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The association between water, sanitation, and hygiene infrastructure, environmental contamination, and neonatal sepsis at two healthcare facilities in Amhara, Ethiopia

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Abstract

Background: Neonatal sepsis rates are high in Ethiopia where there is often inadequate water, sanitation, and hygiene (WASH) capacity in healthcare facilities (HCF). This leads to increased environmental contamination which can be passed to neonates via multiple routes, including contaminated hands, surfaces, and invasive medical devices, and cause healthcare-associated (HA) infections. The main objectives of this study are to determine if there is an association between HA neonatal sepsis, environmental contamination, and WASH capacity at two HCF in Amhara, Ethiopia.

Methods: A modified WASH Conditions Assessment Survey (WASHCon), was deployed over 32 weeks in five neonatal units of two Ethiopian HCF. Surveys were collected in conjunction with environmental and neonatal clinical samples. Multivariable logistic regression was conducted to determine an association between these variables.

Results: Felege Hiwot Hospital had a higher prevalence of neonatal sepsis, antimicrobial resistant (AMR) sepsis, and mortality. Debre Tabor Hospital had a higher frequency of environmental contamination, and environmental AMR isolates. Sepsis due to *Klebsiella spp.* was associated with hospital of birth (aOR: 0.11 (95%CI: 0.02-0.64); p=0.002), detection of environmental contamination in the NICU (aOR: 35.31 (95%CI: 1.54-808.85), p=0.03), and contaminated hands in the delivery unit (aOR: 5.50= (95%CI: 1.16-25.77), p=0.03). Sepsis due to *S. aureus* was associated with detection of environmental contamination in the NICU (aOR: 0.01 (95%CI: <0.01-0.50), p=0.024), and the frequency of hand contamination in the delivery (0.036 (95%CI: 0.004-0.33), p=0.003) and KMC units (aOR: 19.60 (95% CI:2.16-177.52), p=0.0081).

All cases of lab-confirmed neonatal sepsis were resistant to one or more antibiotics, and rates of resistance in environmental contamination isolates were high in both HCFs, making multivariable logistic regression impossible for this outcome. No association was found between WASH capacity and environmental contamination.

Conclusions: This study is the first to report an association between environmental contamination of hands and surfaces and HA neonatal sepsis in two HCF in Ethiopia. The prevalence of AMR environmental contamination was high in the clean and safe healthcare (CASH) certified facility, and resistance was 100% for all lab-confirmed sepsis cases. WASH Capacity did not align with contamination which warrants further investigation into facility cleaning and hand hygiene behaviors.

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Contents

Literature Review

Scope and Purpose

Topic: Healthcare-associated neonatal sepsis, environmental contamination, and healthcare facility (HCF) water, sanitation, and hygiene capacity Ethiopia.

Scope: This literature review will provide background on the morbidity and mortality of healthcare-associated (HA) neonatal sepsis and the importance of water, sanitation, and hygiene (WASH) capacity in HCF to prevent the environmental contamination found in medical devices, and on hands and surfaces, that contributes to HA neonatal sepsis and the associated mortality. This review will not include the topics of community-related neonatal sepsis, or WASH capacity or environmental contamination in the home. This review will also not address the topic of home births, maternal risk factors for neonatal sepsis, maternal sepsis and mortality, or community-associated antimicrobial resistant bacteria.

Exposure: WASH capacity, infrastructure, and behaviors, and environmental contamination from healthcare-associated bacteria.

Outcome: Neonatal sepsis, early onset sepsis (sepsis within the first 72 hours of birth), late onset sepsis (sepsis after the first 72 hours), pathogen-specific sepsis, antimicrobial resistant sepsis, and mortality.

Hypothesis: Poor WASH infrastructure and behaviors contribute to environmental contamination in the hospital, and newborn exposure to environmental contamination is associated with neonatal sepsis in HCF. Understanding the association between healthcare-associated neonatal sepsis, environmental contamination, and WASH capacity will guide the development of WASH-focused infection prevention and control recommendations for facilities to implement to prevent HA neonatal sepsis and ultimately reduce neonatal mortality.

Neonatal Sepsis

The global neonatal sepsis rate is estimated to be 2,202 per 100,00 live births (95% CI 1,099–4,360), or approximately 3 million cases annually.[1-4] Relative to other infectious diseases, neonatal sepsis causes 1.6 times the number of childhood deaths caused by malaria, and over 4 times the number caused by HIV globally.[5] Approximately 0.421 million neonatal deaths per year [6·7%; range 4·3–11·0%] are attributed to neonatal sepsis.[1, 6] And while the incidence of neonatal sepsis is declining at 2.6% per year, this a slower reduction than seen for other causes of deaths among neonates and children under five.[6] The United Nations (UN) Sustainable Development Goal (SDG) 3.2 targets the reduction of neonatal mortality to at least as low as 12 per 1000 live births in all countries by 2030.[5, 7, 8] The current rate of decline in deaths attributed to neonatal sepsis is not sufficient to achieve this goal, and increased annual reductions need to occur.

Neonatal healthcare-associated infection (HAI) rates are three to 20 times higher in low- and middle-income countries (LMIC) than in high-income countries (HIC).[9] Under half of sub-Saharan neonatal deaths occur in healthcare settings. However, mortality in the healthcare setting is expected to increase as the number of facility-based births also increases in order to achieve SDG 3.2.[10] This rise in institutional deliveries and referrals will intensify pressure on LMIC healthcare facilities (HCF) already struggling under the weight of inadequate infrastructure and insufficient human and financial resources.[3, 11]

Neonatal Sepsis in Ethiopia

Between 2014 and 2019, the Ethiopian neonatal mortality rate was 30 deaths per 1000 live births.[12] Sepsis is associated with mortality, and in Ethiopia, one third of neonatal deaths are due to sepsis.[13] A recent meta-analysis reported a national neonatal sepsis prevalence of 45% for Ethiopia, and 64.44% for the Amhara region specifically.[14] Of note, the I^2 value was 99.2% for

each of these meta-analyses indicating very high heterogeneity in the results. This heterogeneity likely arose from differences in the definitions for sepsis onset in each study, and whether diagnosis was determined via signs and symptoms or lab confirmation. None of the included studies defined the sepsis as either healthcare-associated (HA) or community-associated (CA), and most included both. Importantly, the included studies reported data from healthcare facilities (HCF) and neighborhoods with differing WASH capacities.[15-19] Despite this heterogeneity and bias, it is clear Ethiopian neonatal sepsis rates are high.

Neonatal Sepsis Definitions

The difficulty in providing consistent and precise estimates of the burden of neonatal sepsis stems from inconsistency in both the symptomatic and lab-based criteria for diagnosing sepsis, and a lack of consensus for a sepsis definition.[4] Neonatal sepsis is typically defined as a systemic inflammatory response due to infections of bacterial, viral, or fungal origin that is associated with hemodynamic changes occurring in the first 28 days of life.[4, 20-22] In many cases, these systemic changes are precipitated by an infection of the blood, urine, or spinal fluid, and while any sterile body fluid can be used for diagnosis, blood cultures are the gold standard. However, in LMIC, laboratory (lab) infrastructure in HCF is often insufficient or nonexistent which necessitates a sign and symptom-based algorithm for diagnosis that increases diagnostic sensitivity and decreases diagnostic specificity.

Once defined, neonatal sepsis is further categorized by time of onset in order to characterize risk factors and probable transmission routes. Early onset sepsis (EOS), which frequently is acquired via vertical transmission from the mother before or during birth, is defined as occurring in the first 72 hours of life.[4] EOS is also defined as occurring in the first 7 days of life. Late onset sepsis (LOS) is often defined as sepsis occurring after day four or seven until day 28, or even day 90, of life. LOS generally is acquired via horizontal transmission from the

environment and unhygienic practices.[3, 10, 23] Finally, very-late onset sepsis is variably defined as occurring after 28, 90, or 120 days and represents vertical or horizontal transmission to medically complex, often premature, neonates.[4, 20,24] These definition categories based on date of sepsis onset, are applied to categorize different risk factors for hospitalized infants in NICUs, where < 72 hours is the cutoff for preterm infants compared with <7 days in term infants.[25, 26] These definitions are unevenly applied across both HIC and LMIC settings and populations, and when hospital delivery can be confirmed relative to delivery at home. However, it is common for infants to be delivered at home in LMIC, which can result in sepsis in the early days of life and subsequent hospital admission. Community-associated sepsis can be caused by different pathogens and risk factors compared with HA sepsis, but these lines are increasingly blurred in LMIC.[10] These differences have resulted in the use of a different set of sepsis onset definitions for LMIC: CA or community-acquired sepsis is defined as occurring in the first two to seven days of life, and healthcare-acquired or HA sepsis occurs after day three or seven, and up to day 28 of life.[27-29] There is a wide range of variability in definitions, and in the application of these definitions. This variability increases the difficulty in the diagnosis and management of sepsis, the attribution of sepsis, and in ensuring adequate neonatal sepsis surveillance.[3, 26, 27] To simplify this variability, it has been proposed that any infection occurring within the first three days of life in an infant born at a LMIC HCF may be considered HA EOS.[3]

Until recently, Group B Streptococcus was the most common etiologic agent of EOS, but due to vaccinations in HIC, these rates are declining.[30] Globally, *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Klebsiella* species, and other gram-negative rods (*Pseudomonas spp.* and *Acinetobacter spp*.) are the most frequent causes of EOS. [10, 31] In LMIC, causal pathogens are similar for LOS including *S. aureus, Klebsiella,* and *coagulase*-negative staphylococci (CoNS), further blurring the EOS vs. LOS definitions. Environmental

contamination has frequently been implicated in the transmission of pathogens that cause neonatal sepsis in both healthcare and home settings.

Almost 70% of these infections do not respond to the empiric regimen of ampicillin and gentamicin, which not only makes these infections untreatable if resources are limited, but also encourages the development of resistance in these bacteria. This high prevalence of antimicrobial resistance (AMR) contributes to *E. coli* being one of the most common causes of EOS mortality.[32]

Antimicrobial-Resistant Neonatal Sepsis

The availability of diagnostic resources, treatment options, and AMR surveillance data resulted in the leveling off of AMR rates in high-income countries; however due to numerous reasons, these rates continue to rise in LMIC in both healthcare and community settings. Many LMIC do not have sufficient resources to designate to AMR surveillance. If a LMIC country has enacted AMR surveillance, it is common for data to be inconsistent or inaccurate. This is due to unreliable data management, the non-representativeness of local data, inconsistent laboratory training, inconsistent lab resources and management, scarce microbiological diagnostic capabilities, and infrequent quality assurance of results. These challenges are attributable to low financial and well-trained human resources.[33, 34] Despite these difficulties, it is estimated that almost 70% LMIC neonatal sepsis cases are resistant to the antibiotics the World Health Organization (WHO) recommends as empiric treatment, and *Klebsiella* spp. have the highest rates of resistance.[3, 9] These high rates of resistance stem from unregulated, over-the-counter purchasing of antibiotics in the community, and empiric treatment of illness with antibiotics due to the previously mentioned scarcity of laboratory and diagnostic resources in LMIC.[35, 36]

Specifically, sub-Saharan Africa is plagued by unregulated availability of antibiotics, scarce diagnostic resources, and inadequate availability of clean water, all of which contribute to

increases in AMR and AMR-associated mortality.[37, 38] Neonatal sepsis cases are frequently caused by AMR pathogens. Neonatal sepsis-causing pathogens with high resistance rates include *E. coli* and *Klebsiella*, both associated with EOS and LOS, and the pathogen most commonly associated with resistance,, *S. aureus*, which is most frequently associated with LOS.[25, 33] Understanding the true HA-AMR burden is difficult in sub-Saharan Africa due to issues of unclear attribution to setting and the use of different sepsis definitions. Over 50% of LMIC births occur in the home, and when reporting data, most studies do not clearly differentiate whether infants were born in the facility or admitted after birth when reporting sepsis; whether infants were born at a healthcare facility (HCF) and acquired sepsis after being sent home and were readmitted with CA sepsis; or whether these infants were born in a HCF and developed HA sepsis.[33, 39]

A longitudinal study conducted in Ethiopia found particularly high rates of resistance in neonatal sepsis.[40] Gram-positive bacteria resistance rates were 91.3% (ceftriaxone) to 98.9% (penicillin), and gram-negative bacteria resistance rates were 83.2% (gentamicin and ceftriaxone). These are rates are for antibiotics commonly used in the empiric treatment of neonatal infections, and this level of resistance to first- and second-line antimicrobials requires a complete revision of a HCF's empiric antimicrobial therapy plan.

Costs Associated with Neonatal Sepsis

Neonatal sepsis causes 1.6 times the number of childhood deaths compared with malaria, and four times the deaths due to HIV.[6] In sub-Saharan Africa an estimated 50% of morality in children under five is attributed to neonatal sepsis. This disproportionate burden of mortality is not reflected in investment made to prevent sepsis, when compared with investments made to prevent infectious diseases such as HIV or malaria.[41] The estimated economic burden associated with neonatal sepsis in sub-Saharan Africa ranges from $10 - $469 billion annually. This wide

range can be attributed to uncertain estimates in the prevalence of disease and the associated burden of mortality. It is also estimated that 5.29-8.73 million disability-adjusted life years (DALYs) are lost every year to neonatal sepsis.[5] These high estimates, and wide ranges of certainty, demonstrate the need for public health action and increased investment in neonatal sepsis research and prevention in this region.

Centers for Disease Control and Prevention (CDC) and WHO have formulated recommendations for the prevention of infections in neonates in HCF and neonatal intensive care units (NICU).[9, 42-45] While CDC develops recommendations intended for high-resource settings, the WHO recommendations are formulated to be implemented in LMIC and can either serve as a benchmark when developing facility policies, or as targeted recommendations for improvement when a root cause or risk assessment uncovers gaps in capacity or practice.

Healthcare Facility Environmental Contamination

Environmental contamination has been tied to numerous outbreaks of neonatal sepsis in HCF in both HIC and LMIC.[3, 46] However, it is difficult to determine the true attribution of environmental contamination of water, surfaces, or hands, to the incidence of HAI in general and healthcare-associated neonatal sepsis in particular, whether endemic or outbreak in nature.[47, 48] This is because infection does not result solely from the presence of contamination, but also from the amount of contamination, type of exposure, virulence of the pathogen, and susceptibility of the host. All of these factors can converge in neonatal units, making these healthcare units priority areas for targeting interventions. Specifically, improvements in clean delivery practices and compliance with the WHO “Six Cleans of Delivery”, are associated with reductions in neonatal sepsis and mortality.[45, 49, 50]

Environmental persistence is different for each pathogen and is based on the conditions of the physical environment. Gram-positive (GP) organisms generally persist in dry conditions

on dust and surfaces, gram-negative (GN) organisms can grow and persist in moist, soiled environments.[47, 48, 51] Persistence can be lengthy for GN bacteria, and Klebsiella in particular has been shown to survive up to 30 months on surfaces.[51, 52] This, in combination with the diversity of growing environments for fungi and persistence conditions for viruses, make cleaning and disinfection a priority in healthcare environments. Safe environmental conditions in HCF is identified by the cleanliness of surfaces and hands. The foundations that ensure these safe environmental conditions include infrastructure, supplies, daily behaviors and practices, and the administrative dedication, feedback to all personnel, and accountability .[53]

Adequate quantity and quality of water is essential to the basic functioning of any HCF.[49, 54-56] Contaminated water has been linked to outbreaks in LMIC HCF where contaminated water was used during maternal and child care and to fill a medical device reservoir or clean an invasive medical device. Additionally, inadequate water quantity and quality can deter a mother from seeking medical care at HCFs or cause a mother to leave a facility early if there is insufficient water quantity or quality for bathing and personal hygiene. These factors could result in mothers postponing care until an infection is so severe it becomes untreatable which contributes to increased mortality from neonatal sepsis.

