UNIVERSITY OF CAPE COAST

THE INTERPLAY BETWEEN MICROBIAL AIR QUALITY AND VERNIX CASEOSA ON THE HEALTH OF NEONATES DURING THE FIRST SEVEN DAYS AFTER BIRTH

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BY

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DECLARATION

Candidate's Declaration

I hereby declare that this dissertation is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: ……. Date: …….

Name: Mary Fouah

Supervisor's Declaration

I hereby declare that the preparation and presentation of the dissertation were supervised under the guidelines on supervision of dissertation laid down by the University of Cape Coast

Candidate's Signature: ……. Date: …….

Name: Dr. Cynthia A. Adinortey

ABSTRACT

Healthcare-Associated Infections are gradually becoming a new public health challenge in healthcare settings, particularly in maternity wards. To explore the interplay of microbial air quality and vernix caseosa on the health of neonates during the first seven days after birth, a cross-sectional study was conducted using 845 samples obtained from the maternity ward of the University of Cape Coast hospital in Cape Coast from October to December 2023. These included air samples, swabbing of 17 items, nurses' palms and 59 neonates who were born in the maternity block within a five week- period of the study. Indoor air was sampled at the various rooms with agar plates placed at different height, 1.5 m above the ground level for the delivery room, and at 84 cm above the ground level in the lying-in rooms. Categorical analysis of samples from the palms of nurses, mothers and the bodies of the neonates indicated 22.0%, 67.8% and 79.7% bacterial growth respectively. *Staphylococcus epidermis* had the highest infection rate among the organisms isolated from the neonates, nurses, and mothers, with 29 neonates, 6 nurses, and 14 mothers affected. A multivariate analysis was conducted on the categorical data from swabs taken from the neonates, nurses' palms, and mothers' palms to assess the likelihood that the neonates were infected by organisms originating from either the nurses' palms, the air, or the mothers. It was found that the organisms from the palm of nurses were significantly associated with the infection of the neonates (p-values of 0.040). Three to seven days after discharge, the number of neonates that were susceptible were 11 (18.6%) of those infected and out of this, Staphylococcus *epidermis* still recorded the highest number out of the organisms isolated (54.5%). A cross-tabulation and Pearson's chi- square analysis was performed to study the relationship between the categorical variable of organisms isolated right after birth and those isolated three to seven days after birth. it was found that, all the babies that had no infection right after birth still had no infection after 3 to 7 days of infection. The relationship showed a p-value of 0.063. This study highlights that despite the delivery ward being regarded as a critical area where high levels of cleanliness and sterility are essential, it is still prone to microbial contamination. Significant microorganism was detected in the air, signifying that the maternity ward environment can be a potential reservoir for bacteria and other microorganisms. This highlights the need for stringent infection control measures, including the regular maintenance of ventilation systems, the use of appropriate personal protective equipment, and adherence to aseptic techniques.

DEDICATION

To my amazing family, Prof. Anthony Kwabena Twum and our children for their immense love and support throughout my MPhil journey.

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LIST OF ABBREVIATIONS

WASH	Water, Sanitation and Hygiene
IPC	Infection Prevention and Control
Sp	Species
MA	MacConkey Agar
UCC	University of Cape Coast
UCCIRB	University of Cape Coast Institutional Review Board
O&G	Obstetrics and Gynaecology
CCTH	Cape Coast Teaching Hospital
CCMH	Cape Coast Metropolitan Hospital
WHO	World Health Organization
ELISA	Enzyme Linked Immunoassay
CAUTIs	Catheter-Associated Urinary Tract Infections
HAIs	Healthcare Associated Infections
HCFs	Health Care Facilities
Ec	*Escherichia coli*
Sa	*Staphylococcus aureus*
Bs	*Bacillus* species
Ms	*Micrococcus* species
Sp	*Streptococcus pneumoniae*
Kp	*Klebsiella pneumoniae*
Se	*Staphylococcus epidermidis*
Mc	*Moraxella catarrhalis*
Pa	*Pseudomonas aeruginosa*
Sc	*Stachybotrys chartarum*
Afl	*Aspergillus flavus*

An	*Aspergillus niger*
NICU	Neonate Intensive Care Unit
SDA	Sabouraud Dextrose Agar
NA	Nutrient Agar
ENT	Ear and Throat
Hc	Histoplasma capsulatum
Ms	*Mucor* species
Bd	*Blastomyces dermatitidis*
At	*Aspergillus terreus*
Ca	*Candida albicans*
CDC	Centre for Disease Control and prevention
Af	*Aspergillus fumigatus*
Pg	*Penicillin glabrum*
Fs	*Fusarium solani*
VAP	Ventilator- associated Pneumonia
CLABSIs	Central Line Associated Blood Stream Infections
RCH	Reproduction and Child Health
NMF	Natural Moisturizing Factors

CHAPTER ONE

INTRODUCTION

1.0 Background

Healthcare-Associated Infections (HAIs) in neonates, also known as neonatal nosocomial infections, are significant concern in healthcare settings, particularly in maternity wards and neonatal intensive care units (NICUs). Neonates are particularly susceptible to HAIs due to their immature immune systems and their underdeveloped skin barrier (Guen et al., 2007). The infections can have serious consequences, leading to prolonged hospital stay, increased healthcare cost, and, in severe cases, mortality (Guen et al., 2007). Neonates are considered to be among the group of people who are immunosuppressed in the society. This is because at birth their organs would not be fully developed to stand any kind of infection.

The air around us, although not sterile, typically contains acceptable levels of microorganisms. However, if the concentration of microbes exceeds a certain threshold, or if harmful species are present in the air, neonates can develop infections such as those affecting the skin, lungs, eyes, mouth, ears, umbilical cord, or bloodstream when exposed. Healthcare-associated infections (HAIs) are particularly difficult to treat or manage because many of the organisms

involved are multidrug-resistant strains (C.D.C, 2019). These microbes may be resistant to first-line antibiotics and antifungals, leading to significant complications for both the mother and the neonate.

Moulds are filamentous fungi composed of hyphae and are typically found on plant and animal matter. They thrive in damp, poorly ventilated areas and reproduce by releasing spores. Airborne mould spores are present both indoors and outdoors, producing mycotoxins—volatile biological compounds—and other by-products found in the air (Brewer et al., 2013). People exposed to these environments face a wide range of acute and chronic health issues, including respiratory diseases, neurological conditions, persistent fatigue, and skin infections.

