe if the following resources/supplies used for infection control are available today in the ward: **Environmental Disinfectant**	Yes		
17.3	Observe if the following resources/supplies used for infection control are available today in the ward: **Soap/Detergent**	Yes		
17.4	Observe if the following resources/supplies used for infection control are available today in the ward: **Hand sanitizer**	Yes		
17.5	Observe if the following resources/supplies used for infection control are available today in the ward: **No supplies available**			Yes
**18.1	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Soap/detergent**	Yes		
**18.2	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Hand Sanitizer**	Yes		
**18.3	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Gloves**	Yes		
**18.4	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Environmental Disinfectant**	Yes		
**18.5	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Clean blade**	Yes		
**18.6	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Clean cord**	Yes		
**18.7	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Clean towel**	Yes		
**18.8	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Clean delivery surface**	Yes		
**18.9	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Clean diaper**	Yes		
19.1	Is waste safely segregated into at least three labeled bins, including sharps, infectious waste and non-infectious?: **Yes, and met all requirements**	Yes		
19.2	Is waste safely segregated into at least three labeled bins, including sharps, infectious waste and non-infectious?: **Yes but did not meet all requirements**		Yes	

19.3	Is waste safely segregated into at least three labeled bins, including sharps, infectious waste and non-infectious?: **No**					Yes
*28	Are you required to wear a mask, shoe covers, and a gown?:	Yes				No
30	Does the PPE appear to be clean?:	Yes				No
*31	Are you required to wash your hands before passing through the control access point? (NICU) **Yes**	Yes				No
*32.1	What materials are used for handwashing (control access point): **Water**	Yes				
*32.2	What materials are used for handwashing (control access point): **Soap**	Yes				
*32.3	What materials are used for handwashing (control access point): **Hand Sanitizer**	Yes				
*32.4	What materials are used for handwashing (control access point): **Water only**			Yes		
*32.5	What materials are used for handwashing (control access point): **No supplies available**					Yes
*33	Do staff put on fresh gloves before entering the NICU?	Yes				
*34	Do staff re-contaminate hands before entering the NICU?					Yes

f. Environmental Cleanliness

Did not observe coded as missing						
* NICU Only						
**Delivery Only						
used in multiple categories						
		Responses				
Question # (WASHCon Lite)	**Question**	**GOOD**		**MODERATE**		**POOR**
17	Observe if the following resources/supplies used for infection control are available today in the ward	Soap/detergent				No supplies available
		Mop & bucket				
		Broom				
		Environmental Disinfectant				
**18	Observe if the following resources/supplies used for infection control are available today in the delivery room	Soap/detergent				
		Mop & bucket				
		Broom				
		Environmental Disinfectant				
		Clean delivery surface				
19	Is waste safely segregated into at least three labeled bins, including sharps, infectious waste and non-infectious?	Yes, and met all requirements		Yes but did not meet all requirements		No
21	Are there uncleaned spills from bodily fluids?	No				Yes
22	Are the floors clean?	Yes				No
23	Is environmental disinfectant used in the ward?	Yes		Yes sometimes		No
36	Is the staff toilet for this ward visibly clean with no presence of feces, blood or bodily fluids?	Yes				No
						No staff toilet
37	Is the patient toilet for this ward visibly clean with no presence of feces, blood or bodily fluids?	Yes				No
						No patient toilet

g. Environmental Cleanliness (LONG)

Long format (1 Yes / 0 No) for coding purposes. Responses broken down by question in cases of non-binary answers.

Did not observe coded as missing

* NICU Only

**Delivery Only

used in multiple categories

Question # (WASHCon Lite)	Question	Responses GOOD	MODERATE	POOR
17.1	Observe if the following resources/supplies used for infection control are available today in the ward: **Soap/Detergent**	Yes		
17.2	Observe if the following resources/supplies used for infection control are available today in the ward: **Mop & bucket**	Yes		
17.3	Observe if the following resources/supplies used for infection control are available today in the ward: **Broom**	Yes		
17.4	Observe if the following resources/supplies used for infection control are available today in the ward: **Environmental Disinfectant**			
17.5	Observe if the following resources/supplies used for infection control are available today in the ward: **No supplies available**			Yes
**18.1	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Soap/detergent**	Yes		
**18.2	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Mop & Bucket**	Yes		
**18.3	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Broom**	Yes		
**18.4	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Environmental Disinfectant**	Yes		
**18.5	Observe if the following resources/supplies used for infection control are available today in the delivery room: **Clean delivery surface**	Yes		
19.1	Is waste safely segregated into at least three labeled bins, including sharps, infectious waste and non-infectious?: **Yes, and met all requirements**	Yes		
19.2	Is waste safely segregated into at least three labeled bins, including sharps, infectious waste and non-infectious?: **Yes but did not meet all requirements**		Yes	
19.3	Is waste safely segregated into at least three labeled bins, including sharps, infectious waste and non-infectious?: **No**			Yes
20	Is the ward visibly clean from dust and soil?	Yes		No
21	Are there uncleaned spills from bodily fluids?	No		Yes
22	Are the floors clean?	Yes		No
23.1	Is environmental disinfectant used in the ward?: **Yes**	Yes		
23.2	Is environmental disinfectant used in the ward?: **Yes sometimes**		Yes	
23.3	Is environmental disinfectant used in the ward?: **No**			Yes
23.4	Is environmental disinfectant used in the ward?: **Don't know**			Yes