The hands of those who care for infants in healthcare settings, frequently healthcare personnel and mothers, are implicated as sources of pathogens, and pose a risk, especially to vulnerable infants in NICUs.[57, 58] Inadequate quantity and quality of water can impede hand hygiene, and inadequate hand hygiene has been linked to neonatal HAI. Adequate hand hygiene supplies, training, monitoring, and feedback have been implemented as hand hygiene interventions, and these have suggested a preventive association between hand washing and neonatal infection prevention.[58] However in some instances, such as ongoing transmission of

AMR pathogens such as *Klebsiella*, soap and water may not be enough, and alcohol-based hand rub may need to be used to eliminate hand contamination and interrupt transmission.[59, 60]

Surface contamination has been implicated in several outbreaks, and studies have suggested that clean birth settings and practices can reduce mortality; however due to the complex nature of care, poor resource availability, and the multiple possible pathways for transmission in any healthcare setting, the causal nature of surface contamination has not been confirmed for transmission of pathogens causing neonatal sepsis in LMIC.[49, 52, 54] Despite this, general wisdom indicates that a clean environment reduces the likelihood of hand contamination and subsequent infection in neonates. CDC has formulated recommendations specific to environmental cleaning in low-resource healthcare settings, and these recommendations can be used to develop facility cleaning policies, engage cleaning staff, and ensure a safe environment for patients.

Healthcare Facility Environmental Contamination in LMIC

Very little data is available on the environmental contamination and conditions in neonatal healthcare units in low-and middle-income (LMIC) countries in sub-Saharan Africa, and specifically Ethiopia. Subsequently, there are almost no data demonstrating the impact of this contamination on HAI in general, or neonatal sepsis in particular. One narrative review examined 186 published articles on controlling environmental contamination in LMIC HCF. This study reported multiple physical pathways for environmental contamination including poor or intermittent infrastructure; inadequate potable water; inadequate toilets and showers for both healthcare personnel and caregivers; misuse of handwashing sinks which stemmed from inadequate sinks for designated environmental cleaning tasks; inadequate or incorrect environmental cleaning practices; and mismanaged facility waste. Several behavioral pathways were also summarized, such as administrative and leadership support; adequate training for the

conduct of hand hygiene and environmental cleaning; and monitoring and feedback on the conduct and implications of these behaviors.

While not in sub-Saharan Africa, one study conducted in a low-income setting found high rates of environmental surface contamination in Nepalese NICUs. This study found the largest percentage of contamination on frequently touched objects (including, but not limited to, incubators, doorknobs, and bedsheets) was due to *E. coli*, *Klebsiella*, and *S. aureus* with a 33.3% AMR rate among the *S. aureus* isolates. This descriptive study also examined neonatal sepsis, and the most commonly isolated pathogens were *Klebsiella*, and *S. aureus*; however, authors did not attempt to find an association between the environmental contamination and sepsis.

Environmental cleaning and disinfection are crucial components of WASH capacity. Frequently, cleaning supplies are inadequate or completely absent, and environmental cleaning staff are overlooked and inadequately trained.[11] Not only are cleaning staff not trained on how to appropriately clean and disinfect facility units, they are also not trained on basic infection prevention and control, a crucial component to executing their tasks. Barriers to adequate cleaning are rarely assessed. And finally, a crucial component of infection prevention and control, a clean environment, is entrusted to the staff who are paid the least and given limited funds for WASH and infection prevention and control (IPC). Given cultural norms, it is unlikely that these frontline workers will be paid more. All of these issues prevent appropriate cleaning and disinfection of the healthcare environment.

Antimicrobial Resistant Microorganisms and Environmental Contamination

Antimicrobial resistant, and especially multi-drug resistant organisms are known for their persistence in the healthcare environment. [51, 61-63] Given the proliferation of AMR in LMIC, it is important for HCF to consider the possibility of AMR pathogens on surfaces when formulating cleaning policies and procedures. As with other topics in this review, there is a paucity of data on

environmental contamination with AMR bacteria in sub-Saharan Africa. Studies have reported significant environmental contamination with AMR in healthcare settings in South Africa, Tanzania, and Morocco, and each highlighted the need for improved cleaning and disinfection infrastructure, training, and engagement of cleaning staff. [64-66] Disinfection, augmented cleaning, and reduction of opportunities for cross contamination and spread are crucial for the prevention of these infections given the longevity of these pathogens in the environment. CDC recommendations for the prevention of AMR pathogens highlight these prevention strategies, and while the recommendations are formulated for high-resource settings, they can be adapted for use in LMIC.

Water, Sanitation, and Hygiene (WASH)

Healthcare WASH programs are a way of assessing and addressing the interrelated capacity of water quality and quantity, sanitation, and removal of waste, and environmental and hand hygiene as they contribute to infection control in these settings. In HCF this also encompasses IPC capacity, training, and behaviors. In 2019, the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) Joint Monitoring Programme (JMP) published harmonized baseline estimates for water, sanitation, hand hygiene, health care waste management, and environmental cleaning (WASH) services in health care facilities. The global baseline report found that 26% of health care facilities lacked basic water services, and 21% of health care facilities had no sanitation service.[67] Environmental contaminants that can cause HAIs can survive for hours on hands and months on surfaces. What is unclear is the association between basic WASH capacities, environmental contamination, and HA infection, specifically neonatal sepsis.

WASH in Ethiopia

In a 2020 survey of HCF in Ethiopia, the WHO and UNICEF JMP found that only 30% of Ethiopian HCF had basic water services, 59% had basic sanitation services, 29% coverage for basic environmental cleaning, and 65% hand hygiene coverage. This sets Ethiopia among the sub-Saharan African countries with the poorest WASH coverage overall in HCF. One cross-sectional study conducted in rural HCF in sub-Saharan Africa, and specifically 534 clinics and health centers in Ethiopia, reported inadequate access to improved water sources and hand hygiene supplies, and inadequate waste facilities.[68]

Clean and Safe Health Facilities (CASH) Initiative

In 2014, the Ethiopian Ministry of Health launched the "Clean and Safe Health facility" (CASH) campaign. The goal of this program is to assess the infrastructure, cleanliness, and IPC capacity in HCF nationwide and ensure a national standard of cleanliness and safety not unlike the Joint Commission in the United States. Further, this effort is to reduce HAI by establishing HCF guidelines, training staff on infection prevention and control and adequate WASH management and conducting national audits for routine upkeep of these trainings. This program is supported by nationally allocated human and financial resources for planning and execution of assessment and improvement of all Ethiopian HCF. Via celebrity engagement and routine audits, the goal was to not only improve safety and quality but also to change the engagement and culture of healthcare personnel.

CASH Certification entails a passing a series of audits intended to assure facility infrastructure, WASH, and IPC capacity. While these audits provide an overarching checklist for the HCF hygiene, there are few markers of true cleanliness in the checklist. Also, the checklist does not require dedicated cleaning equipment in units; separate sinks for hand hygiene, laundry, device reprocessing, and cleaning supplies (i.e. a mop sink); and does not define what

"adequate" means for cleanliness or availability of supplies. If disinfectant is present, it does not assure that the disinfectant is being used, or more importantly that it is being used in the correct concentrations, on the correct surfaces, or at the correct frequencies. These gaps could result in significant proliferation of AMR despite the perception of cleanliness and safety.

As of 2020, 150 HCF are CASH Certified. Despite the CASH manual and audit tool provided by the CASH program, the level of cleanliness within each facility is unclear. Additionally, the manual specifies that recertification should occur every six months, but it is also unclear if this occurs. While these efforts are laudable, without routine surveillance of HAI and environmental conditions, it is unclear whether these efforts truly have an impact on patient safety. Future refinement of this program, could entail routine surveillance of HAI and contamination, monitoring of healthcare and environmental cleaning personnel behaviors, and feedback to employees to ensure targets are not only met, but also maintained.

Research Questions

Evidence exists on high rates of neonatal sepsis, high frequencies of environmental contamination, and low WASH capacity in LMIC. However, to date, there have been no studies examining the association between these variables. This study was conducted to examine the association between neonatal sepsis, environmental contamination, and WASH capacity in LMIC HCF.

The research questions this study addresses are: What is the association between lab-confirmed neonatal sepsis, environmental contamination, and WASH capacity in two healthcare facilities in Amhara, Ethiopia?

Specific Research Questions:

- What is the prevalence of healthcare-associated sepsis and resistant sepsis at each facility?
- What is the association between sepsis and the contamination of hands, medical devices, and frequently touched surfaces in the delivery, kangaroo mother care, post-natal care, maternity surgical theater, and neonatal intensive care units of each hospital?
- What is the association between contamination of hands, medical devices, and frequently touched surfaces in the delivery, kangaroo mother care, post-natal care, maternity surgical theater, and neonatal intensive care units of each hospital?

Public Health Implications

There is a need to reduce the incidence of neonatal sepsis, and sepsis associated with AMR bacteria in Ethiopia. In order to do this, it is important to understand the drivers of neonatal sepsis rates in Ethiopia, and specifically, the association between neonatal sepsis, environmental contamination, and WASH capacity in HCF.

First, this work can be used to determine neonatal sepsis prevalence, environmental contamination, and WASH capacity in two Ethiopian HCF to provide feedback to facility administration to improve monitoring and feedback capacity in these facilities. Similarly, the results of this study will highlight gaps in water, sanitation, and hygiene infrastructure and practice that can be used to draft WASH-specific recommendations and interventions that can be used in HCF to reduce environmental contamination and the outcomes of neonatal sepsis, resistant sepsis, and neonatal mortality. And finally, on a larger scale, the results of this study and the associated recommendations can be used to create a WASH-based IPC algorithm for root cause analysis in LMIC HCF.

Manuscript

Title, Authors, & Abstract

Title: The association between water, sanitation, and hygiene infrastructure, environmental contamination, and neonatal sepsis at two healthcare facilities in Amhara, Ethiopia.

Authors: Erin Stone, Kun Zhao, John Cranmer, Kelly Geith, Kristin Carr, Habib Yakubu, Lamesgin Alamineh, Mulusew Belew, Abebe Gebremariam, Gizachew Yismaw, Yesewbelay Minale Negesse, Addis Derbew Adugna, and Christine L. Moe

Abstract

Background: Neonatal sepsis rates are high in Ethiopia where there is often inadequate water, sanitation, and hygiene (WASH) capacity in healthcare facilities (HCF). This leads to increased environmental contamination which can be passed to neonates via multiple routes, including contaminated hands, surfaces, and invasive medical devices, and cause healthcare-associated (HA) infections. The main objectives of this study are to determine if there is an association between HA neonatal sepsis, environmental contamination, and WASH capacity at two HCF in Amhara, Ethiopia.

Methods: A modified WASH Conditions Assessment Survey (WASHCon), was deployed over 32 weeks in five neonatal units of two Ethiopian HCF. Surveys were collected in conjunction with environmental and neonatal clinical samples. Multivariable logistic regression was conducted to determine an association between these variables.

Results: Felege Hiwot Hospital had a higher prevalence of neonatal sepsis, antimicrobial resistant (AMR) sepsis, and mortality. Debre Tabor Hospital had a higher frequency of environmental contamination, and environmental AMR isolates. Sepsis due to *Klebsiella spp.* was associated with hospital of birth (aOR: 0.11 (95%CI: 0.02-0.64); p=0.002), detection of environmental contamination in the NICU (aOR: 35.31 (95%CI: 1.54-808.85), p=0.03), and contaminated hands in the delivery unit (aOR: 5.50= (95%CI: 1.16-25.77), p=0.03). Sepsis due to *S. aureus* was associated with detection of environmental contamination in the NICU (aOR: 0.01 (95%CI: <0.01-0.50), p=0.024), and the frequency of hand contamination in the delivery (0.036 (95%CI: 0.004-0.33), p=0.0033) and KMC units (aOR: 19.60 (95% CI:2.16-177.52), p=0.0081).

All cases of lab-confirmed neonatal sepsis were resistant to one or more antibiotics, and rates of resistance in environmental contamination isolates were high in both HCFs, making multivariable logistic regression impossible for this outcome. No association was found between WASH capacity and environmental contamination.

Conclusions: This study is the first to report an association between environmental contamination of hands and surfaces and HA neonatal sepsis in two HCF in Ethiopia. The

prevalence of AMR environmental contamination was high in the clean and safe healthcare (CASH) certified facility, and resistance was 100% for all lab-confirmed sepsis cases. WASH Capacity did not align with contamination which warrants further investigation into facility cleaning and hand hygiene behaviors.

Introduction

Neonatal sepsis is the third leading cause of neonatal mortality in low- and middle-income countries (LMIC), and neonatal sepsis in LMIC accounts for over 95% of sepsis-related neonatal deaths worldwide.[6, 23] The United Nations (UN) General Assembly set the Sustainable Development Goal (SDG) of ending preventable deaths of newborns by 2030 with all countries aiming to reduce neonatal mortality to 12 per 1000 live births.[7] Despite global declines in neonatal sepsis rates, over the last 20 years, Ethiopia's neonatal sepsis prevalence continues to be high, and one meta-analysis estimated a pooled national prevalence of 45% (range 17% to 78%.).[14] Further, the Amhara region of Ethiopia is estimated to have a pooled neonatal sepsis prevalence of 64.4%, which is the highest in the country.[14] Sepsis was not uniformly defined across the studies included in this meta-analysis. Because laboratory capacity in Ethiopia is still expanding so many of the sepsis cases included in the meta-analysis were determined via signs and symptoms and lab-confirmation. Additionally, this prevalence included HA and community-associated (CA) sepsis cases. Debre Tabor General Hospital (DT) and Felege Hiwot Regional Referral Hospital (FH) are located in the Amhara region, and the estimated 2016 neonatal sepsis prevalence was 23.9% at FH.[16]

Neonatal sepsis is a systemic inflammatory response that is often the result of a suspected or proven bacterial, viral, or fungal infection in the neonate.[4, 10] Neonatal sepsis is classified into early onset sepsis (EOS) and late onset sepsis (LOS). While there is not consensus on definitions for these categories, frequently EOS is frequently defined as sepsis in the first 72 hours of life, and LOS is defined as occurring between 3 and 28 days of life. In low-resource settings, EOS is

frequently attributed to vertical transfer of pathogens from the mother or horizontal transfer of pathogens from the birth environment due to inadequate resources for implementing aseptic technique.[4, 26] EOS in LMIC is often caused by *Escherichia coli* (*E. coli*), coagulase-negative *Staphylococcus* (CoNS), *Klebsiella spp*., and *Listeria monocytogenes* . LOS is attributed to the horizontal transfer of pathogens from the environment, most often healthcare settings, and the same etiologic agents are associated with LOS in low-resource settings, further blurring the distinction of classification in LMIC. However, these similar pathogens and pathways make the prevention of these infections possible.[21, 26, 29] Neonatal sepsis leads to increased neonatal mortality due to the weaker immune systems of neonates, and this is even more acute for low birthweight (LBWT), premature, and unstable neonates. These weakened immune systems can also lead to a reduction in the clinical manifestations of infections, specifically sepsis, making diagnosis especially difficult.