The air we breathe is laden with microorganisms known as bioaerosols, which are suspensions of liquid droplets and solid particles that may contain viruses, fungal spores (conidia), bacterial endospores, pollen, and plant fragments (Kim et al., 2018). Moulds, a diverse group of fungi, pose a health risk in enclosed spaces such as homes, schools, cinemas, libraries, and hospitals. Even small amounts of mould can be hazardous, and prolonged exposure can lead to serious health consequences (Weinhold, 2007). Mould toxicity is characterized by consistent exposure, which may lead to allergy symptoms like sore throat and

coughing in immunocompromised individuals (Pizzorno, 2016). Additionally, exposure to mould can trigger breathing difficulties and asthma attacks in people with chronic lung conditions (Mendell et al., 2011). Long-term exposure can result in infections, memory loss, fatigue, and lung problems (Mendell et al., 2011).

Nevertheless, not only fungi cause infection or diseases, bacteria are also not innocent about hospital acquired infections. Different kinds of bacteria found indoor produce volatile and biological substances also found in the air. When the host's immune system is impaired, opportunistic bacterial infections begin. Most antibiotic-resistant bacteria can be located in healthcare institutions due to regular drug exposure and major hands-on treatment provided to patients (C.D.C, 2019). The most regular causative species of hospital infection on neonates are *Escherichia coli, Klebsiella pneumoniae*, *Staphylococcus epidermidis Staphylococcus aureus* and *Candida albicans* (Kucova et al., 2021). These species are most common microbes that affects the development of early-onset neonatal infections (Shane et al., 2017). Also, multidrug-resistant characteristics are found in 20 % - 40 % of all known HIA infections (Boucher et al., 2009).

Different care settings in European hospitals showed varying rates of at least one HAI: primary care had a rate of 4.4 %; tertiary care had a rate of 7.1 %; intensive care units had a rate of 19.2 %; and long-term care had a rate of 3.7 % (Szabó et al., 2022). In the USA, the average rate of HAI among hospitalized patients was 3.2 % in 2015, a rate that is considerably less than the 4% discovered in 2011 research (Szabó et al., 2022). In US healthcare institutions, HAI occurred in 6.1 % of step-down or specialized medical units, 36.4 % of critical care sites, 57.5 % of ward and nursery locations, and 6.4 % of mixed severity locations. HAI seems to be more common in developing countries. The prevalence of HAI approximated 15.5% in these less resourced countries, with infections in infants and neonates of who vernix caseosa is expected to protect them; making up the majority of the cases (Allegranzi et al., 2011).

Vernix caseosa is a whitish, cheesy, waxy substance that covers the skin of a neonate at birth. It consists of an oily substance called sebum, water, and some shed skin cells. Vernix caseosa has many important benefits and various positive effects on the neonate, contributing to their maturity and growth. Pickens et al. (2000) indicated that vernix caseosa was a moisturizer for the stratum corneum (Rawlings et al., 1994). Vernix caseosa is responsible for

maintaining the suppleness and elasticity of the stratum corneum (Saijo & Tagami, 1991). In theory, the high-water content in vernix caseosa could create an optimal high-humidity micro environment (Hoath et al., 2006). New-born frequently encounter bacterial skin infections (Hooven & Polin, 2019). The critical point in the defence mechanisms of new-born lies in their underdeveloped adaptive immunity, which relies on antimicrobial peptides and proteins (Wilson et al.,2015) to the presence of antimicrobial components, vernix caseosa may play a significant role in protecting the foetus from acute or sub-acute chorioamnionitis and promoting the colonization of the skin by microorganisms after birth (Marchini et al., 2002; Visscher et al., 2005). Vernix caseosa substitutes have the potential to be useful in medicine, particularly for treating the underdeveloped skin barrier of premature infants, due to their unique protective and healing properties (Visscher et al., 2005) or aiding in the healing of wounds on adult skin (Haubrich, 2003).

However, a significant challenge lies in the fact that the biological material itself cannot be directly employed in treatment. Consequently, researchers have sought to create synthetic biofilms that replicate the distinctive composition and attributes of natural vernix (Rissmann et al., 2009). A key obstacle in this effort is the limited knowledge of *Vernix caseosa's* composition.

Furthermore, artificial vernix caseosa could have valuable uses in the cosmetics industry, potentially serving as a natural skin cleanser. (Moraille et al., 2005).

1.1 Statement of the Problem

Infections occur in the process of delivering health care, such as during surgery or procedures like endotracheal intubation, where pathogens may enter the body through invasive techniques, potentially leading to healthcare-associated infections (HAIs), also known as nosocomial infections or nasal intubation, central venous catheterization, during delivery and suctions in the labour ward, cutting of cord, surgical drains, nasogastric tube, tracheostomy, urinary catheter, and treatment procedures like blood transfusion, parenteral nutrition, stress-ulcer prophylaxis, length of stay. Microorganisms including Fungi and bacteria naturally exist on our skin and the hands of healthcare professionals (Keri et al., 2021). Through mucosal membranes, cuts, and wounds, these fungi and bacteria enter the body and cause infections that can range in severity from minor to life-threatening while patient is in the hospital (Keri et al., 2021). Some neonates start developing skin rashes and acute to severe upper respiration infections after twenty-four hours or within seven days of delivery (Kutlubay et al., 2017). This

can affect the baby's sleeping pattern, feeding and gross development of the brain (Singh et al., 2018).

Hospital acquired infections are difficult to treat as compared to community ones because HAIs are mostly multi drug resistant (C.D.C, 2019). Neonates are particularly vulnerable to microbial infections during the first 48 hours of life, as their delicate skin can easily become susceptible to various pathogens. This susceptibility is exacerbated by factors such as the presence of water, proteins, and glucose in the amniotic fluid, which provide a nutrient-rich environment that facilitates the growth and proliferation of microbes. The optimal temperature surrounding the newborn also promotes microbial survival and increases the likelihood of infection. In Ghana, several studies have investigated nosocomial infections, particularly in maternity wards and Neonatal Intensive Care Units (NICUs). Researchers have documented the prevalence of these infections in major hospitals, such as Korle-Bu Teaching Hospital, where studies by Enweronu-Laryea and Newman (2007) and Fenny et al. (2022) highlight the ongoing challenges in managing neonatal infections. Similar research conducted at Cape Coast Teaching Hospital by Ocran and Tagoe (2014) emphasizes the need for improved infection control measures. Internationally, studies by Pessoa-Silva et al. (2007) and Randle et al. (2006)

have contributed to the broader understanding of nosocomial infections in NICUs, demonstrating that neonatal infection prevention is a global concern. These findings underscore the critical importance of protecting newborns from early exposure to harmful microorganisms, particularly in hospital environments where they are most vulnerable. There is currently no information available regarding the nosocomial infection on the neonate in the University of Cape Coast Health facility. This made it crucial to check the air quality for bacteria and fungi in order to determine any potential nosocomial illness that neonates could contract in the maternity block of University Health Services in Cape Coast, Ghana.