36.1	Is the staff toilet for this ward visibly clean with no presence of feces, blood or bodily fluids?: **Yes**	Yes				
36.2	Is the staff toilet for this ward visibly clean with no presence of feces, blood or bodily fluids?: **No staff toilet**					Yes
36.3	Is the staff toilet for this ward visibly clean with no presence of feces, blood or bodily fluids?: **No**					Yes
37.1	Is the patient toilet for this ward visibly clean with no presence of feces, blood or bodily fluids?: **Yes**	Yes				
37.2	Is the patient toilet for this ward visibly clean with no presence of feces, blood or bodily fluids?: **No staff toilet**					Yes
37.3	Is the patient toilet for this ward visibly clean with no presence of feces, blood or bodily fluids?: **No**					Yes

g. Drinking Water

Did not observe coded as missing	
* NICU Only	
**Delivery Only	
used in multiple categories	

Availability

Question # (WASHCon Lite)	Question	Responses GOOD	MODERATE	POOR
3	Is water piped in ward?	Yes		No
4	What type of water is currently available in this ward?	Treated		No water available
		Untreated		
		Both treated and untreated		
5	Is water piped into this ward functional?	Yes		No
6	Is (flowing) water available during the visit?	Yes		No
7	How is water accessed in this ward?	Pipe taps	Covered buckets	Other
			Uncovered buckets	
			Covered buckets with tap on bottom	
			Uncovered buckets with tap on bottom	
			Jerrycan	
8	Is water stored in the health facility?	Yes		No
10	Is water stored in the ward?	No (if available piped water in 5)	Yes	No (if no available piped water in 5)

Quality

Question # (WASHCon Lite)	Question	Responses GOOD	MODERATE	POOR
4	What type of water is currently available in this ward?	Treated	Treated and untreated	Untreated
				No water available
7	How is water accessed in this ward?	Piped tap	Covered buckets	Uncovered buckets
		Covered bucket with taps on bottom	Jerrycan	Uncovered buckets with tap on bottom
				Other
11	How is water stored in this ward?	Storage tank	Covered container	Uncovered container
			Jerrycan	Other
12	What kind of (stored) water is currently available in this ward?	Treated	Treated and Untreated	Untreated
				No water available

h. Drinking Water (LONG)

Long format (1 Yes / 0 No) for coding purposes. Responses broken down by question in cases of non-binary answers.

Did not observe coded as missing

* NICU Only

**Delivery Only

used in multiple categories

Availability

Question # (WASHCon Lite)	Question	Responses GOOD	MODERATE	POOR
3	Is water piped in the ward?	Yes		No
4.1	What type of water is currently available in this ward?: **Treated**	Yes		
4.2	What type of water is currently available in this ward?: **Untreated**	Yes		
4.3	What type of water is currently available in this ward?: **Both treated and untreated**	Yes		
4.4	What type of water is currently available in this ward?: **No water available**			Yes
5	Is water piped into this ward functional? **Yes**	Yes		No
6.1	Is (flowing) water available during the visit?	Yes		No
7.1	How is water accessed in this ward?: **Pipe taps**	Yes		
7.2	How is water accessed in this ward?: **Covered buckets**		Yes	
7.3	How is water accessed in this ward?: **Uncovered buckets**		Yes	
7.4	How is water accessed in this ward?: **Covered buckets with tap on bottom**		Yes	
7.5	How is water accessed in this ward?: **Uncovered buckets with tap on bottom**		Yes	
7.6	How is water accessed in this ward?: **Jerrycan**		Yes	
7.7	How is water accessed in this ward?: **Other**			Yes
8.1	Is water stored in the health facility? **Yes**	Yes		No
10.1	Is water stored in the ward?: **Yes**		Yes	
10.2	Is water stored in the ward?: **No (piped water in q5)**	Yes		
10.3	Is water stored in the ward?: **No (no piped water in q5)**			Yes

Quality

Question # (WASHCon Lite)	Question	Responses GOOD	MODERATE	POOR
4.1	What type of water is currently available in this ward?: **Treated**	Yes		
4.2	What type of water is currently available in this ward?: **Both treated and untreated**		Yes	
4.3	What type of water is currently available in this ward?: **Untreated**			Yes
4.4	What type of water is currently available in this ward?: **No water available**			Yes

7.1	How is water accessed in this ward?: **Pipe taps**	Yes				
7.2	How is water accessed in this ward?: **Covered buckets with tap on bottom**	Yes				
7.3	How is water accessed in this ward?: **Covered Buckets**			Yes		
7.4	How is water accessed in this ward?: **Jerrycan**			Yes		
7.5	How is water accessed in this ward?: **Uncovered buckets**					Yes
7.6	How is water accessed in this ward?: **Uncovered buckets with tap on bottom**					Yes
7.7	How is water accessed in this ward?: **Other**					Yes
11.1	How is water stored in this ward?: **Storage tank**	Yes				
11.2	How is water stored in this ward?: **Covered container**			Yes		
11.3	How is water stored in this ward?: **Jerrycan**			Yes		
11.4	How is water stored in this ward?: **Uncovered container**					Yes
11.5	How is water stored in this ward?: **Other**					Code to 0?
12.1	What kind of (stored) water is currently available in this ward?: **Treated**	Yes				
12.2	What kind of (stored) water is currently available in this ward?: **Treated and untreated**			Yes		
12.3	What kind of (stored) water is currently available in this ward?: **Untreated**					Yes
12.4	What kind of (stored) water is currently available in this ward?: **No water available**					Yes