Healthcare settings, such as neonatal intensive care units (NICU) and kangaroo mother care units (KMC), are places where premature, low-birthweight, immunocompromised, and unstable infants receive care. This puts these infants at a greater risk of infection because these settings are frequently overcrowded and contaminated in LMIC. Neonatal sepsis, and all infections, may be acquired in the healthcare setting via multiple routes including contaminated hands, surfaces, and invasive medical devices. High rates of bacterial contamination have been found on the surfaces of LMIC NICUs, including *E. coli, S. aureu*s, and *Klebsiella spp.* [69] All of these species are causal pathogens for neonatal sepsis.

The goal of SDG 6.2 is to ensure access to water and sanitation for all and includes institutions like healthcare facilities and schools in addition to households. A 2019 World Health Organization (WHO) report highlighted the lack of adequate water, sanitation, and hygiene (WASH) in healthcare facilities in LMIC. This report indicated that, while data was incomplete,

overall WASH coverage for Ethiopia was moderate. Ninety-three percent of healthcare facilities reported having an improved water source on premise, while only 79% of facilities reported the presence of basic sanitation services, 75% reported the presence of adequate waste management, and data was unavailable for basic hand hygiene services at point of care for patients. This report also noted that urban healthcare facilities, such as those in this study, reported having higher WASH capacity than rural healthcare facilities. This coverage indicates Ethiopia still has a long way to go to ensure adequate WASH capacity in all healthcare facilities.

This improved coverage may, in part, be attributable to Ethiopia's Clean and Safe Health Facilities Initiative (CASH). CASH is a multimodal government program intended to improve WASH conditions in healthcare facilities. This initiative includes a framework of support from regional health bureaus, community engagement, healthcare facility (HCF) culture change, and routine evaluation of facility performance. This program implemented minimum national cleaning standards that are reinforced by routine audits in HCF. One of the two facilities included in this study, DT, is a CASH-certified HCF.

Water, sanitation, and hygiene capacity, coupled with infection prevention and control strategies, can mitigate the spread of neonatal sepsis, and prevent morbidity and mortality.[49, 63, 70, 71] Contamination increases when WASH capacity and practices are scarce or insufficient, and the interrelationship is complex between the environmental contamination transmission pathways, the exacerbating or mitigating factors of WASH capacity and behaviors, and infection prevention and control strategies in healthcare facilities. Figure 1 illustrates these complexities in a conceptual model of environmental contamination, etiologic agent transmission, and their impact on sepsis morbidity and mortality. It can be a challenge for facility administration and leadership to determine where to target limited financial, human, and material resources. Frequently, the significant long-term cost-savings of infection prevention and control are difficult to

contextualize in the short-term constraints of budgets, supply chain issues, and human resource schedules and trainings.[5] Understanding the associations between observed gaps in WASH and IPC capacity and practices and environmental contamination and neonatal sepsis will enable the development of a targeted plan of action for each HCF.

To date, no studies have been conducted that clearly identify an association between WASH, environmental contamination, and neonatal sepsis in LMIC healthcare facilities. The objective of this study is to determine the prevalence of lab-confirmed healthcare-associated (HA) neonatal sepsis and sepsis attributable to antimicrobial resistant (AMR) bacteria, and to determine the association between sepsis; the contamination of hands, medical devices, and environmental surfaces; and the water, sanitation and hygiene capacity in two HCF in the Amhara region of Ethiopia.

Methods

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

Setting

This study was conducted in the delivery (DEL), neonatal intensive care (NICU), kangaroo mother care (KMC), mother surgical theater (MST), and post-natal care (PMC) units of FH and DT in the Amhara region of Ethiopia. As one of the largest referral hospitals in the region, FH is located in the capitol city of Amhara, Bahir Dar. Serving 5-7 million people, it is staffed by approximately 740 health care workers. The facility is crowded, receiving between 450-500 deliveries per month and cares for 7-25 babies per day in the NICU. Located in Debre Tabor City in the South Gondar Zone of Amhara, DT serves over 2.5 million people with, on average, 260 monthly deliveries. This facility is designated a “Clean and Safe Healthcare

facility" (CASH) which indicates a high standard of cleanliness by the Ethiopian government and is staffed by approximately 314 healthcare workers.

Population Sampling Methods

Infants were recruited into the study if their families lived inside the catchment area and provided informed consent and were born in one of the two study facilities during the study period between August 2018 to June 2019. Infants were excluded if they did not have blood drawn for suspected sepsis or did not have a confirmed discharge date. (Figure 2) Infants in stable condition and ≥ 2,000 grams were recruited in the PNC Unit. Infants in stable condition and <2,000 grams were recruited in the KMC Unit. Unstable babies of any weight were recruited in the NICU. Neonatal data, including familial background data, and 7 and 14 day follow up survey data on neonatal outcomes were collected via a combination of maternal interviews and facility records. Mothers of infants were interviewed at the time of study recruitment using a standardized questionnaire and also via questionnaires during a home visit at 7 days post-discharge and a phone call at 14 days post discharge. Infants were considered lost to follow up if mothers were not able to provide either a 7 day or 14 day interview.

Caregivers and healthcare personnel in the units were randomly selected for hand rinse samples during environmental surveys. Healthcare personnel consisted of physicians, nurses, midwives, and anesthesiologists. Caregivers were almost entirely mothers with the exception of one "caregiver."

Clinical Sample Collection and Testing

All clinical and environmental samples were transported to and processed at the Amhara Public Health Institute (APHI) in Bahir Dar.

The primary outcome is the incidence of any laboratory-confirmed neonatal sepsis. Secondary outcomes include laboratory-confirmed neonatal sepsis with evidence of AMR, and laboratory-confirmed sepsis caused by *E. coli, S. aureus*, and *Klebsiella spp.*, and mortality. Microbiological testing of blood specimens from neonates with signs and symptoms of sepsis is standard of care for public healthcare facilities. Blood samples were taken by doctors or nurses from any infants admitted to the NICU for suspected sepsis during the first 28-days of life. Blood culture vials are incubated at 35-37°C for 5 days and are examined daily for the presence of growth, for example, signs such as turbidity or hemolysis.

The blood samples were dispensed into broth media containing 0.025% SPS which is a polyanionic anticoagulant which is also anticomplementary & antiphagocytic. Throughout the 7 days of aerobic incubation at 35 –37°C, plates were examined for growth, and sub-culture plates are selected if incubation has shown the presence of bacteria. The Broth media were determined for bacterial growth with daily visual examination. Sub-culture plates were incubated at 35 – 37°C in 5% CO_2 and examined for growth after 24 hours of incubation. Susceptibility testing was performed on selected isolates.

Environmental Sampling Methods

At the onset of the study, a baseline WASHCon assessment[72, 73] was conducted and structured observations were performed in each HCF to guide sample collection locations. Four types of environmental samples were collected from each HCF units as close as possible to the WASHCon Lite assessment. Hand rinse, tap water, surface swabs, and medical device water samples were collected and tested for bacterial contamination by *E. coli, S. aureus*, and other coliforms including *Klebsiella*. These environmental bacteria targets were chosen because they are common etiologic agents that cause HA neonatal sepsis including EOS and LOS. *E. coli* is also used as an indicator of fecal contamination in the environment. Finally, due to the limited

regional environmental testing capacity, *Klebsiella* could not be tested for as an individual bacterium in environmental samples, so the bacterial test for "other coliforms than *E. coli*" was selected to approximate this outcome. Determining the frequency of contamination of these bacterial targets in the environment will aid in determining the association between environmental contamination and lab-confirmed neonatal sepsis.

Environmental Sample Collection and Testing

Environmental samples were collected by swabbing high-touch surfaces, fomites, and linens in each of the five units. Hand rinse samples were collected by submerging both hands sequentially in Whirl-pak® bags containing 100 mL sterile water. Tap water samples (100 mL) were collected from the point of use in sterile containers. Water from medical devices in the NICU was collected as a 1 mL volume.

Hand rinse and tap water samples were tested for *E. coli* using the membrane filtration technique and quantitative results were obtained using m-ColiBlue24® Media and filters (Hach|VWR, USEPA Method #10029). Membrane filters were incubated at 35ºC for 24 hours. In this method *E. coli* colonies appear dark blue on the media and other coliforms appear red. Samples were incubated at 35˚C for 24 hours. For membrane filtration tests, only the presence of E. coli was recorded. The hand rinse samples, and the environmental swab samples, were also tested by 1 mL volume for the presence of *E.* blue *aureus*, and other coliforms using CompactDry™ XSA and EC plates (Hardy Diagnostics, 2018a, 2018b). For the XSA plates pink colonies were recorded as *E. coli*, and the number of red colonies were recorded as *S. aureus*. Water from medical devices was tested by 1 mL volume with the compact dry plates using the same technique as "other coliforms". 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 study refers to coliforms other than *E. coli* that were detected.

WASH Capacity Assessment

The WASH conditions at each HCF were assessed using the Center for Global Safe Water, Sanitation, and Hygiene's (CGSW) WASH Conditions in Healthcare Facilities Assessment Tool (WASHCon)..[72, 73] The tool is a validated survey that uses interviews and direct observations to assess the water supply, hand hygiene facilities, environmental cleanliness, and sanitation capacity, and waste management in healthcare settings.[73] The WASHCon survey tool was deployed to establish baseline conditions in each HCF at the outset of this effort on April 26, 2018 at FH, and April 27, 2018 at DT in Ethiopia. Baseline assessments consisted of interviews with the director and administrators of each facility guided by questions to gather general information about the facility. The baseline survey established facility size and patient volume, the number of clinical and environmental staff and the health and cleaning services provided, whether policies and guidelines are in place, and the general WASH conditions at each HCF. A subset of questions from the full WASHCon survey, named WASHCon Lite, was deployed routinely, via unannounced visits, between September 2018 and June 2019 in each of the five units at both facilities. (Appendix) Survey responses were recorded using the CommCare mobile data collection platform WASHCon Lite was deployed at regular intervals on a mobile device by a program associate using the CommCare mobile data collection platform (Dimagi Inc., Cambridge, Massachusetts) in the DEL, KMC, PNC, NICU, and MST units of each study HCF. The median duration between WASHCon Lite assessments was 16 days (range 8-50 days). Ten WASHCon Lite assessments were completed at FH and 12 were completed for DT.

Antimicrobial Susceptibility Testing

Bacterial isolates identified from all blood specimens and environmental samples were tested for antimicrobial susceptibility using the Vitek 2 Microbial ID-Susceptibility testing system (Biomerieux).

Data Maintenance, Cleaning, and Analysis

Maternal and neonatal data, and environmental 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).

Neonatal data was cleaned using STATA version 1.0, and (StataCoro, College Station, Texas), and WASHCon Lite assessment and environmental sample data were cleaned using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina). Neonates were matched to environmental sample data and WASHCon Lite data by HCF and unit to the closest WASHCon Lite assessment by the closest available date.

Basic descriptive analyses were conducted for the neonatal, environmental sample, and WASHCon survey data. Two-sided chi square test for independence was conducted for categorical variables, and Fisher's exact test was employed when cell counts were low

Multivariable logistic regression was conducted to test the association between neonatal sepsis, unit-level environmental contamination and WASHCon elements for the DEL, NICU, and KMC units; and to test the association between the incidence of pathogen-specific neonatal sepsis and sources of environmental contamination. Neonates included in this analysis were those with a known discharge date. The fundamental assumptions in considering which environmental sample types and which units to include in the model are as follows: 1) all infants were exposed to the DEL unit at the time of birth; 2) to the NICU because this is where infants were sampled for sepsis; and 3) to the KMC units for many of these infants based on stability at birth. This is because infants who were diagnosed with suspected sepsis in the first 28 days of life and prior to discharge were likely the infants in most critical condition. Backwards elimination was conducted for the multivariable logistic regression analyses to determine the final model for the sepsis outcome. The receiver operating characteristic (ROC) curve was

calculated, and the area under the curve (AUC) score was used to assess model fit. All analyses used $p<0.05$ to determine significance.

Descriptive statistics, including proportions, chi square test, fisher's exact test, and multivariable logistic regression analysis of the association between sepsis, environmental contamination, and WASH capacity were all conducted using SAS version 9.4.

Results

Neonatal sepsis

Seventy infants with blood specimens were included in this study. Forty infants (57.1%) were born at FH, and 30 infants (42.9%) were born at DT. (Figure 3) Of the infants born at FH, 27 (67.5%) were diagnosed with lab-confirmed sepsis from any pathogen, and of those cases, all 27 (100.0%) were resistant to an antimicrobial agent. Twenty-four infants (80.0%) were diagnosed with lab-confirmed sepsis from any pathogen at DT, and 21 cases of sepsis (91.3%) were resistant to any antimicrobial agent.

The most prevalent etiologic agents found in blood samples from both hospitals were *S. aureus* (28%), *Klebsiella spp.* (20.0%), CoNS (12.9%), and *E. coli* (4.3%). (Table 1) Proportionally, twice as many neonatal sepsis cases were positive for *S. aureus* at DT compared with FH (40.0% vs. 20.0%), and approximately three times as many neonates tested positive for *Klebsiella spp.* and CoNS at FH compared with DT (Klebsiella: 27.5% vs. 10.0%, and CoNS: 17.5 vs. 6.7%). The frequency of *E. coli* positive neonatal sepsis cases was similar at both HCF. (FH: 5.0% vs. DT: 3.3%) Finally, seven infants had lab-confirmed poly-microbial sepsis, and 85.7% of these infants were born at FH. Seventy percent of all included infants were LBWT, weighing ≤2500g, and 79.5% of lab-confirmed sepsis cases were LBWT.

Sepsis Onset

Fifty-five infants (78.6%) had blood samples taken due to probable sepsis in the first three days of life (EOS), and 70.9% were lab-confirmed positive. (Table 2) Fifteen infants (21.3%) were considered to have probable late-onset sepsis (LOS) and had blood samples taken between days 4 and 28 of life and prior to discharge. Eighty percent of probable LOS blood samples were lab-confirmed positive, and 36 of 39 lab positive infants (92.3%) were resistant to any pathogen. One hundred percent of lab-confirmed LOS sepsis cases were resistant to any pathogen Significantly more LBWT infants were lab-confirmed positive in both the EOS and LOS groups. (79.5% and 90.0%, respectively).

Neonatal Mortality

Seven infants (10.0%) were deceased at 14 days follow-up and of these, six died from lab-confirmed sepsis. All seven infants were born at FH, had a birthweight ≤2500g, and died in the facility, as opposed to being discharged and then died at home. (Table 3) Three infants who died from lab-confirmed sepsis were positive for *Klebsiella spp.* (50%), three were positive for *S. aureus* (50%), and three were positive for CoNS sepsis (50%). Of the six infants who died from lab confirmed sepsis, 50% had polymicrobial infections.

Environmental Contamination

Five hundred environmental samples were collected from randomly selected high-touch surfaces, fomites, sink taps, medical devices, and hands in the five units of the two hospitals during surveys from October 22, 2018 and June 3, 2019. Of these, 227 samples were collected at FH and 273 were collected at DT. (Table 4.) At FH, 27.9% of all samples were positive for any of the target bacteria, and 40.3% of all samples were positive for any of the target bacteria at DT. At both FH and DT, the KMC units had the highest prevalence of contamination, with 39.4% and 71.0% of all samples testing positive for one or more of the target bacteria, respectively.