1.2 Research Questions

i. What are the most prevalent bacterial and fungal contaminants at University of Cape Coast's hospital maternity block?

ii. Which nosocomial infections are emerging at the health facility?

iii. What nosocomial infections are prevalent in the facility?

iv. Are the neonates susceptible to nosocomial infections in the ward?

1.3 Hypothesis

Nosocomial infections in healthcare institutions have no effects on neonates.

1.4 Objective of the Study

This study seeks to assess microbial indoor air quality of the UCC Hospital Maternity block and its effect on the neonate in the first seven (7) days of their life and beyond.

1.5 Specific Objectives

Specifically, the research sought to:

i. isolate and identify bacteria and fungi from labour and lying-in rooms of the UCC Hospital Maternity block over a five-week period

ii. assess any infection on the neonates after six hours of delivery

iii. assess any infection on the neonates after seven days of discharge

iv. identify the kind of microbes responsible for the infection on the neonates

CHAPTER TWO

LITERATURE REVIEW

2.0 Background

Healthcare-Associated Infections (HAIs) in neonates, also known as neonatal nosocomial infections, are significant concern in healthcare settings, particularly in maternity wards and neonatal intensive care units (NICUs) (Kumar et al., 2018). Neonates are particularly susceptible to HAIs due to their immature immune systems and underdeveloped skin barrier (Guen et al., 2007). Common HAIs in neonates include ventilator-associated pneumonia (VAP), central line-associated bloodstream infections (CLABSIs), and catheter-associated urinary tract infections (CAUTIs). These infections can have serious consequences, leading to prolonged hospital stays, increased healthcare costs, and, in severe cases, mortality (Guen et al., 2007).

Neonates are considered to be among the group of people who are immunosuppressed in the society. This is because at birth their organs would not be fully developed to stand any kind of infection (Camacho-Gonzalez et al., 2013). The air surrounding us is not sterile since there is always allowable microorganism in the atmosphere. However, if the microbe's concentration exceeds certain threshold, the neonates can have infections such as skin, lungs, eye, mouth, ear, cord, blood and so on (Reuter et al., 2014).

Most often than not, delivery occur at the hospitals, clinics and maternity homes, which are normally enclosed. Such places happen to be the neonates first point of contact to the outside world. After delivery, some of the neonates are sent to the NICU due to complications for further management and those with no complication are sent home with their mothers. If their first point of contact has microbial contamination, the neonate might pick these infection as they go home or the NICU (Cason et al., 2021). As the neonate is sent to these places and proper cleaning is not ensured, the infection begin to manifest itself as skin, cord, eye, throat, lung and mouth infections (Cason et al., 2021). HAIs are very difficult to treat or to manage since most of these organisms are the multidrug resistant strains (C.D.C, 2019).

They might have resistance against first line antibiotics and antifungal. The infection can bring about a lot of inconvenience to the mother and the neonate. In terms of epidemiology, research from Europe and America show relatively comparable outcomes in surveillance programs, notwithstanding the fact that the precise global frequency in HAI remains unknown due to the lack of credible data and monitoring methodologies. (Sikora & Zahra, 2020).

2.1 Microbial Indoor Air Quality

Microbial indoor air quality in maternity ward can have implications on the health and well-being of neonates after delivery. The presence of certain microorganisms in the air can increase risk of HAIs and respiratory issues in these vulnerable neonates. Maternity wards are high-risk areas for microbial contamination due to the presence of many people, including healthcare workers and visitors and the continuous flow of patients. Airborne microorganisms in the maternity ward can come from various sources, including human shedding (skin, respiratory secretions), construction or renovation activities, and contaminated medical equipment. Ongoing construction or renovation work can generate airborne particles and microorganisms that can potentially contaminate the indoor air. Improperly cleaned or non-sterilized medical equipment can be a source of microbial contamination in the environment.

Airborne pathogens, such as *Staphylococcus aureus,* Enterobacteriaceae, and respiratory viruses, can be major contributors to HAIs in the maternity ward (Coffin & Zaoutis, 2011). Poor indoor air quality, especially with high levels of particulate matter or airborne pathogens, can exacerbate respiratory problems in neonates. Premature infants or those with pre-existing respiratory conditions may be more vulnerable negative effects of indoor air pollution (Esposito et al.,

2014). Exposure to environmental pollutants, including microbial contaminants, can increase the risk of respiratory distress in these infants. World Health Organization (WHO) recommends that indoor air quality in healthcare facilities should be monitored regularly to ensure that it meets acceptable standards. The WHO also recommends that healthcare facilities should have a comprehensive infection control program in place to prevent the spread of infections (Harun et al., 2022). Maintaining proper indoor air quality in the maternity ward requires strict adherence to infection control measures. Implementing ventilation systems that provide sufficient air exchange and efficient filtration can help reduce the concentration of airborne pathogens (Elsaid & Ahmed, 2021).

2.2 Common Bacterial Species that can Cause HAIs

Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Proteus vulgaris, Klebsiella pneumoniae, Streptococcus pneumoniae, and *Micrococcus* are different types of bacteria that can have varying effects on neonates (Sharma et al., 2013). The impact of these bacteria on neonates largely depends on the site of infection, the route of transmission, the neonate's immunological state and the prompt provision of suitable medical therapies (Singh et al., 2018). Acinetobacter baumanii is related with significant

mortality in intensive care units due to its innate multidrug resistance (Elbehiry et al., 2023). Multidrug-resistant bacteria are frequent in HAI and are associated with high mortality rates (Jernigan et al., 2020). According to one study (Sievert et al., 2013), around 20% of all documented pathogens exhibit multidrug resistance.