While the PNC and MST units were similarly contaminated by all target bacteria at both HCF (32.3% and 36.4% respectively), the proportion of samples with bacterial contamination in the NICU at DT was 1.5 times higher than the proportion of samples with bacterial contamination in the FH NICU (40.3% vs 23.2%, respectively). The DEL units had the fewest samples with any bacterial contamination at both FH and DT (21.5% and 18.0%, respectively).

Hand Rinse Samples

One hundred and twenty seven paired hand rinse samples were collected from staff and mothers at both HCF: 58 at FH, and 69 at DT. (Table 4) Hand rinse samples were more frequently contaminated by any target bacteria at FH; however, this difference was not significant (51.7% vs. 40.6%). At FH, the highest frequency of bacterial hand rinse contamination was in the PNC (70.0%) and the lowest was in the NICU (47.37%). It is important to note that at FH, three of the four units had hand rinse bacterial contamination frequencies greater than 50%. At DT, the highest frequency of any bacterial hand rinse contamination was found in the KMC unit (77.8%), and the lowest in the PNC unit (11.1%). Only one unit (the KMC) had a bacterial hand rinse sample contamination frequency greater than 50%.

The detection of AMR bacteria in hand rinse samples was most frequent in samples collected at DT with frequencies ranging from 100.0% in the DEL and PNC units (note, the sample size was one for each unit), to 80.0% - 88.9% from the KMC and NICU. While detection of AMR bacteria was less frequent in hand rinse samples at FH, the lowest frequency reported was 45.5% in the NICU. The highest frequency of AMR bacteria in hand rinse samples was found in the PNC (66.7%) and the KMC (57.1%) at FH.

Coliforms other than *E. coli* were the most frequently detected target bacteria in hand rinse samples (22.0%), followed by *S. aureus* (21.0%), and finally *E. coli* (16.3%) (Tables 8, 9 and 10). *E. coli* and other coliforms were present in varying frequencies across units, with the

highest frequencies of both bacteria reported for the NICU and KMC. units *S. aureus* was detected at similar frequencies in hand rinse samples from the KMC, PNC and MST units at both hospitals (21.8%, 33.3%, and 35.7% respectively), and lowest in the NICU and DEL units (12.2% and 15.0%).

Hand rinse samples collected from mothers had the highest frequency of any bacterial contamination (76.2%), and samples from nurses and midwives had the lowest frequency of contamination by any bacteria (37.5% and 38.9%) (Table 5). Almost 50% of the samples from physician hands were contaminated with the target bacteria. Overall, mother and physician hand rinse sample bacterial contamination were more frequent at DT, while nurse and midwife hand contamination were more frequent at FH. Of note, only one midwife hand rinse sample was contaminated at DT (7.14%). Bacterial hand rinse sample contamination was more frequently detected in samples from women than from men (56.9% vs. 31.5%), however this is likely influenced by the preponderance of contamination by target bacteria found in the hand rinse samples of mothers.

Hand rinse samples collected from hands of mothers and physicians had the highest frequency of bacterial isolates with AMR (83.3% and 63.6%, respectively) (Table 5). Healthcare personnel and caregiver hand rinse samples at DT had a higher frequency of AMR target bacteria overall except for Midwives, and no AMR target bacteria was found in midwife hand rinse samples at DT. Overall, hand rinse samples from females had slightly higher frequency of AMR pathogen detection (64.5% vs. 58.8%). Between facilities, there was no difference in frequency of AMR found in target bacteria of contaminated hand rinse samples for men compared with women.

Environmental Surface Swabs

Of the 261 environmental surface swabs collected, 121 were at FH, and 140 were at DT. Samples were randomly collected from a diverse array of pre-determined, frequently-touched surfaces and from multiple surface types. (Table 6) Swabs collected at DT were contaminated with any target bacteria at more than two times the frequency of swabs collected at FH (48.6% vs. 23.1%). The highest frequency of environmental swab samples that were positive for any of the target bacteria was in the KMC of DT (82.4%). The NICU at DT was also highly contaminated and 58.5% of environmental swabs were positive for any of the target bacteria. The lowest frequency of bacterial-positive swabs was found at DT in the MST (16.8%). At FH, the highest frequency of positive environmental swabs was also in the KMC unit (36.8%). All other units exhibited similar frequencies of swabs that were positive for bacterial contamination (approximately 20%), except for the PNC which had the lowest frequency of swabs with bacterial contamination (17.6%). (Table 4)

Bacterial isolates from 104 swabs collected at FH were tested for antimicrobial susceptibility, and bacterial isolates from 51 swabs collected at DT were tested. A greater frequency of AMR bacteria was found in environmental samples from FH, where the highest frequency of AMR isolates was detected in the KMC unit (73.9%) and the NICU (65.9%). At DT, the highest frequency of AMR isolates from environmental swabs was also found to be in the KMC unit (50.0%), and the NICU (25.0%). It should be noted that while the DEL and PNC units of both facilities had lower frequencies of AMR isolates detected in environmental swabs, (FH: 37.5% and 47.4%, and DT: 23.1% and 9.1% respectively), these detection rates for AMR target bacteria from environmental samples are still considered very high. At both facilities, there were no detectable AMR isolates in the MST environmental swabs that were tested for resistance. (Table 4)

The individual swabbing sites that were most frequently contaminated with any target bacteria were linens (including bedsheets, blankets, and towels) in the KMC unit and NICU at 86.8% and 76.9%, respectively. (Table 6) The rates of bacterial detection from linens were similar for both facilities. Swabs from bedrails were also frequently contaminated with the target bacteria, with 72.2% of samples from DT positive for one or more of the target bacteria, and specifically in the NICU (68.8%) and the DEL unit (100.0%). (Table 7) Not surprisingly, bacterial isolates from these surfaces were also more frequently found to be resistant to any antimicrobial agent. These surfaces are frequently touched by both caregivers and healthcare personnel and are in contact with infants. Sites that would be touched less often in the course of routine care, and likely only touched by healthcare personnel such as IV tubing, and cabinets had the lowest overall rates of bacterial contamination of any sites sampled (13.3% and 13.6%, respectively).

Medical Device Water

Samples of water from medical devices (such as reservoirs connected to oxygen tanks or oxygen concentrators) were only collected in the NICU at both facilities. Twenty one samples were collected at FH and 31 samples were collected at DT. (Table 4) The overall frequency of target bacteria detection from medical device water was 19.2%, however this detection rate was 2.7 times higher at DT than at FH (25.8% vs. 9.5%). Two positive samples of device water from FH and six positive samples of device water from DT were tested for antimicrobial susceptibility. Bacterial isolates from all 8 of these samples were resistant to one or more antibiotics.

Tap Water

Tap water was the least frequently contaminated of all the environmental samples collected and analyzed from these two hospitals. Twenty seven tap water samples were collected

at FH, and 33 samples were collected at DT. At FH, 3.7% of the collected samples were positive for one or more of the target bacteria. This was only one sample retrieved from the NICU. At DT, 18.2% of the tap water samples were contaminated with the target bacteria. One contaminated tap water sample was detected from each ward except for the PNC where two contaminated samples were detected (40.0%). None of the bacteria isolated from these samples were resistant to antibiotics.

Detection of specific bacterial targets from environmental samples

Overall, *E. coli* were more frequently detected in environmental samples from DT (16.6%) compared to FH (7.14%). At FH, *E. coli* contamination was most frequently detected in environmental samples from the PNC unit (12.0%) and least frequently detected in samples from the NICU (6.0%) and DEL unit (6.3%). *E. coli* contamination was most frequently detected in samples collected from the KMC unit (37.5%) and the NICU (18.5%) at DT, and least frequently detected in environmental samples from the MST (5.13%) and the DEL unit (9.7%).

Of the types of environmental samples analyzed, *E. coli* were most frequently detected in surface swab samples from the KMC unit (22.6%) and least frequently detected in swabs collected from the MST (3.6%). (Table 8) Hand rinse samples exhibited a similar range of *E. coli* frequencies of detection, from a maximum of 22.5% in the NICU samples to a minimum of 5.3% in the PNC. *E. coli* was the only target bacteria detected in tap water samples. Only one tap water sample was found to be contaminated with *E. coli* at FH (3.7%), and six samples from DT (18.2%) were positive for *E. coli*, one from each unit, except the PNC which had two contaminated samples (40.0%) No medical device water samples were positive for *E. coli*.

Other coliforms were the most frequently recovered bacterial group from environmental samples at both facilities. Overall, the detection rate for other coliforms was 24.8.H%. However, these organisms were detected in environmental samples from DT more than twice as often as

from samples at FH (39.1% vs. 18.1%, respectively). (Table 9) The highest proportion of coliform-positive samples was observed in the KMC unit for both environmental swabs (56.7%) and hand rinse samples (52.6%). Samples from the NICU also had high frequencies of contamination with other coliforms in both environmental swabs (33.3%) and hand rinse samples (29.8%). The lowest frequencies of environmental swab contamination by other coliforms were found in the DEL unit (7.5%) and the MST (7.1%). The lowest rates of other coliform contamination in hand rinse samples was observed in the PNC (0) and the MST (0). Other coliforms were the only target bacterial group retrieved from medical device water samples in the NICU, and 20.5% of samples were positive for this bacterial group. No tap water samples were positive for other coliforms.

S. aureus was detected in the lowest overall frequency of the three target bacterial groups (11.8%) across environmental sample types and facilities. (Table 10) *S. aureus* detection frequency was highest in samples collected in the PNC and NICU of FH (28.0% each), and lowest in the DEL unit (9.38%). At DT, *S. aureus* were detected most frequently in samples from the KMC unit (26.1%), and least frequently in the samples from the PNC unit (3.7%). Hand rinse samples were more frequently contaminated with *S. aureus* than environmental swabs. *S. aureus* detection in environmental swabs ranged from 12.5% in samples from the KMC units to 4.9% in samples from the DEL units. Detection of *S. aureus* in hand samples ranged from 35.7% in MST samples to 12.2% in NICU samples. No tap water or medical device water samples were positive for *S. aureus.* shown

Antimicrobial Resistant Environmental Contamination

Antimicrobial resistance testing of bacterial isolates from environmental samples determined that 90.4% of bacterial isolates from DT were resistant to at least one antibiotic, and at FH, antimicrobial AMR was detected in 29.2% of the environmental isolates. This is a three-

fold difference in detection rates of AMR bacteria from the environments of these two facilities. At FH, the highest detection rates of AMR isolates were in the samples from the KMC (47.6%) with similar detection rates in samples from the NICU (24.5%), DEL (26.1%), and PNC (25.0%) units. The FH MST samples were not analyzed for AMR. At DT, the highest detection rates of AMR environmental isolates were found in the DEL (100.0%) and PNC (100.0%) units, and samples from the KMC and NICU units had similar high detection rates of AMR bacteria (93.8% and 97.4%, respectively).

Water Sanitation and Hygiene Capacity: WASHCon Lite Surveys

During the study period of August 2018 to June 2019, eight WASHCon lite surveys were deployed at FH and 12 were deployed at DT. (Table 11) Surveys were conducted as close as possible to the environmental sampling survey. At FH, the median time between WASHCon survey and environmental sampling was 5 days (range 0 – 22 days). At DT, the median time between WASHCon survey and environmental sampling was 0.5 days (range 0-12 days). The WASHCon Lite survey was not deployed in the MST, limiting the ability to determine any association between environmental sample results and WASH capacity for this unit.

Water Quality and Quantity

Piped water was available in both DT and FH; however, it was not always functional at a facility level, or in all five units surveyed. The WASHCon lite survey indicated that water was stored in the facility, however this water was not used for drinking like tap water was. Notes in the WASH con survey indicated that when piped water was not functional in units or in the entire facility, the surveyor made notes to indicate “we get” – suggesting that water was fetched from another location and brought to the facility or unit. At DT, water was either treated (95.8%) or unavailable (4.2%). At FH, a combination of treated and untreated water was frequently available (84.4%), however it is unclear in what proportion, their sources, or storage. Water was

unavailable less frequently than it was available at FH (12.5%) Water was stored at the facility-level in both facilities, and in the wards frequently at DT (89.6%) and infrequently at FH (34.3%).

Hand Hygiene

Hand hygiene stations at point of care were observed for both healthcare personnel and caregivers in all wards during WASHCon surveys. FH healthcare personnel hand hygiene stations were functional during 68.8% of the surveys across wards, however this did not align with the hand hygiene supplies observed during surveys. Soap and water, the bare minimum to effectively execute hand hygiene, were available only 28% of the time across wards. Only water was available during half of the surveys across wards, and during these surveys, 81.3% of healthcare personnel hand hygiene stations were listed as functional. No supplies were seen during 15.6% of surveys. At DT, healthcare personnel hand hygiene stations were observed to be functional in the wards for 85.4% of surveys. However, soap and water, or soap and sanitizer were available across wards in less than half of the surveys (45.8%). Where healthcare personnel hand hygiene supplies were insufficient to adequately conduct hand hygiene, the hand hygiene station was indicated to be functional in 73.1% of surveys, again conflicting with the definition of what constitutes a functional hand hygiene station.

Caregiver hand hygiene stations at FH were indicated as functional during 65.6% of surveys, however hand hygiene supplies were sufficient to adequately conduct hand hygiene (at least soap and water) during only 6.3% of the surveys. At DT, caregiver hand hygiene stations were observed to be functional during 68.8% of surveys; however, supplies were never sufficient to adequately conduct hand hygiene across all units (no soap and water, and no hand sanitizer). No visual assessments of hand hygiene behaviors or compliance were conducted during the WASHCon Lite Surveys.

Environmental Cleanliness and Waste Management

The units of FH were visibly clean during 96.9% of surveys, as were the floors. However, bodily fluids were observed during 12.5% of all unit surveys, and during surveys where the units were determined to be visibly clean. Waste at FH was appropriately separated into bins during 37.5% of visits despite the availability of appropriate separate bins during an additional 50% of the visits to the units. FH healthcare personnel toilets were observed to be visibly clean during 87.5% of unit observations, however caregiver toilets were observed to be visibly clean during only 50.0% of visits at FH.

The units of DT were visibly clean during only half of the unit-level observations. Bodily fluids were present during 29.2% of observations in the units, and during none of these instances was the unit observed to be visibly clean. Floors were observed to be visibly clean during 50% of unit observations. Waste was appropriately separated into bins during 85.4% of observations in the units. The places waste was not separated appropriately were in the DEL and PNC units during the months of November and December of 2018 and January of 2019. Healthcare personnel toilets were observed to be visibly clean during 85.4% of surveys, and caregiver toilets were visibly clean in 41.7% of observations.

Infection Prevention and Control Supplies

At both facilities, IPC supplies were not always observed in each unit. At FH, gloves (87.5%) and disinfectant (81.3%) were the supplies most frequently available in the units. A mop and broom were less frequently available (65.6%), and soap was rarely available (12.5%). Separate personal protective equipment (PPE) was available for both care givers and healthcare personnel during only two unit-level visits, and PPE was visibly clean in 48.5% of unit observations.