Particularly notable pathogens include methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-intermediate Staphylococcus aureus (VISA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), Enterobacteriaceae with extended-spectrum cephalosporin resistance corresponding to extended-spectrum beta-lactamase (ESBL) production, vancomycin-resistant *Enterococcus aureus* (VRE), carbapenem-resistant *Enterobacteriaceae* as well as *Acinetobacter* species, and multi-drug resistant *Pseudomonas aeruginosa* (Sievert et al., 2013)

In neonates, *Staphylococcus aureus* can cause infections of the skin and soft tissues, infections of the bloodstream (sepsis), and pneumonia. MRSA infections can be particularly difficult to manage since they have antibiotic resistance (Hornik et al., 2012). Staphylococcal scalded skin syndrome (SSSS) is an uncommon but severe skin illness caused by specific strains of

Staphylococcus aureus that causes blistering and exfoliation of the skin (Ross & Shoff, 2017). Other bacterial species that can cause HAIs are:

- ***Proteus vulgaris:*** *Proteus vulgaris* is known for its association with urinary tract infections (UTIs). In neonates, UTIs caused by *Proteus vulgaris* can lead to irritability, poor feeding, fever, and possible kidney involvement (Leung et al., 2019).
- ***Klebsiella pneumoniae:*** *Klebsiella pneumoniae* can cause severe infections in neonates, including pneumonia, bloodstream infections, and urinary tract infections. Neonatal infections with *Klebsiella pneumoniae* may lead to respiratory distress, sepsis, and other systemic manifestations.
- ***Streptococcus pneumoniae:*** Streptococcus pneumoniae is a common cause of respiratory infections in children, including neonates. In neonates, *Streptococcus pneumoniae* infections can lead to pneumonia, sepsis, and meningitis. Pneumococcal meningitis is a serious and potentially life-threatening infection that can occur in neonates and requires prompt medical intervention.
- ***Micrococcus*:** *Micrococcus* species (*luteus* and *roseous*) are typically considered non-pathogenic and are part of the normal skin flora. They

normally cause body odour. In rare cases, *Micrococcus* species can cause opportunistic infections in neonates, particularly in those with compromised immune systems. It is essential to note that neonates have immature immune systems, making them more susceptible to infections. Additionally, premature neonates and those with underlying health conditions are at higher risk of severe infections. Early recognition, prompt diagnosis, and appropriate treatment with antibiotics are crucial in managing bacterial infections in neonates.

2.3 Common Moulds and Yeast that are Found Indoors

2.3.1 Types of Moulds

Moulds are categorized into three types, each of which has a particular impact on humans. They are, allergic moulds, pathogenic moulds, and toxigenic moulds (Kuhn & Ghannoum, 2003). Allergenic moulds are the least dangerous to most individuals and cause seasonal allergy symptoms. Pathogenic moulds are moulds that might be harmless for certain individuals but dangerous for others with pre-existing conditions. They can infect those with immune systems that are impaired (such as premature infants) or those who suffer from asthma or similar breathing disorders. The toxigenic mould, occasionally referred to be

black mould, is the most dangerous because they can create and spread deadly spores, these moulds are exceedingly harmful. This mould species can cause serious illnesses and even death, particularly in the most fragile patients (Mehler et al., 2022).

2.3.2 Possible Sources of Mould Exposure

Mould can grow wherever there is an excess of moisture. Flooded basements, moist carpets, improperly maintained air conditioning units, plumbing leaks, roof leaks, and windows that do not seal properly are all major causes of mould growth indoors. There are over 1,000 different varieties of mould that can develop inside, but the majority of them are not dangerous to human health (Hardin et al., 2003).

2.3.3 Common Moulds in Buildings

Alternaria, Blastomyces, Cryptococcus, Emmonsia, Ganoderma, Aspergillus Histoplasma, Microsporum, Mucor, Cladosporium, Penicillium, Candida, Rhizopous, Stachybotris, and Trichophyton are some of the species of moulds that are frequently discovered in buildings (Hardin et al., 2003).

- ***Chaetomium:*** This particular allergic mould frequently develops in areas that have experienced flood damage. In the aftermath of a flood,

Chaetomium may likely grow in the structure. If there was previously a leak, the humid, warm environment that results make it possible for mould to develop. Typically, it starts out as a sort of fuzzy white outgrowth that later goes black, leading to confusion with *Stachybotrys.*

- ***Alternaria:*** It is a type of allergenic mould that is commonly found in bathrooms around the sink, shower, toilet, and other sources of water. This is because this particular type of mould is frequently an indicator of leaks. When the circumstances are favourable, they spread swiftly. It has a velvety feel and is dark green or brown tint. When exposed to it, it can induce sneezing, watery eyes, and other symptoms (Money et al., 2004).
- ***Cladosporium:*** This is a type of allergic mould that grows in unusual settings. While most moulds prefer warm conditions, this mould can thrive in both warm and cold temperatures. It can thrive on ruined food stored in a refrigerator or cold storage unit. It has a one-of-a-kind appearance, with a suede-like texture and an olive or brown tone. It can also be found in fabrics, carpets, and floor boards (Money et al., 2004).
- ***Mucor:*** Another allergenic mould that can be discovered indoors is Mucor. It has the potential to be harmful due to its rapid growth. It is

distinguished by its white or grey coloration, which develops in large patches. Mucor also likes dampness because it develops in moist areas with a lot of condensation. They thrive on leaky roofs and air-conditioning rooms.

- ***Stachybotrys:*** This is a toxicogenic mould that is commonly referred to as "black mould" due to its dark brown colour. Moisture is also required for the growth of this mould. It is commonly seen in restrooms and other wet areas (Money et al., 2004).
- ***Aspergillus:*** According to Money et al. (2004), this is a type of pathogenic mould; however, not all aspergillus moulds cause sickness. Aspergillus is widespread in the environment, including the air and buildings. It is distinguished by its thick grey or green spores. It can, however, come in a variety of colours. Because of the absorbent characteristics of wood, they are commonly seen on it. They spread by dispersing spores. These little spores are picked up by the air and drift away, hoping to land and flourish somewhere.

2.3.4 Toxic Mould Infections and Its Associated Risk

Mycotoxins can be harmful to people' health if they are inhaled or otherwise exposed to them. These poisons can cause the following effects depending on the type of exposure and the individual's vulnerability: skin infections, sinus infections, lung infections, liver difficulties, kidney impacts, and other organ damage are all possibilities (Kraft et al., 2021). Nervous system damage, immune system suppression and cancer can also be attributed to toxic moulds. Not everyone becomes ill when exposed to mycotoxins. Infants, the elderly, and those with mould sensitivity or allergy, those with compromised immune systems, those with underlying lung disease, and those receiving hospital care are more vulnerable. Several investigations have discovered a link between toxic mould and catastrophic health consequences. In the late 1990s, the CDC published the findings of an examination into acute pulmonary haemorrhage (lung bleeding) in ten infants from Cleveland, Ohio (Dearborn et al., 1999). All the infants resided in the same neighbourhood and suffered from haemorrhage between January 1993 and December 1994. One of the babies perished. The CDC linked the children's illnesses to toxic mould exposure, namely *Stachybotrys*, which was thought to have occurred as a result of substantial household water damage that occurred six months before the infants

became ill. Researchers linked toxic black mould to wet building-related ailments in 2008, including respiratory, immunologic, and neurologic problems. They also stated that laboratory research has revealed that mycotoxins from this mould induce lung irritation (Pestka et al., 2008).

The repercussions of exposure to dangerous moulds could be hard to foresee. In most individuals, exposure does not result in illness. However, some groups are more exposed than others. These include children, the elderly, individuals on immunosuppressive medications, people with asthma, mould allergies, or previous lung conditions, people with compromised immune systems. Chronic bronchitis, sinusitis, and/or chronic coughing are all symptoms of mould-related health difficulties (Mendell et al., 2011). Others are persistent sneezing and coughing. chronic fatigue, pain in the joints and muscles, skin rashes, nosebleeds, ear infections, persistent headaches, nausea, vomiting, and/or diarrhoea, and palpitations with weight loss (Mendell et al., 2011). Mould exposure can, in severe cases, lead to memory loss, liver failure, kidney damage, and lung damage (Kraft et al., 2021). There is great deal of misinformation about the symptoms of toxic mould exposure, which could lead to some people getting the wrong diagnosis. It is possible to mistake mould

exposure for conditions including fibromyalgia, lupus, Lyme disease, rheumatism, or chronic obstructive pulmonary disease (Alone, 2014).

The impact of these microbes on neonates largely depends on the site of infection, route of transmission, neonate's immune status, and timely administration of appropriate medical interventions. However, the skin happens to be the mode of transmission in neonates.

2.3.5 Yeast

Yeasts are fungi that grow as single cells and produce daughter cells by budding or binary fission. They vary from most fungi, which form thread-like hyphae. However, this distinction is not important, because some fungi can switch between a yeast phase and a hyphal phase depending on the environment (Chander, 2017). Such fungi are known as dimorphic (having two morphology), and numerous species cause sickness in humans. Common baker's yeast. *Saccharomyces cerevisiae* belongs to the genus Cryptococcus, which contains *Cryptococcus neoformans*, a human disease. *Candida albicans*, a dimorphic fungus, can be a significant human pathogen (Köhler et al., 2017). Yeast, like mould, thrives in a damp environment rich in simple, soluble nutrients such as sugars.

Cryptococcus albidus, a type of budding yeast, has a sturdy polysaccharide capsule that can be seen as a distinct nimbus. They are commonly found on the surface of leaves, fruits, roots, and food. However, they cannot degrade polymers like starch and cellulose, unlike many other hyphal fungi. The disease occurs in about 7-8% of AIDS patients in the US and a slightly smaller percentage (3-6%) in Western Europe (Chang and Chen, 2015). The capsule plays a critical role in C. neoformans pathogenicity because it prevents cells from being detected and gulped by white blood cells. *Cryptococcus neoformans* additionally produces phenoloxidase, which is unusual among *Cryptococcus species*. This enzyme converts phenolic chemicals into melanin, which may safeguard cells from the antimicrobial effects of oxidants in host tissues (Casadevall et al., 2019). *Cryptococcus neoformans* thrives on old, "weathered" bird droppings in cities, but struggles to compete with microorganisms in damp ones. It attacks the lungs, causing moderate or chronic persistent pneumonia, depending on the individual's immunity. However, in a small percentage of the population, the fungus might spread "inaudibly" in the central nervous system and cause death.

It is characteristic of the fungal subgroup Basidiomycota (which incorporates fungi), although it is minuscule and causes the discharge of small

(approximately 3 micrometers) airborne basidiospores. This is the optimal size for lung storage (DeLeon, 2023). *Candida albicans* is a dimorphic fungus that thrives at 37 °C. Its natural habitat is the mucous membranes of humans together with other warm-blooded animals, where it grows as a yeast and poses minimal damage. According to research, it can be isolated from the mucosal of up to 50% of people, including the mouth, gut, vagina, and, less commonly, the skin's surface (Wilson 2005). Under certain conditions, the same strains of *Candida albicans* that develop as harmless commensal can become pathogenic invading the mucosal and inflicting extensive harm. This usually happens when a variety of predisposing circumstances allow the yeast population to increase and out-compete the resident bacteria that keep the yeast population in control. The yeast cells then produce a hyphal extension that locally penetrates the mucosal barrier, causing tissue irritation and exfoliation.

One of the finest instances is the condition thrush, which causes white spots on the tongue and back of the throat that mimic spots on a bird's chest. This is prevalent in neonates, presumably caused by going via an infected birth canal or the environment in which they were born (Borkar & Shinde, 2023). It is also frequent in AIDS patients and persons who have undergone a sustained Antibacterial therapy lowers the typical resident bacterial population.

Candida albicans can also cause vaginitis, which is inflammation and invasion of the vaginal lining, particularly in the third trimester of pregnancy and in women using pills. The predisposing variables appear to be hormonal, with alterations in the balance of cell types in the feminine epithelium. The individuals who use dentures are prone to get stomatitis, a related illness. Candida can stick to denture resin, and a high sugar diet can also promote adherence by increasing the creation of a lactoprotein glue on the yeast cell surface. Systemic candidiasis is a more dangerous illness in which yeast cells grow throughout the circulatory system. This can happen following invasive surgical procedures, including the procedure for the introduction of intravenous tubes, to which yeast cells cling, forming a foundation from which the cells may sprout and spread.

All of these cases demonstrate that *Candida albicans* is a classic opportunistic infection that is typically maintained under control but can resurface under certain predisposed conditions. It is quite easy to distinguish from candida species by its capacity to sprout hyphae whenever yeast cells are inoculated to test tubes containing plasma/ serum and treated at 37°C for 3-5 hours. Only *Candida albicans* and a few other pathogenic Candida species achieve this.

However, the fungus has a strong tendency to revert to the yeast phase after only a brief period of hyphal development. The hyphae themselves have a beaded appearance and produce to budding yeast cells, where other fungi hyphae generate branches.

2.4 Morphology Composition of Vernix Caseosa

The biology and role of vernix cannot be understood without reference to the stratum corneum. The stratum corneum constitutes the primary barrier of the mature epidermis. The lipids of the stratum corneum provide the primary barrier to trans epidermal water loss. In contrast, vernix is a simpler system composed of a nonlamellar lipid matrix that contains embedded corneocytes without desmosomal connections. This architecture supports the concept that vernix functions as "mobile-phase" stratum corneum. Measurements of the water content of vernix have revealed major differences between this natural barrier cream and native stratum corneum, as well as between vernix and standard wound ointments used in new-born care.

Phase-contrast and standard light microscopy techniques expose the notably cellular nature of *Vernix caseosa*. Agorastos et al. (1988) were the first to systematically examine the cellular composition of vernix. The cells within

vernix are typically of polygonal or ovoid shape, and they lack nuclei, although remnants of nuclei, known as nuclear ghosts, are frequently observed. The authors also detected acid phosphatase activity within granules inside the cells and within the amorphous material that surrounds the cellular elements. Furthermore, lanugo hairs, often trapped within the vernix substance, exhibit strong and positive acid phosphatase activity, particularly in the papillae. A more comprehensive analysis of the corneocytes present in vernix caseosa reveals that these fatal cells can be distinguished from corneocytes found in the mature stratum corneum by their absence of desmosomal attachments (Pickens et al., 2000).