At DT, IPC supplies were present during more observations than at FH. Gloves (95.8%) and a mop (93.8%) were available most often, followed by disinfectant (89.5%), and soap (72.9%), while a broom (66.7%) was available during the fewest observations. Waste was appropriately separated in only 10.4% of observations, despite the availability of separate bins during an additional 50.2% of observations. Separate PPE was available for caregivers and healthcare personnel in 85.4% of observations, and PPE was clean in 72.9% of unit-level observations.

Unit-specific Practices

IPC practices such as visitor restrictions and aseptic technique during delivery are the unit-specific practices observed at both facilities. Across both HCF, the highest number of the "Six Cleans" reported was four in any single observation in the DEL units. This level of cleanliness was observed during only 35.0% of observations. NICU visitor control access was enforced during all visits at both facilities, and during 40.0% of observations, caregiver entry was observed beyond the control point. It is unknown if caregivers donned PPE and performed adequate hand hygiene before entry.

Multivariable Logistic Regression: Healthcare-associated Neonatal Sepsis

The following model was run for each of the following neonatal sepsis outcomes: any lab-confirmed sepsis, sepsis positive for *S. aureus*, sepsis positive for CoNS, and sepsis positive for *Klebsiella spp.* These pathogens were chosen for analysis due to prevalence in this study, and our objective to determine if there was an association between target bacteria found in environmental samples, and pathogens found in clinical samples. All infants were assumed to have exposure to the DEL because of birth location and the NICU because of blood sampling location. KMC exposure was also assumed for a proportion of these infants due to the location flow found in Figure 2. Because of the overall 87.3% antimicrobial resistance rate among

bacterial isolates associated with the neonatal sepsis cases, the model was not run for this outcome.

Equation 1. Full Logistic regression model predicting the odds of lab-confirmed neonatal sepsis

$$\ln(Odds\ of\ Neonatal\ Sepsis) = \alpha + \beta_1\ Healthcare\ Facility + \beta_2 Birthweight \leq 2500g + \beta_3\ Age\ at\ culture + \beta_4\ NICU\ hand\ \%\ pos + \beta_5 NICU\ swab\ \%pos + \beta_6\ NICU\ device\ \%pos + \beta_7 KMC\ hand\ \%\ pos + \beta_8\ KMC\ swab\ \%\ pos + \beta_9\ DEL\ hand\ \%\ pos\ \beta_{10} DEL\ swab\%pos + \varepsilon$$

For the outcome of any sepsis, no independent variables were associated with the dependent outcome variable.

For the outcome of *Klebsiella spp.*-positive sepsis, the following variables were significantly associated with the dependent outcome variable, and the AUC score for the model was 0.832:

- Facility (FH ref): aOR: 0.11 (95%CI: 0.02-0.64); p=0.002
- 10% increase in NICU swab contamination: aOR: 35.31 (95%CI: 1.54-808.85), p=0.03
- 50% increase in DEL hand contamination: aOR: 5.50= (95%CI: 1.16-25.77), p=0.03

For the outcome of *S. aureus*-positive sepsis, the following variables were significantly associated with the dependent outcome variable, and the AUC score for the model was 0.786:

- 10% increase in NICU swab contamination: aOR: 0.01 (95%CI: <0.01-0.50), p=0.024
- 50% increase in DEL Hand contamination: aOR: 0.036 (95%CI: 0.004-0.33), p=0.0033
- 50% increase in KMC Hand contamination: aOR: 19.60 (95% CI:2.16-177.52), p=0.0081

For the outcomes of CoNS-positive sepsis and AMR sepsis, no independent variables were associated with the dependent outcome variable.

Multivariable Logistic Regression: Healthcare Environmental Contamination

The following model was run for each of type of neonatal sepsis: any positive sepsis, sepsis positive for *S. aureus*, sepsis positive for CoNS, and sepsis positive for *Klebsiella spp.* These pathogens were chosen for analysis due to prevalence and the ability to determine if there is an association between the target bacteria found in environmental samples (*E. coli, S. aureus,* and other coliforms), and pathogens found in clinical samples. Environmental samples included hand rinse samples, surface swab samples, and medical device water samples. All infants were assumed to have exposure to the DEL because of birth location and the NICU because of blood sampling location. KMC exposure was also assumed for a proportion of these infants due to the location flow found in Figure 2. Because of the overall 87.3% antimicrobial resistance rate among bacterial isolates associated with the neonatal sepsis cases, the model was not run for this outcome.

Equation 2. Full Logistic regression model predicting the odds of environmental contamination

$$\ln(Odds\ of\ any\ contamination) = \alpha + \beta_1\ Healthcare\ Facility + \beta_2 unit + \beta_3 SampleSite + \beta_4\ Sex + \beta_5 healthcare\ personnel\ hand\ hygiene\ supplies + \beta_6\ caregiver\ hand\ hygiene\ supplies + \beta_7 soap\ present + \beta_8\ gloves\ present + \beta_9\ healthcare\ personnel\ toilet\ visibly\ clean + \beta_9\ caregiver\ toilet\ visibly\ clean + \varepsilon$$

For the outcome of hand contamination by any of the target organisms (*E. coli, S. aureus,* and other coliforms) in the NICU, the following variables were significantly associated with an AUC score for the model of 0.7682:

- Sex (male sex was used as the reference category): aOR: 2.45 (95%CI: 1.05-5.71); p=0.038
- Separation of waste: aOR: 0.896 (95%CI: 0.896 -0.4315), p=0.0216

No independent variables were associated with the dependent outcome variables for detection of any of the target organisms from hand rinse samples in the KMC and DEL, and for detection of any of the target organisms from environmental swab samples in the NICU, KMC, and DEL.

Discussion

This is the first known study to directly examine the burden of lab-confirmed HA neonatal sepsis and its association with hand rinse and environmental swab samples contaminated by target pathogens (*E. coli, S. aureus,* and other coliforms) in the context of two HCF with limited, but differing, WASH and IPC capacity in Ethiopia.

Neonatal Sepsis and Environmental Contamination

The logistic regression model indicated a 35 time increase in the adjusted odds of *Klebsiella spp.*-positive sepsis with every 10% increase in the prevalence of target bacteria contamination of NICU swabs (aOR: 35.31 (95%CI: 1.54-808.85), p=0.03). It must be noted that the confidence interval is extremely wide for this measure of effect, limiting the generalizability of these results. This wide confidence interval is likely due to the small sample size.

The prevalence of FH NICU environmental samples positive for any target bacteria was moderate (20.8%). The NICU at DT was among the units with the highest prevalence of contamination by any target bacteria at both HCF. The KMC ward was the unit with the highest frequencies of environmental samples with evidence of contamination by one or more of the target bacterial indicators for both facilities, however infants in these units did not have invasive medical devices like the NICU infants. Infants are sent to the NICU if they require more

intensive care, including invasive medical devices, and they possibly are exposed to more frequent contact with healthcare personnel to ensure the health and safety of these unstable infants. The increased risk of bloodstream infections and sepsis associated with invasive medical devices is well known.[25, 74] And while this risk is associated with LOS in HIC, healthcare facilities in HIC do not contend with the prevalence of general bacterial contamination, and prevalence of contamination with AMR-bacteria reported at these facilities. This study did not examine central line insertion and maintenance practices at either hospital, and suboptimal insertion and maintenance practices could be a source of sepsis in these infants.

The sites with the highest frequency of samples with one or more of the target bacterial indicators in the NICU were linens, such as blankets, bedsheets, and towels (76.9%), and bedrails (68.9%) which are two types of fomites in close proximity to the infants, and in the case of linens, in direct contact with the infants. There are multiple examples of outbreaks caused by contaminated bed linens.[75] One study, examining hospital laundry in Nigeria reported a high frequency of bacterial isolates was found in dirty linen, including each of the target bacteria in this study (*E. coli*, *S. aureus,* and other coliforms).[76] Freshly laundered linens were also found to be contaminated, however this was not at the same frequency as the dirty linens. Another basic science study examining transmissibility of methicillin-resistant *S. aureus,* found this target bacteria in transmissible concentrations for up to 14 days in bed sheets.[77] The bedlinens sampled in the NICUs and KMCs at both HCF in this study were frequently brought from home by mothers. It is unclear whether the frequency or type of laundering is insufficient to completely decontaminate the neonatal linens sampled in this study, or whether the contamination of bed linens in these units originates in the HCF or at home.

Invasive medical devices were also frequently contaminated, including radiant warmers and ambubags (38.5%), CPAP machines (33.3%), and intravenous tubing and its stand (23.1%).

While these items are not in direct contact with infants, they are frequently touched by healthcare personnel who very likely touch infants before and after touching these devices in the course of care.

Hand contamination of DEL personnel was also a predictor of *Klebsiella spp.*-positive sepsis. There was a 5.5 increase in the adjusted odds of neonatal sepsis associated with *Klebsiella* with every 50% increase in the proportion of hand rinse samples from DEL personnel that had evidence of contamination with one or more of the target bacterial indicators (aOR: 5.50= (95%CI: 1.16-25.77), p=0.03). While the frequency of any target bacteria in hand rinse samples was lower in the DEL unit of both hospitals compared with other units, it was far higher in the DEL at FH (42%) than in the DEL at DT (20%).

Hand washing of birth attendants is associated with a reduction in neonatal mortality[58] and tetanus-associated mortality; [49] and the implementation of healthcare personnel hand hygiene programs in NICUs and nurseries has been associated with a reduction in neonatal infections and sepsis.[78-80] Midwife hand rinse samples at FH had the highest frequency of composite contamination with any target pathogen of any at that facility (58.3%). Midwives play a crucial role in delivery, especially in LMIC. It is important for midwives to receive the same hand hygiene training as other healthcare personnel in order to achieve clean deliveries.[49, 81] These results highlight the importance of adequate hand hygiene and aseptic technique in this unit. The negative association between increasing frequency of NICU hand rinse sample contamination with any target bacteria, and *S. aureus* positive sepsis is similar to what is seen with NICU surface contamination. The negative association suggests a reduction in neonatal sepsis with increasing frequency of hand contamination by any target bacteria and is counterintuitive. This suggests the sepsis frequency is different at different levels of a categorical variable such as hand hygiene supplies or perhaps by facility. Future work will entail stratification within the model by

one of these independent variables for this outcome to explore the possibility of effect measure modification by one of the terms.

Finally, facility of birth was a significant predictor of risk of sepsis, and the odds of *Klebsiella spp.* -positive sepsis for infants born at FH was 9 times higher than the odds for infants born at DT. The proportion of environmental samples with detection of other coliform contamination was higher at DT than at FH in all units, so on the surface, the higher risk of sepsis associated with FH is not intuitive. However, FH is a referral hospital and receives regional referrals for high-risk pregnancies, which may result in more vulnerable neonates being born at this facility. This highlights the susceptibility of these neonates to infection based on their likely medically complex and unstable conditions. The larger population of more vulnerable infants at FH, combined with other coliform contamination frequencies of 44.4% in the KMC unit, and 18% in the NICU, provides adequate opportunity for exposure to microbial contamination and pathogens associated with sepsis.

One study examined environmental samples in the NICU and compared them with the blood culture results from infants with sepsis.[69] *Klebsiella spp.* and *S. aureus* were etiologic agents for neonatal sepsis and also bacteria found in environmental swabs. This study did not examine their results for an association due to small sample sizes, however authors found the bacterial results in environmental samples similar to the bacterial results in neonatal blood culture samples. While the regional laboratory used in this study did not have the ability to test environmental samples for *Klebsiella* it is expected that these bacteria dominate the frequencies of detection for other coliforms.

Interestingly, while the proportion of NICU swabs and DEL hand rinse samples with detection of the target bacterial indicators were significant predictors of neonatal sepsis associated with *S. aureus*, this effect was protective (aOR: 0.01 (95%CI: <0.01-0.50), p=0.024;

and aOR: 0.036 (95%CI: 0.004-0.33), p=0.0033). These results are counterintuitive. This could be due to effect measure modification by a variable that is specific to *S. aureus* and gram-positive bacteria. OR this could be due to a facility-level WASH variable. Future work will entail a thorough examination of *S. aureus* hand contamination in all units to determine if there is an association with a specific unit.

Sepsis Onset

Fifty-five infants had blood specimens that were cultured in the first three days of life (EOS), and 15 infants had blood specimens that were cultured between days three and 28 of life (LOS). A greater proportion of infants were LBWT (≤2500g) in both groups, and sepsis frequency was higher among LBWT infants. LBWT is a known risk factor for neonatal sepsis, regardless of whether the infants developed EOS or LOS.[17, 19, 26, 82, 83]. In both facilities, the KMC unit and the NICU were the units with environmental samples that were most frequently contaminated with one or more of the target bacterial indicators, and it is important to target infection prevention and control recommendations to these units that have the most vulnerable infants. There were no statistically significant differences between infants with lab-confirmed EOS compared with LOS in terms of prevalence of lab-confirmed neonatal sepsis, sepsis due to AMR bacteria, or pathogen-specific sepsis.

Mortality Associated with Neonatal Sepsis

Of the infants who died, mortality was higher in infants who were born at FH compared to infants born at DT; infants of LBWT compared to infants of NBW; infants who were twins compared twin status, and who had sepsis. All cases of sepsis that resulted in mortality were associated with AMR-pathogens. However, among the study infants, AMR-pathogens accounted for 94.1% of the lab-confirmed sepsis cases. may have AMR-prevalence of This study shows that there is high frequency of contamination with AMR- target bacteria in all neonatal wards in

both facilities. Lab-confirmed HA neonatal sepsis attributable to AMR bacteria was more frequent at FH, as was mortality attributable to lab-confirmed AMR sepsis. The high frequency of AMR target bacteria in environmental contamination at both facilities is an important contributor to the frequency of sepsis, however because FH is a referral hospital, the births are more complicated and the neonates more fragile. This results in a higher likelihood of infection and mortality despite a lower frequency of contamination. The variable of birth complexity was not collected during the study and thus could not be assessed in this model.

Environmental Contamination, IPC, and WASH

An association was found between detection of one or more of the target bacteria in hand rinse samples and sex of the person in the NICU who provided the samples. Females were 2.45 times more likely to have a hand rinse sample with bacterial contamination compared to males (aOR: 2.45 (95%CI: 1.05-5.71); p=0.038). While hands are frequently implicated in the transmission of infectious agents, microbial contamination found on hands can be transient or resident, and importantly, both types of contamination can cause neonatal sepsis.[50, 70, 84] This association with sex may be a proxy for the proportion of hand rinse samples collected from persons with different roles within the units. In both NICUs combined, hand rinse samples were collected from 12 physicians, 27 nurses, 12 midwives, and one mother. The significant association between hand contamination and sex is likely dominated by the high frequency of bacterial contamination detected in hand rinse samples from midwives at FH (Table 5). Hand hygiene is one of the most important steps that can be taken to prevent infection, and one of the most complex to execute. The combination of supplies, human factors, and behaviors makes hand hygiene compliance difficult even in HIC.[58, 83, 85] Due to the high frequency of hand rinse samples with detection of AMR-bacteria in all units, and the possibility of low hand hygiene compliance at DT despite adequate supplies in the NICU and KMC, both hospitals may consider

investing in alcohol-based hand rub to reduce the human factors and conditions that may be barriers to practicing good hand hygiene in these units.[59, 60] Additionally, ABHR may aid in reducing the frequency of AMR bacteria in both sepsis and in the environment.[60, 86]

An association was found between hand contamination by any target bacteria and adequate separation of waste into appropriately marked containers in the NICU. Appropriate waste separation was associated with an 0.90 reduction in the odds of hand contamination compared to inadequate waste separation. (aOR: 0.896 (95%CI: 0.896 -0.4315, p=0.022). While the possibility exists that inadequate waste separation would result in increased frequency of hand contamination due to improper disposal of biological material, or other contaminated material, it is more likely that this association reflects periods of overcrowding, or improper hygiene training. During periods of overcrowding, it can be difficult to maintain proper hand and environmental hygiene and take the steps necessary to ensure basic IPC standards are maintained. Increased numbers of patients can also create more waste, which can overwhelm cleaning and waste removal staff and the recommended frequencies that may have been determined for lower patient volumes may be inadequate. It is important to establish contingency plans for a change in the frequency of waste removal, cleaning, and disinfection during periods of overcrowding when patient volumes may overburden existing resources.