The *Vernix corneocytes*, which are about 1–2 micrometers thick, are enveloped by a thick layer of shapeless lipids that do not possess the usual structured arrangement found in the *Stratum corneum*, as described in a study by Rissmann et al. (2006). To pinpoint the abundant water content in freshly collected *Vernix caseosa*, researchers employed cryoscanning electron microscopy coupled with X-ray beam analysis, as detailed in a study by (Pickens et al., 2000). This analysis reveals that carbon (representative of lipids) is distributed around the corneocyte, while the corneocyte interior contains a notably higher concentration of oxygen (indicating water).

This distribution pattern suggests that the exceptionally high-water content in vernix primarily resides within the corneocyte population. Moreover, the water content within these corneocytes is influenced by the surrounding osmotic conditions, as elucidated in research by Hoath et al. (2004). Initially, early research into vernix composition primarily concentrated on characterizing its lipid content by employing organic solvents for extraction, as documented by Stewart et al. (1982). It was established that vernix contains an approximate 10% ($^{w}/_{w}$) lipid content, as reported by Hoeger et al. (2002). A closer examination of this intricate mixture has disclosed the presence of various compounds, including wax and sterol esters, ceramides, squalene, cholesterol, triglycerides, and phospholipids. It's noteworthy that vernix is exclusively produced by humans and not by any other animal species, rendering it a distinctive skin barrier film. Studies by Sumida et al. (1998) and Hoeger et al. (2002) have offered comprehensive insights into the lipid constituents found within vernix. This includes the presence of ceramides and cholesterol, which are typically associated with stratum corneum development, alongside triglycerides, wax and sterol esters, squalene, and phospholipids, which are typical components of sebum. These findings bolster the idea that the lipid

matrix of vernix consists of a blend of stratum corneum lipids and sebaceous lipids.

Notably, Sumida et al. (1998), conducted experiments wherein they artificially reconstituted this distinctive lipid matrix of vernix. They discovered that these reconstituted vernix lipids exhibited greater hygroscopic properties compared to reconstituted sebaceous lipids on their own.

Rissmann et al. (2006) conducted a more comprehensive examination of vernix lipids, revealing new insights into their composition. Notably, they were the first to confirm the presence of lipids adhering to vernix corneocytes and found lower levels of barrier lipids than previous studies had reported. It's worth mentioning that the method used to collect vernix during delivery could be a significant factor in these findings. For example, scraping the stratum corneum surface may increase the presence of barrier lipid components.

Unlike the well-characterized lipid constituents, the protein components of vernix are not as thoroughly understood. However, recent research has identified numerous antimicrobial peptides in vernix, including Lysozyme, lactoferrin, human neutrophil peptides 1–3, secretory leukocyte protease inhibitor, LL-37, cystatin A, UGRP-1, and calgranulin A, B, and C (Yoshio et al., 2004).

An analysis of free amino acids after chloroform–methanol lipid extraction and acid hydrolysis of the residue revealed a significant abundance of asparagine and glutamine (Baker et al., 1995). This finding is particularly noteworthy because vernix is known to detach from the fetal skin surface before birth and is subsequently ingested by the foetus (Narendran et al., 2000).

Glutamine is presently being investigated as a potential growth-promoting factor for the developing fetal gut, as indicated by Buchman in 1996. When amino acids are extracted from water, they can yield varying proportions, as noted by Utturkar (2005). The primary constituent of vernix is water, and a comparison was made between the dry weight-to-wet weight ratios of *Vernix caseosa* and standard topical creams used in the new-born nursery by Pickens et al. (2000). Their findings revealed that approximately 80–81% (w/w) of vernix is volatile. Karl–Fischer titration analysis pinpointed that this volatility is solely attributable to water content. When exposed to a dry ambient environment, similar to the transition that vernix experiences at birth, vernix gradually releases its water content. In utero, vernix typically exists in the amniotic fluid, which is tightly regulated in terms of its ionic composition, as highlighted by Albuquerque et al. (1999). Studies conducted outside the womb have demonstrated that *Vernix corneocytes* can expand or contract in response

to hypo-osmotic or hyper-osmotic environments, as described by Hoath et al. (2004). Collectively, these data support a mechanism in which vernix plays a role in osmoregulation. Following birth, the regulation of water on the body's surface is crucial for normal thermoregulation, as well as to prevent desiccation and maintain the flexibility of the stratum corneum, as discussed by Hoath et al. (2006).

2.4.1 Benefits of Vernix

The presence of a hydrophobic vernix layer on the emerging epidermis is likely to alter the water distribution between the skin's surface and the surrounding amniotic fluid. As vernix and the underlying stratum corneum develop concurrently, they significantly modify the electrical characteristics of foetal skin, resulting in the creation of a high-resistance skin surface (Wakai et al., 2000). This electrical isolation of the foetus in the womb is presumably a crucial aspect of the foetus gaining independence. In theory, vernix may play a role in regulating water movement across the developing epidermis and maintaining water levels within the stratum corneum at the time of birth. The epidermal barrier is distinct because it appears to have evolved as a means of limiting evaporative water loss in preparation for terrestrial life while simultaneously

utilizing water as its primary plasticizing agent. For instance, research conducted by Proksch et al. (1993) clearly highlights the importance of the trans epidermal water gradient as a potential regulator of DNA synthesis and lipid production .

Additional molecular components found in vernix imply potential biological roles relevant to the moment of birth. For instance, an ELISA assay detected surfactant protein D in vernix extracts and localized it to the sebaceous glands in the foreskin of new-born humans (Narendran et al., 2000). Surfactant protein D is a molecule present in tracheal fluid responsible for maintaining the sterility of airways (LeVine & Whitsett, 2001). This molecule belongs to the broader collecting family, and its presence in vernix and sebaceous glands suggests it may play a crucial role on the skin's surface. Recent reports also indicate the presence of additional antimicrobial peptides organized in granules within vernix, further supporting its potential primary function in infection control (Yoshio et al., 2003).

Apart from exposure to a microorganism-rich environment, birth represents a period of heightened oxidative stress. Thiele et al. reported that human skin exhibits antioxidant properties characterized by elevated levels of alpha tocopherol in the stratum corneum and sebum (Thiele & Packer, 1999).

Alpha tocopherol can also be found in vernix (Visscher et al., 2005). Unlike full-term infants, very low birth weight preterm infants do not display an overgrowth of sebaceous glands and typically have minimal or no vernix present at birth. Consequently, these preterm infants may potentially lack vernix and its associated molecules, while also possessing a stratum corneum that lacks proper barrier function. Another suggested biological function of vernix during birth is its potential role in regulating body temperature. Early clinical observations linked the removal of vernix at birth to the development of below-normal temperatures in prematurely born infants (Saunders, 1948). Currently, vernix is frequently wiped off the skin and discarded.

Vernix' high-water content suggests a possible adverse effect on increasing evaporative heat loss. Studies have shown that washing the skin surface after birth can reduce evaporative heat loss when compared to leaving vernix intact on newborns (Riesenfeld et al., 1986). The hydrophobic nature of vernix suggests a potential function in repelling external water. Conversely, the elevated water content within vernix might have an apparently contradictory impact, as it could help maintain moisture in the outermost layer of the skin, known as the stratum corneum, by promoting slow and controlled drying following birth. This gradual drying process is crucial for the breakdown of

filaggrin into small hydrophilic molecules collectively referred to as natural moisturizing factors (NMF), as proposed by Rawlings et al. (1994). It's plausible that the high water content in vernix creates an ideal, highly humid micro-environment for this purpose. Saijo and Tagami (1991) noted that newborns experience significant skin drying and reduced stratum corneum moisture retention during the initial days after birth in 1991.

The inadequate water-absorption properties of the stratum corneum and the common occurrence of skin peeling in newborns may be attributed, in part, to the combination of rapid drying, particularly when placed under radiant warmers, and exposure to harsh cleansing agents during bathing. Leaving vernix in place at birth could potentially mitigate such effects, as suggested by Visscher et al. (2005).

The analysis of earlier literature offers intermittent accounts of vernix being considered as a potential ointment for wound healing (Zhukov et al., 1992). Other sources discuss the mechanical barrier qualities of vernix concerning the prevention of bacterial intrusion (Joglekar, 1980). The direct anti-infective properties of vernix and its potential roles in hindering or directing bacterial colonization during birth remain uncertain (Baker et al., 1995). It is hypothesized Vernix present on the fetal skin surface could be transferred to the

mother's perineum during childbirth. Therefore, it is reasonable to speculate that vernix might have a positive impact on the healing of epidermal wounds and could potentially be used as a therapeutic barrier cream for very low birth weight infants. Some data has revealed that vernix operates as a natural skin cleanser. When tested on human skin contaminated with fine carbon particles, vernix exhibited comparable effectiveness to standard commercial skin cleansers (Moraille et al., 2005).

The idea that a new-born arrives in the world covered in a substance with inherent cleansing abilities is fascinating. Unlike conventional soaps, this substance is comprised of biologically relevant lipids that seamlessly blend with the skin's surface and pores. Any residue left behind after cleansing is likely to provide additional benefits, such as anti-oxidation, moisturization, and infection control. From this perspective, vernix primarily functions in a manner similar to the self-cleaning properties of the stratum corneum, where the shedding of skin cells continually renews and 'cleans' the interface between the Neonate and the environment.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Cape Coast, which is a metropolitan assembly and also capital of the Central Region of Ghana covers a total land area of approximately 122 km^2, it is located on longitude 1 ˚ 15'West and a latitude of 5 ˚ 06'North and has a population of 189,925 according to 2021 census (Britannica.com., 2023). Farming and fishing are the common occupation of the inhabitants and Fante is the common language spoken. The healthcare facilities include Cape Coast Teaching Hospital (CCTH), which is a teaching hospital, as well as primary health centers like Cape Coast Metropolitan Hospital (CCMH), University of Cape Coast Hospital (UCCH), and other polyclinic such as Ewim Polyclinic.

The UCC Hospital has six (6) wards and ten (10) units (department) including Eye, Ear and Throat (ENT), Obstetrics and Gynaecology(O&G), Neonate Intensive Care Unit (NICU), Reproduction and Child Health (RCH) departments. In all, the facility has 77 beds of which the surgical and medical has forty (40) beds, accident and emergency has ten (10) beds, the pediatric ward has eleven (11) beds (four beds and seven cots). The maternity ward has sixteen (16) lying-in beds, two first-aid beds and four (4) delivery beds. The

staff strength of the maternity ward is twenty (20) of which twelve (12) are midwives, two (2) gynaecologist, one (1) ward assistant, four (4) orderlies and one youth employment person. UCC hospital is positioned such that not only the staff and their dependant as well as students assess this facility for healthcare, the inhabitants of Cape Coast and its surrounding communities also seek health care services from the Hospital.

3.1.2 Study Design

A longitudinal study design was used. This study design help to compare the various variables at the same time. These include, economic background in terms of occupation in relation to infection and treatment. This research design allows the detection of changes over the period of study. It also allows the establishment of the cause-and-effect relationships, avoiding recall bias and tracking individual development over the period.

3.2 Methods

3.2.1 Ethical Considerations

Ethical approval was sought from the University of Cape Coast Ethics Committee with Ethical approval (UCCIRBCANS/2023/11). Permission was

sought from the management of the facility through the Director before data was collected. Participants were briefed on the objective of study and allowed to voluntarily consent to it or decline. Confidentiality was maintained.

3.2.2 Sample Collection

A purposive sampling method was used to recruit all the labour cases in the facility between October and December 2023 who were born within the air sampling days into the study. Indoor air was passively sampled twice a day by gravitation, three times a week (Monday, Wednesday and Friday) for five weeks. Indoor air was sampled at the three rooms with agar plates (Figure1) placed on different heights. For labour or delivery room, air samples were collected at a height of 1.5 m above the ground level which happens to be the breathing zone for adult humans. And at the lying-in rooms, at a height of 84 cm which happens to be the breathing zone for the neonates on their waiting beds (since the hospital is baby friendly there are no baby cots). Specific areas of the indoor air of the buildings were sampled. Samples were taken on neonates who were delivered six hours and beyond within the day and who had not taken their first birth. Hand swabs were taken from midwifes who attended to the mother during delivery and the palms of mothers.

3.3 Study Period and Study Site

The study was conducted at the Maternity block of the University of Cape Coast Hospital. The study commenced from 30th October to 2nd December, 2023. Samples were taken twice a day, three times a week from both delivery and lying-in rooms of maternity block for a period of five weeks. Neonates who were born in the Maternity block within the five weeks of the sample collection were also sampled. A week after discharge and in consultation with the midwife on duty, when mothers reported at the hospital for their first review, observations were made to look-out for any kind of infections, thus; skin, upper respiratory, cord, etc.