Alternately, new providers, or providers who have been inadequately trained on waste management standards and protocols, may improperly dispose of waste. In this instance, implementing regular or routine refresher trainings may increase compliance with good waste management practices. This would be especially beneficial at FH where waste was not adequately separated despite the availability of appropriate bins in 50% of observations.

If the hand rinse sample contamination with target bacteria was due to transient contamination, the higher frequencies of hand rinse samples with the target bacterial indicators,

and AMR-bacteria, in both the NICU and the KMC unit may be due to the presence of unstable and fragile infants who are more susceptible to these infections. These infants will require longer facility stays which increases healthcare personnel contact and visits to the healthcare facilities by mothers who will stay in the facility with their sick infants. This additionally increases the risk of introducing bacterial contamination from the community and transmitting this bacterial contamination to others. Additionally, understaffing of cleaning personnel, as suggested by the WASHCon baseline, in conjunction with overcrowding can also cause an increase in environmental contamination in NICU and KMC units, which could also result in increases in the frequency of bacterial contamination in hand rinse samples.

Overcrowding

The increasing demand on neonatal services in healthcare facilities has an impact on facility capacity and resources.[87] Healthcare facilities in LMIC are often overcrowded, and FH and DT are no exception. Multiple infants are cared for in single bassinets and shared blankets, resulting in an increased likelihood of cross-contamination from infected infants while sharing a bed. Additionally, a constant rotation of babies into the unit could mean that these cribs are never vacant. If the beds are never empty, the linens may never be cleaned or changed. Compounding this, if contaminated linens are brought from home, then that contamination may persist for months in a single bassinet in a facility. This is reflected in the high frequency of detection of the target bacterial indicators in the swabs of neonatal bed sheets, blankets, and towels.

If the number of infants exceeds the available beds in the NICU, then it also follows that the number of caregivers and mothers will also exceed facility capacity. It may be difficult for facilities to maintain adequate hand hygiene supplies and adequate cleanliness of caregiver toilets as seen in both facilities. Space limitations may result in the mothers of hospitalized neonates living, cooking, and sleeping on the floors of hallways and KMC units in order to stay

near their children. This overcrowding is common in LMICs and can impede the ability for the environmental cleaning staff to adequately clean the units and their surrounding environments. Even if a facility has sufficient numbers of well-trained cleaning staff, it may be challenging for this staff adequately clean and disinfect the facility due to the impact of overcrowding.[63, 87]

Cleaning

At DT, the frequency of surface swabs that were positive for target bacteria was twice as high as the frequency of swabs with bacterial contamination at FH. Additionally, at DT, the frequency of surface swab samples with isolates of target bacteria that were resistant to any antibiotic were four times more frequent than the frequency of isolates of target bacteria that were resistant to any antibiotic at FH. This is despite the certification of DT as a CASH Hospital which is also in contrast to the lower frequency of WASHCon Lite observations indicating the DT units, healthcare personnel toilets, and caregiver toilets were "visibly clean." The high frequency of environmental samples with bacterial target bacteria positive samples is in direct contrast with the observed availability of cleaning supplies, especially disinfectant, in the units. In light of increasing rates of AMR-bacteria in LMIC, "visibly clean" does not mean a facility is actually clean from a microbiological perspective.[88, 89]

Importantly, during the baseline WASHCon survey, the ratio of cleaning staff to deliveries at DT was 1:7.7, while at FH, the ratio was 1:4.5. These ratios suggest understaffing of cleaners at DT. As in HIC, one of the most crucial functions in HCF, the assurance of a clean environment, is entrusted to those who are paid the least and treated as invisible. Environmental cleaning staff are frequently not included as stakeholders in facility initiatives or in the development of facility policies or trainings. However, increased engagement of these staff has been shown to improve environmental conditions.[11, 52, 90] Environmental cleaning staff are frequently trained only upon hiring, and as with any healthcare personnel, routine trainings,

combined with monitoring and feedback, are required to assure improved outcomes.

Stakeholder engagement is crucial to success in changing the environmental contamination problems observed in this study of overcrowded LMIC healthcare facilities and ensuring all persons in the facility are working towards the goal of a cleaner facility will improve environmental conditions. Nurses, physicians, midwives, mothers, and cleaning staff all need to be engaged to improve the success of an augmented cleaning program.[91] Outreach to the mothers, who are frequently left out of communication about infection rates and education on infection prevention and control, will help improve overall cleanliness and neonatal outcomes.[92, 93]

Strengths & Potential Limitations

This is the first study to examine the relationships between WASH and IPC capacity, environmental contamination, and neonatal sepsis in LMIC, and in Ethiopia specifically. WASHCon is a validated survey tool used to evaluate WASH and IPC capacity in healthcare facilities. This makes the WASH conditions at FH and DT directly comparable to conditions at other facilities where WASHCon surveys have been conducted.

WASH data was paired with microbiological analyses of clinical and environmental samples and neonatal health data to determine if there was an association between these factors. These data can be used to develop a detailed framework of recommendations designed to improve environmental conditions and reduce the incidence of neonatal sepsis at both facilities. The insights gained about the high frequency of environmental microbiological contamination, detection of AMR-bacteria in environmental and clinical samples, prevalence of neonatal sepsis, and sepsis associated with AMR bacteria will enable targeted interventions at both facilities.

In the protocol for this pilot study, environmental samples were planned to be collected at 2-week intervals at the same time as the routine WASHCon Lite surveys. In reality,

governmental instability and physical infrastructure issues delayed these surveys. FH was unable to conduct as many surveys as DT, and the FH environmental sample collection and WASHCon surveys were sometimes over 20 days apart. Given the temporal variability in the environmental conditions, this survey frequency limited our ability to link WASH capacity with the environmental contamination that was detected and to link exposure to environmental contamination with risk of neonatal sepsis.

Environmental swabs were collected from a range of surfaces. However, not all surfaces that have been implicated in previous reports of infection were sampled, such as invasive devices in direct contact with infants in the NICU, and delivery tables and tools in the delivery unit. Future sampling of these surfaces could further illuminate contamination pathways that lead to the development of neonatal sepsis.

Many WASHCon lite survey questions asked personnel their perception of facility practices, including type of water present, whether disinfectant was used, and whether healthcare personnel self-contaminated while donning and doffing PPE. Personnel interviewed may not have been qualified or knowledgeable enough to answer these questions, and if they were, these questions likely resulted in response bias. This would have resulted in an overestimation of WASH and IPC capacity at both facilities.

Because there were no confirmed discharge dates for healthy infants, all infants included in this analysis were considered for suspected sepsis. The inclusion of patients with increased risk of severe disease in this study may have resulted in sick patient bias. This type of selection bias highlights the risk to severely ill and unstable infants and means that these results may not be applicable to healthier infants whose immune systems are stronger and are less likely to develop sepsis.

It is important to note that this was a pilot study, and the overall samples sizes were small

especially for bacterial isolates from environmental samples that were analyzed for AMR at both hospitals. This resulted in very small numbers of samples collected in the PNC, DEL, and MST, compared with those collected in the NICU and KMC units. This may impact the confidence in these results.

Nonetheless, it is clear that environmental contamination provides pathways for transfer of sepsis-causing pathogens to neonates. While the true association between WASH capacity and neonatal sepsis is less clear, it appears that overcrowding and supply issues impacted both hospitals. Future work should assess the behavioral components of infection prevention and control to better understand the impact of WASH infrastructure on IPC practices in healthcare facilities in LMIC. The establishment of an IPC team made up of stakeholders from administration, physicians, nurses, midwives, cleaning personnel, and mothers who, together, can develop and implement an evidence-based bundle of IPC interventions will reinforce positive IPC behaviors and practices and reduce both environmental contamination and neonatal sepsis at both HCF. The success of this effort will entail adequate supply of hand hygiene materials, environmental cleaning materials, recurring trainings on hand hygiene and environmental cleaning, and will assure environmental cleanliness standards for all who enter the facilities.

Summary, Public Health Implications, and Future Directions

Governmental and Global Advocacy: This study contributes to building an evidence-base for Ethiopian national and global advocacy for child and maternal health. It also supports international action by WHO, UNICEF and other organizations to establish safe WASH conditions within healthcare facilities in order to improve neonatal health. In 2015, The Ministry of Health in Ethiopia launched a unique Clean and Safe Health Facilities Initiative (CASH) that included water, sanitation, and implementation of infection prevention standards. The results of

this study will provide targeted data for improving Ethiopia's CASH strategy.[94] Any gaps highlighted by this study can be addressed in the delivery of the program and in improvements to the audit tool to ensure that CASH-certified facilities continue to adhere to the recommendations of program. Synergistic insights from the CASH initiative and this study could be applied throughout sub-Saharan Africa and would provide a crucial, data-informed foundation for future global work toward reaching multiple SDGs including SDG 3.2 and SDG 6.7.

Many healthcare facilities in Ethiopia and other LMIC do not have the laboratory capacity to confirm bacterial infections and identify etiologic agents. Even if laboratory capacity exists, intermittent electricity, and improper storage of laboratory supplies could impact the accuracy of laboratory results. Low resources and low reliability of existing resources contribute to the ubiquity of empiric antibiotic treatment in LMIC. Ethiopia and other countries have adapted the WHO guidelines for Managing Possible Bacterial Sepsis Infections (PSBI) for infants.[43] Empiric treatment can exacerbate resistance in facilities when the symptom algorithm used to define probable sepsis is too sensitive. While the criteria used for syndromic assessment of neonatal sepsis in hospitalized infants at both HCF are unknown in this study, future work should be conducted to find whether these criteria exist in scanned forms within the study archives. This would enable an analysis of the sensitivity and specificity of the syndromic definition used in these facilities for diagnosing sepsis and could inform the development of a sepsis definition that captures all sepsis, not just the more severe cases described in this study. This guidance could help balance appropriate antibiotic treatment while reducing morbidity and mortality in this vulnerable population.

This study reports an association between unit-specific environmental and hand contamination and HA neonatal sepsis, and between environmental contamination and gaps in WASH capacity. The public health implications of these findings suggest that it is possible to

provide a suite of evidence-based IPC interventions for both facilities to implement with the achievable goal of reducing the frequency of contamination, neonatal sepsis, and AMR-sepsis in both facilities. However, the prevalence of AMR-bacteria detected in clinical specimens and environmental samples in both facilities is concerning. This concern stems from the difficulty in reducing or removing this frequency of contamination with AMR target bacteria from both facilities. The possibility exists for community transmission, or a community reservoir of AMR target bacteria and possibly CA AMR infections, which could be indicated by the frequency of bacterial contamination detected on the hands of mothers and on items like the bed linens in cribs that were brought from home.

Future research should include:

- Data on the unit-level movement of each infant was not available for this analysis; however, it may be available in the near future. These data could be used to further refine this analysis and develop a nested case-control study comparing infants with lab confirmed sepsis to infants with probable sepsis, and infants who were discharged home. If possible, infants could be matched on birth date, birthweight status, and home neighborhood given the concerns for AMR-pathogens that are endemic in the community.
- A community-based survey of homes similar to that deployed in these two HCF. This study will conduct WASHCon assessments in conjunction with environmental sample collection and analyses in homes where a family member was recently in one of these two HCF and compare them to families with no healthcare exposures within the last year. Given the high frequency that AMR-Klebsiella and other coliforms were detected in both facilities, this pathogen should be a focus of this research.

- The development of a before-after study where facility-wide IPC initiatives are developed with stakeholder engagement and implemented in each facility. For the six months before the implementation of these recommendations, WASH, environmental conditions, and sepsis frequency will be monitored as in this study. The IPC initiative will be launched concurrently in both hospitals during a one-month washout period. Then surveys will restart and continue for another six months. A follow-up survey will be conducted at 1 year post-intervention to provide accountability and determine the sustainability of this project.

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Tables and Figures

Figure 1. Conceptual flow diagram of healthcare-acquired sepsis, environmental contamination, and water, sanitation, hygiene, and infection prevention and control capacity. (adapted from Laborde et. al. 1993)[95]

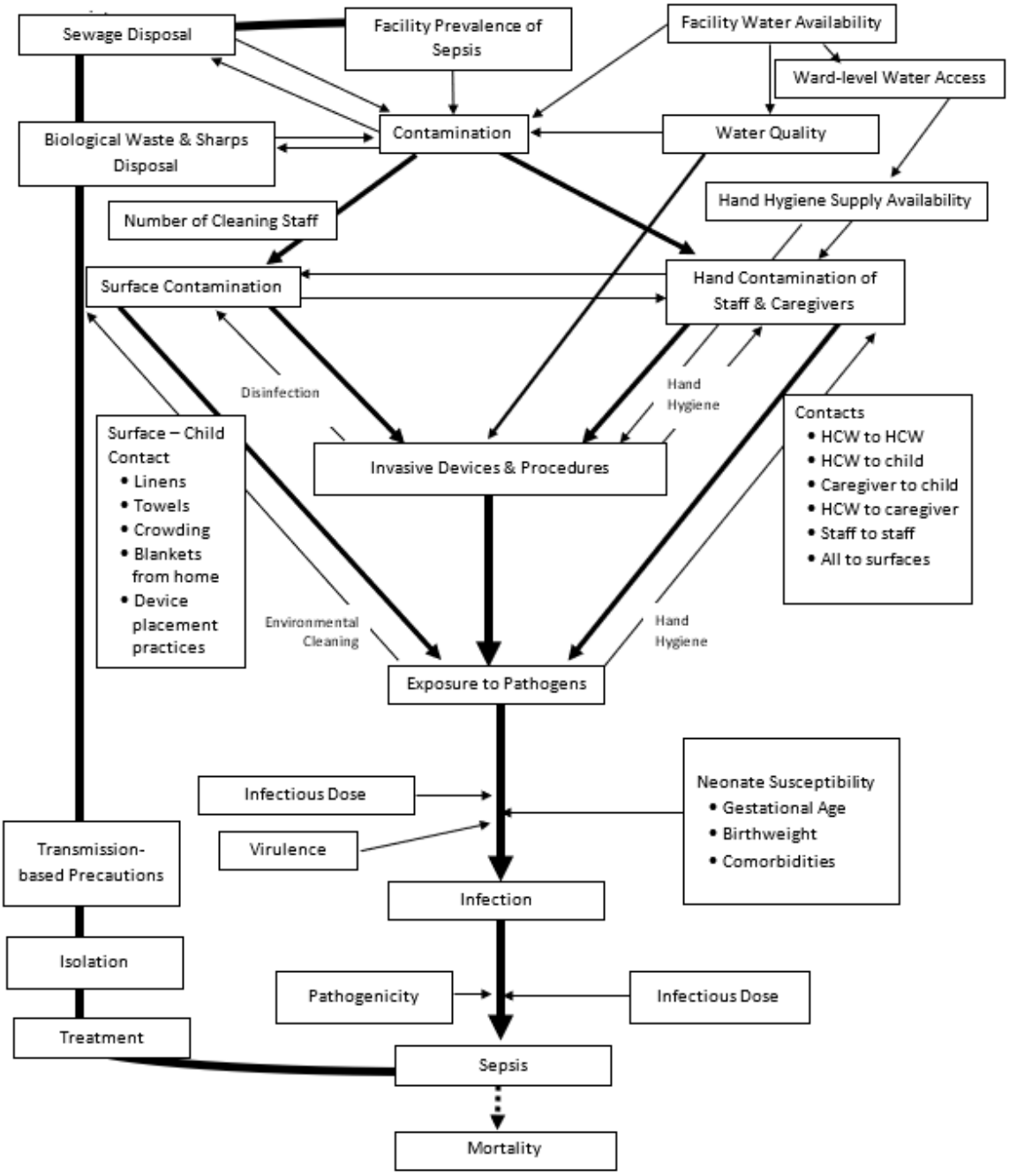

Figure 2. Infant location algorithm

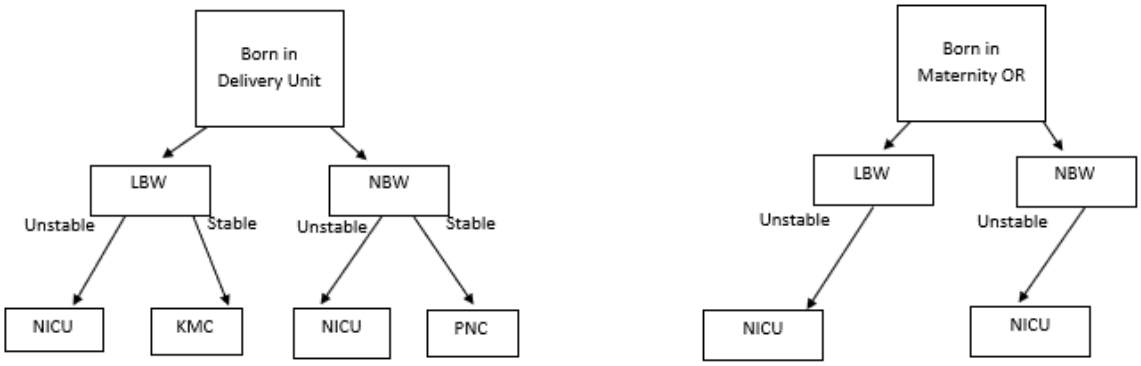

Figure 3. Flow chart of infants, blood culture results, and resistance

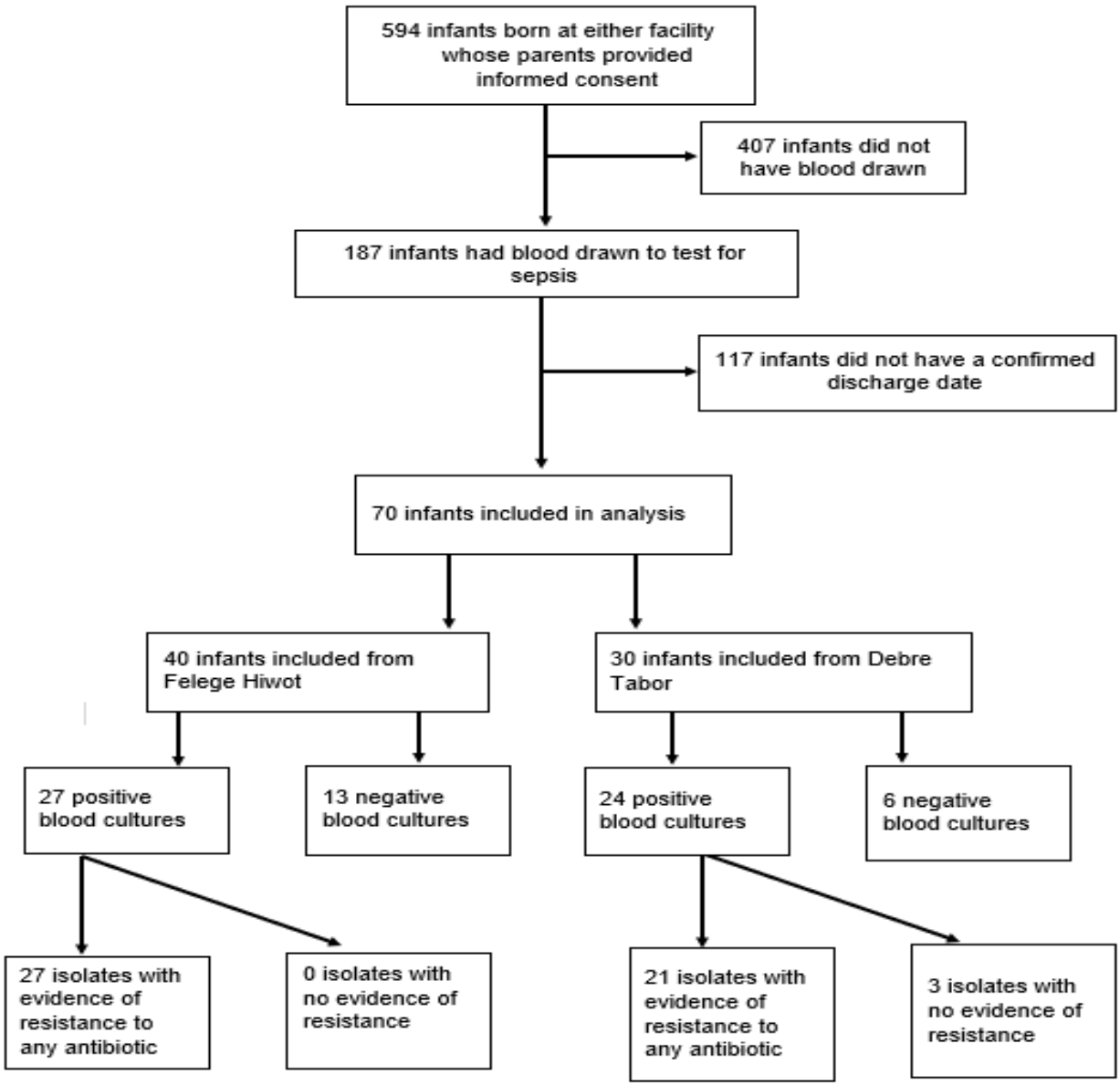

Table 1. Demographic and antenatal characteristics for all 70 study infants with confirmed discharge date according to facility of birth (FH = Felege Hiwot; DT = Debre Tabor)

	Sepsis Positive: n/N (%)		**Resistance Positive: n/N (%)**	
Characteristic	FH: N=40	DT: N=30	FH: N=27	DT: N=24
Lab Positive	27/40 (67.5)	24/30 (80.0)	27/27 (100.0)	21/24 (87.5)
Chi square p-value	p=0.29		p=0.10	
S. aureus positive	8/40 (20.0)	12/30 (40.0)	8/8 (100.0)	12/12 (100.0)
^ p-value	p=0.11		p=NA	
Klebsiella positive	11/40 (27.5)	3/30 (10.0)	11/11 (100.0)	3/3 (100.0)
^ p-value	p=0.08		p=NA	
CoNS positive	7/40 (17.5)	2/30 (6.7)	7/7 (100.0)	2/2 (100.0)
* p-value	p=0.28		p=NA	
E. coli positive	2/40 (5.0)	1/30 (3.3)	2/2 (100.0)	1/1 (100.0)
* p-value	p=1.00		p=NA	
LBWT (<2500g)	19/28 (67.9)	21/21 (100.0)	19/19 (100.0)	18/21 (85.7)
NBWT (≥ 2500g)	8/12 (66.7)	3/9 (33.3)	8/8 (100.0)	3/3 (100.0)
* p-value	p=1.00	p<0.001	p=NA	p=1.00
WHO LBWT (<2000g)	19/28 (67.9)	16/16 (100.0)	19/19 (100.0)	13/16 (81.3)
WHO NBWT (≥ 2000g)	8/12 (66.7)	8/14 (57.1)	8/8 (100.0)	8/8 (100.0)
* p-value	p=1.00	p=0.01	p=NA	p=0. 53
Early Onset Sepsis	20/32 (62.5)	19/23 (82.6)	20/20 (100.0)	16/19 (84.2)
Late Onset Sepsis	7/8 (87.5)	5/7 (71.4)	7/7 (100.0)	5/5 (100.0)
* p-value	p= 0.24	p= 0.60	p=NA	p=1.00
Deceased at 14 days	21/33 (63.6)	0/0	21/21 (100.0)	0/0
Alive at 14 days	6/7 (85.7)	24/30	6/6 (100.0)	21/24 (87.5)
* p-value	p= 0.39	p=NA	p=NA	p=NA

* Chi square test, ^Fisher's exact value, LBWT: low birthweight, NBWT: normal birthweight

Table 2. Characteristics of l-lab-confirmed sepsis cases by timing of blood specimen collection ≤72 hours of life (EOS) vs. >72 hours of life (LOS)

	Sepsis Positive: n/N (%)		Resistance Positive: n/N (%)	
Characteristic	EOS, N=55	LOS, N=15	EOS, N=39	LOS, N=12
Positive samples	39/55 (70.9)	12/15 (80.0)	36/39 (92.3)	12/12 (100.0)
* p-value	p=0.74		p=1.00	
S. aureus positive	16/55 (29.1)	4/15 (26.7)	16/16 (100.0)	4/4 (100.0)
* p-value	p=1.00		p=NA	
Klebsiella positive	11/55 (20.0)	3/15 (20.0)	11/11 (100.0)	3/3 (100.0)
^ p-value	p=1.00		p=NA	
CoNS positive	7/55 (12.7)	2/15 (13.3)	7/7 (100.0)	2/2 (100.0)
^ p-value	p=1.00		p=NA	
E. coli positive	2/55 (3.6)	1/15 (6.7)	2/2 (100.0)	1/1 (100.0)
^ p-value	p=0. 52		p=NA	
Felege Hiwot	20/32 (62.5)	7/8 (87.5)	20/20 (100.0)	7/7 (100.0)
Debre Tabor	19/23 (82.6)	5/7 (71.4)	16/19 (84.2)	5/5 (100.0)
^ p-value	p= 0.14	p=1.00	p=0.11	p=NA
LBWT (<2500g)	31/39 (79.5)	9/10 (90.0)	28/31 (90.3)	9/9 (100.0)
NBWT (≥ 2500g)	8/16 (50.0)	3/5 (60.0)	8/8 (100.0)	3/3 (100.0)
^ p-value	p=0.05	p=0.24	p=1.00	p=NA
WHO LBWT (<2000g)	27/35 (77.1)	8/9 (88.9)	24/27 (88.9)	8/8 (100.0)
WHO NBWT (≥2000g)	12/20 (60.0)	4/6 (66.7)	12/12 (100.0)	4/4 (100.0)
^ p-value	p=0.22	p=0.53	p=0.54	p=NA
Deceased at 14 days	6/7 (85.7)	0/0	6/6 (100.0)	0/0
Alive at 14 days	33/48 (68.8)	12/15 (80.0)	30/33 (90.9)	12/12 (100.0)
^ p-value	p=0. 66	p=NA	p=1.00	p=NA

* Chi square test, ^Fisher's exact value, LBWT: low birthweight, NBWT: normal birthweight

Table 3: Infant characteristics according to mortality status at 14 days follow up

Lab positive blood samples/ all blood samples	n/N (%)	
Characteristic	Deceased N=7	Alive, N=63
	6/7 (85.7)	45/63 (71.4)
^p-value	p=0.67	
Resistance positive bacteria	6/6 (100.0)	42/45 (93.3)
^p-value	p=1.00	
S. aureus positive	2/6 (30.0)	2/2 (100.0)
^p-value	p=1.00	
Klebsiella positive samples	3/6 (50.0)	3/3 (100.0)
^p-value	p=1.00	
CoNS positive samples	3/6 (50.0)	3/3 (100.0)
^p-value	p=1.00	
Felege Hiwot (N=40)	7 (17.5)	33 (82.5)
Debre Tabor (N=30)	0	(100.0)
^p-value	p= 0.02	
LBWT (<2500g)	7/49 (14.3)	42/49 (85.7)
NBWT (≥ 2500g)	0/21	21/21 (100.0)
^p-value	P=0.09	
WHO LBWT (<2000g)	7/44 (15.9)	37/44 (84.1)
WHO NBWT (≥ 2000g)	0/26	26/26 (100.0)
^p-value	p=0.04	

* Chi square test, ^Fisher's exact value, LBWT: low birthweight, NBWT: normal birthweight

Table 4: Environmental samples positive for any of the target bacteria and detection of antimicrobial resistance in environmental isolates by sample type, facility, and unit.

	ALL n/N (%)		**Environmental Swab n/N (%)**			**Tap Water n/N (%)**		**Device Water n/N (%)**		**Hand Rinse n/N (%)**	
	FH N=227	DT N=273	All N=261	FH N=121	DT N=140	FH N=27	DT N=38	FH N=21	DT N=31	FH N=58	DT N=69
Any Positive for target bacteria	61/227 (26.9)	110/273 (40.3)	96/261 (36.8)	28/121 (23.1)	68/140 (48.6)	1/27 (3.7)	6/33 (18.2)	2/21 (9.5)	8/31 (25.8)	30/58 (51.7)	28/69 (40.6)
^p-value	p=0.01			p <0.0001		p =0.22		p=0.17		p= 0.22	
NICU	23/99 (23.2)	61/136 (44.9)	49/118 (41.5)	11/53 (20.8)	38/65 (58.5)	1/8 (12.5)	1/14 (7.1)	2/21 (9.5)	8/31 (25.8)	9/19 (47.4)	14/31 (45.2)
KMC	13/33 (39.4)	22/31 (71.0)	21/36 (58.3)	7/19 (36.8)	14/17 (82.4)	0/3	1/5 (20.0)	--	--	6/11 (54.2)	7/9 (77.8)
Delivery	10/46 (21.5)	7/39 (18.0)	9/43 (20.9)	5/22 (22.7)	4/21 (19.1)	0/10	1/8 (12.5)	--	--	5/12 (41.7)	2/10 (20.0)
PNC	10/31 (32.3)	12/33 (36.4)	12/36 (33.3)	3/17 (17.6)	9/19 (47.4)	0/4	2/5 (40.0)	--	--	7/10 (70.0)	1/9 (11.1)
MST	5/18 (27.8)	8/34 (25.5)	5/28 (17.9)	2/10 (20.0)	3/18 (16.7)	0/2	1/1 (100.0)	--	--	3/6 (50.0)	4/10 (40.0)
^p-value	p=0.34	p<0.0001	p =0.00	p=0.67	P<0.0001	p=0.63	p=0.14	--	--	p= 0.74	p =0.04
Any AMR in target bacteria	33/113 (29.2)	66/73 (90.4)	61/ 104 (58.7)	15/56 (26.8)	46/48 (95.8)	--	--	2/2 (100.0)	6/6 (100.0)	16/30 (53.3)	14/19 (73.7)
^p-value	p<0.0001			p<0.0001				--		--	
NICU	12/49 (24.5)	38/39 (97.4)	29/44 (65.9)	5/20 (25.0)	24/24 (100.0)	--	--	2/2 (100.0)	6/6 (100.0)	5/11 (45.4)	8/9 (88.9)
KMC	10/21 (47.6)	15/16 (93.8)	17/23 (73.9)	6/12 (50.0)	11/11	--	--	--	--	4/7 (57.1)	4/5 (80.0)
Delivery	6/23 (26.1)	4/4 (100.0)	6/16 (37.5)	3/13 (23.1)	3/3	--	--	--	--	3/6 (50.0)	1/1
PNC	5/20 (25.0)	9/9 (100.0)	9/19 (47.4)	1/11 (9.1)	8/8	--	--	--	--	4/6 (66.7)	1/1
MST	0/0	0/5	0/2	0/0	0/2	--	--	--	--	0/0	0/3
^p-value	P=0.25	p<0.001	P=0.004	p=.019	p=0.001	--	--	--	--	p=0.95	p=0.04

FH: Felege Hiwot, DT: Debre Tabor, NICU: Neonatal Intensive Care Unit, KMC: Kangaroo Mother Care, PNC: Post Natal Care, ,MST: Maternity Surgical Theater, AMR: Antimicrobial-resistant target bacteria (including *E. coli, S. aureus*, and other coliforms) *Chi square test, ^Fisher's exact value

Table 5: Hand rinse samples positive for any of the target bacteria[a] by role and gender

	ALL: N=127	**FH; N=58**	**DT; N=69**
Role	^p=0.032	*p=0.48	*p<0.001
Physician	9/19 (47.4)	3/10 (30.0)	6/9 (66.7)
Nurse	18/48 (37.5)	9/16 (56.3)	9/32 (28.1)
Midwife	14/36 (38.9)	13/22 (59.1)	1/14 (7.1)
Mother	16/21 (76.2)	5/9 (55.6)	11/12 (91.7)
GENDER	^p=0.0066	^p=0.80	^p=<0.001
Male	17/54 (31.5)	13/24 (54.2)	4/30 (13.3)
Female	41/72 (56.9)	17/34 (50.0)	24/38 (63.2)
Any Antimicrobial AMR	*p=0.27	*p=0.75	*p=0.41
Physician	7/11 (63.6)	3/6 (50.0)	4/5 (80.0)
Nurse	6/13 (46.2)	3/8 (37.5)	3/5 (60.0)
Midwife	7/13 (26.5)	7/12 (58.3)	0/1
Mother	10/12 (83.3)	3/4 (75.0)	7/8 (87.5)
GENDER	*p=0.51	*p=0.49	*p=0.39
Male	10/17 (58.8)	8/14 (57.1)	2/3 (66.7)
Female	20/31(64.5)	8/16 (50.0)	12/15 (80.0)

FH: Felege Hiwot, DT: Debre Tabor, AMR: Antimicrobial-resistant target bacteria (including *E. coli*, *S. aureus*, and other coliforms to detect bacteria such as *Klebsiella spp.*) *Chi square test, ^Fisher's exact value

[a]: target bacteria included *S. aureus*, *E. coli*, and Other Coliforms (to detect bacteria such as *Klebsiella spp.)*

Table 6. Frequency of target bacteria detection[a] from swabs of environmental surfaces by sampling site, facility, and unit

Site	ALL	FH	DT	NICU	KMC	DEL	PNC	MST
	N=261	N=58	N=69	N=118	N=36	N=43	N=36	N=28
IV tubing and stand	4/30 (13.3)	4/25 (16.0)	0/5	3/13 (23.1)	--		1/9 (11.1)	
CPAP machine	4/11 (36.4)	3/10 (30.0)	1/1 (100.0)	2/6 (33.3)	--	1/2 (50.0)		1/3 (33.3.0)
Sink faucets	2/7 (28.6)	1/3 (33.3)	1/4 (25.0)	0/1	1/2 (50.0)	--	--	1/4 (25.0)
Oxygen cylinder	2/7 (28.6)	2/6 (33.3)	6/18 (33.3)	7/22 (31.8)		--	--	1/2 (50.0)
Linens (bed sheet, blanket, towel)	29/39 (74.4)	5/7 (71.4)	24/32 (75.0)	10/13 (76.9)	13/15 (86.7)	--	6/11 (54.6)	--
Cabinet	3/22 (13.6)	1/14 (7.1)	2/8 (25.0)	1/15 (6.7)	0/1	2/6 (30.0)		
Door & handle	15/50 (30.0)	5/30 (16.7)	10/20 (50.0)	4/10 (40.0)	5/14 (35.7)	3/14 (21.4)	2/8 (25.0)	1/4 (25.0)
Bed rail	16/26 (61.5)	3/8 (37.5)	13/18 (72.2)	11/16 (68.8)		2/2 (100.0)	3/8 (37.5)	--
Chair	5/12 (41.7)	3/10 (30.0)	2/2 (100.0)	2/2 (100.0)	2/4 (50.0)	1/6 (16.7)	--	--
Sterile surgical equipment & drape	0/3	0/3	1/6 (16.7)	0/0	--	--	--	1/9 (11.1)
Monitor and pickup tray	5/13 (38.5)	--	0/3	0/0	--	--	--	0/3
Radiant Warmer & Ambubag	5/13 (38.5)	1/1 (100.0)	4/12 (33.3)	5/13 (38.5)	--	--	--	--
Fetal monitor	0/5	--	0/5	0/0	--	0/5	--	--
Stethoscope or thermometer	3/5 (60.0)	0/2	3/3 (100.0)	3/5 (60.0)	--	--	--	--
Other	1/5 (20.0)	0/2	1/3 (33.0)	1/2 (50.0)	--	--	--	0/3
^p-value	p =0.13	p =0.07	p=0.001	--	--	--	--	--

FH: Felege Hiwot, DT: Debre Tabor, NICU: Neonatal Intensive Care Unit, KMC: Kangaroo Mother Care, DEL: Delivery Unit, PNC: Post Natal Care, MST: Maternity Surgical Theater, IV: Intravenous, CPAP: continuous positive airway pressure, *Chi square test, ^Fisher's exact value

[a]: target bacteria included *S. aureus*, *E. coli*, and Other Coliforms (to detect bacteria such as *Klebsiella spp.)*

Table 7. Frequency of detection of AMR target bacteria[a] from swabs of environmental surfaces in each facility

Site	ALL	ALL FH	ALL DT
Total Swabs	**N=157**	**N=56**	**N=48**
IV tubing and stand	4/15 (26.7)	4/15 (26.7)	--
CPAP machine	1/1 (100.0)	1/1 (100.0)	--
Sink faucets	1/2 (50.0)	1/1 (100.0)	0/1
Oxygen cylinder	2/4 (50.0)	1/2 (50.0)	1/2 (50.0)
Linens (bed sheet, blanket, towel)	23/24 (98.8)	3/4 (75.0)	20/20 (100.0)
Cabinet	1/7 (14.3)	0/6	1/1 (100.0)
Door handle and door	9/24 (37.5)	1/16 (6.3)	8/8 (100.0)
Bed rail	13/16 (81.3)	1/4 (25.0)	12/12 (100.0)
Chair	4/8 (50.0)	3/7 (42.9)	1/1 (100.0)
Radiant Warmer & Ambubag	3/3 (100.0)	--	3/3 (100.0)
^p-value	p<0.001	p<0.01	p<0.01

FH: Felege Hiwot, DT: Debre Tabor, IV: Intravenous, CPAP: continuous positive airway pressure, ^Fisher's exact value

[a]: target bacteria included *S. aureus*, *E. coli*, and Other Coliforms (to detect bacteria such as *Klebsiella spp.)*

Table 8. Frequency of *E. coli* detection from surface swab and hand rinse samples by facility unit

UNIT	ALL, n+/N (%)	FH, n+/N (%)	DT, n+/N (%)
Total	52/417 (12.5)	13/182 (7.1)	39/235 (16.6)
NICU	27/202 (13.4)	5/83 (6.0)	22/119 (18.5)
KMC	11/51 (21.6)	2/27 (7.4)	9/24 (37.5)
Delivery	5/63 (7.9)	2/32 (6.3)	3/31 (9.7)
PNC	6/53 (11.3)	3/25 (12.0)	3/28 (10.7)
MST	3/48 (6.3)	1/15 (6.7)	2/33 (5.1)
*p-value	p=0.14	p= 0.90	p= 0.02

* Chi square test

Table 9. Frequency of other coliform detection from surface swab and hand rinse samples by facility unit

UNIT	ALL, n+/N (%)	FH, n+/N (%)	DT, n+/N (%)
Total	100/403 (24.8)	34/188 (18.1)	66/215 (30.7)
NICU	59/199 (29.7)	16/89 (18.0)	43/110 (39.1)
KMC	27/49 (55.1)	12/27 (44.4)	15/22 (68.2)
Delivery	5/61 (8.2)	5/32 (15.6)	0/29
PNC	7/52 (13.5)	1/25 (4.0)	6/27 (22.2)
MST	2/42 (4.8)	0/15	2/27 (7.4)
*p-value	p<0.0001	p<0.001	p<0.0001

* Chi square test

Table 10. Frequency of *S. aureus* detection from surface swab and hand rinse samples by facility unit

UNIT	ALL, n+/N (%)	FH, n+/N (%)	DT, n+/N (%)
Total	50/416 (11.8)	23/188 (12.2)	26/228 (11.4)
NICU	20/209 (9.6)	7/25 (28.0)	14/120 (11.7)
KMC	9/50 (18.0)	3/27 (11.1)	6/23 (26.1)
Delivery	5/63 (7.9)	3/32 (9.4)	2/31 (6.5)
PNC	8/52 (15.4)	7/25 (28.0)	1/27 (3.7)
MST	7/42 (16.7)	4/15 (26.7)	3/27 (11.1)
*p-value	p=0.25	0.02	p=0.12

FH: Felege Hiwot, DT: Debre Tabor, NICU: Neonatal Intensive Care Unit, KMC: Kangaroo Mother Care, PNC: Post Natal Care, OR: MST: Maternal Surgical Theater, *Chi square test, ^Fisher's exact value

TABLE 11 WASHCon Lite survey results by facility and unit

	Felege Hiwot				Debre Tabor			
	NICU	**KMC**	**DEL**	**PNC**	**NICU**	**KMC**	**DEL**	**PNC**
	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)
Water Availability & Quality								
Functional Piped Water	7/8 (88)	7/8 (88)	8/8 (100)	3/8 (38)	12/12(100)	8/12 (67)	5/12 (42)	8/12 (67)
Water Available	4/8 (50)	6/8 (75)	6/8 (75)	1/8 (13)	12/12 (100)	7/12 (58)	6/12 (50)	7/12 (58)
Treated Water Available	0/8	1/8 (13)	0/8	0/8	12/12 (100)	11/12 (92)	9/12 (75)	11/12 (92)
Unit Water Storage	2/8 (25)	0/8	3/8 (38)	5/8 (63)	11/12 (92)	1/12 (8)	11/12 (92)	11/12 (92)
Hand Hygiene								
Hand Hygiene Station (HCP)	7/8 (88)	6/8 (75)	7/8 (88)	2/8 (25)	12/12 (100)	12/12 (100)	8/12 (67)	9/12 (75)
Soap & Water or ABHR available	6/8 (75)	1/8 (13)	2/8 (25)	0/8	7/12 (58)	9/12 (75)	3/12 (25)	2/12 (17)
Hand Hygiene Station (Caregivers)	7/8 (88)	7/8 (88)	4/8 (50)	3/8 (38)	10/12 (83)	10/12 (83)	6/12 (50)	7/12 (58)
Soap & Water or ABHR available	1/8 (13)	0/8	1/8 (13)	0/8	0/12	0/12	0/12	0/12 (8)
Hand Hygiene Promotion Materials Available	4/8 (50)	0/8	2/8 (25)	0/8	12/12 (100)	12/12 (100)	11/12 (92)	12/12 (100)
Visible Cleanliness								
Segregated Waste	3/8 (38)	2/8 (25)	5/8 (63)	2/8 (25)	3/12 (25)	2/12 (17)	0/12	0/12
Visibly Clean of Dust and Soil	7/8 (88)	8/8 (100)	8/8 (100)	8/8 (100)	10/12 (83)	11/12 (92)	2/12 (16.7)	1/12 (8)
Bodily Fluids Visible	1/8 (13)	1/8 (13)	0/8	2/8 (25)	0/12	0/12	6/12 (50)	8/12 (67)
Floors Visibly Clean	8/8 (100)	8/8 (100)	8/8 (100)	7/8 (88)	8/12 (67)	12/12 (100)	2/12 (17)	1/12 (8)
Staff Toilet Visibly Clean	8/8 (100)	6/8 (75)	7/8 (88)	7/8 (88)	10/12 (83)	9/12 (75)	11/12 (92)	11/12 (92)
Caregiver Toilet Visibly Clean	2/8 (25)	4/8 (50)	4/8 (50)	6/8 (75)	9/12 (75)	8/12 (67)	1/12 (8)	1/12 (8)
Environmental Cleaning Supplies Available								
Gloves Available	8/8 (100)	5/7 (71)	8/8 (100)	7/8 (88)	11/12 (92)	12/12 (100)	11/12 (92)	12/12 (100)
Disinfectant Available	7/8 (88)	4/7 (57)	8/8 (100)	6/8 (75)	11/12 (92)	11/12 (92)	10/12 (83)	11/12 (92)
Soap Available	1/8 (13)	2/7 (29)	1/8 (13)	1/8 (13)	9/12 (75)	9/12 (75)	9/12 (75)	8/12 (67)
Mop Available	6/8 (75)	4/7 (57)	6/8 (75)	5/8 (63)	11/12 (92)	12/12 (100)	11/12 (92)	11/12 (92)
Broom Available	5/5 (100)	4/7 (57)	7/8 (88)	5/8 (63)	8/12 (67)	8/12 (67)	8/12 (67)	8/12 (76)
PPE separate	1/8 (13)	0/7	1/8 (13)	0/8	12/12 (100)	12/12 (100)	9/12 (75)	8/12 (67)
PPE Visibly Clean	8/8 (100)	1/7 (14)	4/8 (50)	0/8	6/12 (50)	9/12 (75)	11/12 (92)	9/12 (75)
Infection Prevention and Control (IPC)								
≥4 of the Six Cleans (Delivery)	--	--	1/8 (13)	--	--	--	6/12 (50)	--
Control Access Point at Entry to NICU Enforced	6/8 (75)	--	--	--	12/12 (100)	--	--	--
Persons Beyond Access Point	8/8 (100)	--	--	--	0/12	--	--	--
PPE Required	6/8 (75)	--	--	--	7/12 (58)	--	--	--
Hand Hygiene Required at Entry	0/8	--	--	--	0/12	--	--	--
Fresh Gloves at Entry	1/8 (13)	--	--	--	0/12	--	--	--
Recontamination of Hands at Entry	0/8	--	--	--	0/12	--	--	--

NICU: Neonatal Intensive Care Unit, KMC: Kangaroo Mother Care, PNC: Post Natal Care,

Appendix

Appendix A. WASH Conditions "WASHCon" Lite Assessment Tool

#	Survey Question	Answer Options
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

#	Survey Question	Answer Options
		□ 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

#	Survey Question	Answer Options
		□ 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?	□ Water □ Soap

#	Survey Question	Answer Options
	Select all that apply	□ 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*	