3.4 Study Participants and Sample Collection

This included the air samples, swabbing of 17 items which directly or indirectly come in contact with the neonates including delivery beds, weighing balance, baby warmer and dressing station, lying-in and labour ward floors, lying -in room, labour ward, nursing station windows, lying-in window, nurses-station sink, delivery room sinks, nurses station table and 59 Neonates who were

born in the maternity block within five weeks of the study. In all 845 samples were collected in the period.

3.5 Inclusion and Exclusion Criteria

3.5.1 Inclusion criteria

Neonates who were delivered within the five-week period at the period of air sample taken were included.

3.5.2 Exclusion criteria

Neonates who were born before or after the sampling period were excluded.

3.6 Data Collection Instruments

Questionnaire for demographics of recruits' mothers were required. Four questions were asked. These questions were asked privately. The questionnaire was administered after the neonate’s skin sample was swabbed.

3.7 Inoculation of Microbiological Media

Petri plates with 25-ml aliquots of the tetracycline - supplemented Sabouraud agar (SA) (Oxoid, UK), and Nutrient agar (NA) (Biomark laboratories, India), MacConkey (MA) (Biomark laboratories, India) were

exposed to indoor air for 30 minutes. The SA Petri plates were incubated at 25°C for five to seven days for colonies of fungi. Petri dishes containing enriched Nutrient Agar (NA) and Malt Extract Agar (MA) were incubated at 37°C for 18 to 24 hours. NA was enriched with whole blood. The dishes were then observed for bacterial growth.

Surfaces including floors, sinks, delivery beds, nursing station table, baby warmer and dressing station, lying-in beds, weighing balance, mothers palm, nurses palm and windows were swabbed for culture as well. The hair, nose, the neck and the face of the neonates were also sampled for culture on all the various media. In order to have a control for this experiment, plates that were exposed to the indoor were considered as the test and those that were not exposed and incubated serve as the control. After the incubation period the cultures were inspected and the outcomes of Sabouraud agar (SA) for fungi, enriched Nutrient agar (NA) and MacConkey agar (MA) media for bacteria and controls were recorded on excel data sheet and in table form. (Examples of culturing outcome is seen in Figure:8,9 and 10).

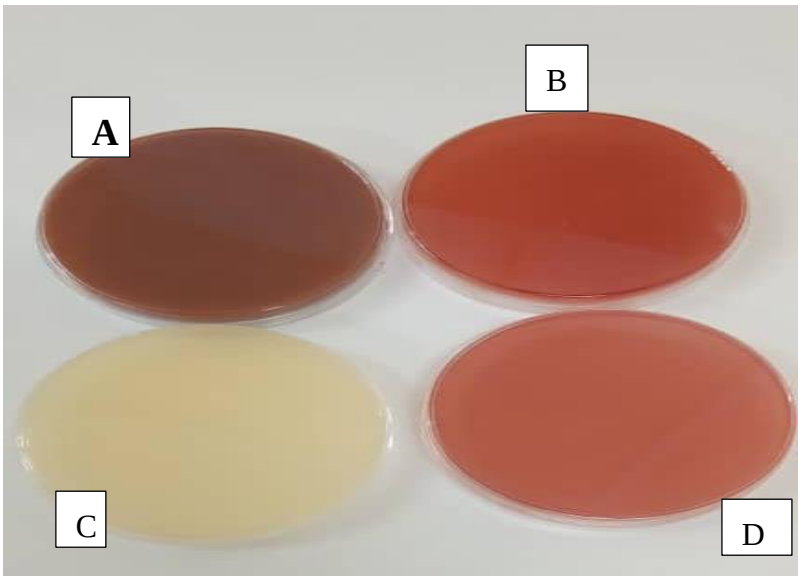

Figure 1: Sterile agar plates used in the study (A: Chocolate agar; B: Blood agar; C: Sabouraud agar; D: MacConkey agar)

3.8 Incubation of Inoculated Agar Plates

All inoculated agar plates were incubated at 37°C for 18 to 24 hours for bacterial isolation and seven (7) days at a temperature of 25°C for fungi isolation respectively. Samples from participatory neonate and the indoor air were cultured in sterile media labelled with unique identification. As seen in Figures 8, 9 and 10 showing some of the growth on agar plates. After the laboratory investigations have been performed and findings recorded on the raw data, the used media and samples were disposed properly using laboratory laid down guidelines and protocols. Within the seven days of discharge, in order not to lose some important information, there were follow up correspondence to fish out any development as far as the neonates health is concerned. This is because

some mothers may resort to local medicines before their review date which can affect the results. On arrival for review, observations were made to record any infection from acute to advanced one. The observations included, cord, eye, skin, mouth, ear, and if there were any kind of cough symptoms.

3.9 Examination of Bacterial and Fungal Cultures

For fungal identification, phenotypically identification method was used. Staining, microscopy and picture identifications. An amount of fungal isolate were removed with an inoculation needle onto an absolute ethanol-sterilized glass slide, after which the fungal culture was covered in a drop of lactophenol cotton blue dye for 3 minutes. The sample was then covered with a cover slip. Excess dye was removed with a Whatman No. 1 filter paper and sample allowed to dry. All prepared slides were observed under an Olympus Microscope (Takachiho Manufacturing Co. Ltd., Japan) (Figure 2 and 3).

Figure 2: A-stained slide for fungal identification

Figure 3: Micrograph of *Fusarium solani* spores

For bacterial identification, Gram's stain was first done to ascertain whether they were Gram-positive or Gram-negative (Figure 4).

3.10.1 Gram Staining

A clean and greased-free glass slide was well labelled with the sample ID. A colony of isolate was then smeared about $\frac{1}{3}$ the size of a glass slide with normal saline for thinning and air-dried. Thermal fixation was also performed. The slide was then flooded with crystal violet staining solution for 1 minute and washed with a gentle indirect stream of tap water for 2 seconds. Lugol iodine solution was used as a mordant to form crystal violet- iodine complex to lock up the crystal violet stain for 1 minute. This forms a complex that maintains the crystal violet stain inside the Gram-positive cell. The slide was washed for 2 seconds and decolonized for 15 seconds with spirit acetone. This only dehydrates Gram-positive cells due to their thicker cell walls, while the thin walls of Gram-negative cells allow all of the crystal violet stain to pass through to remove stains. The slides were counter-stain by flooding with safranin for one minute. The slides were washed with an indirect stream of tap water until no colour appeared in the effluent and dried with absorbent paper. After the staining process was completed, a bright field microscope was used to observe the

results. Gram negative stained pink/red while Gram positive stained blue/purple as showed in Figure:4. (Tripathi & Sapra,.2020).

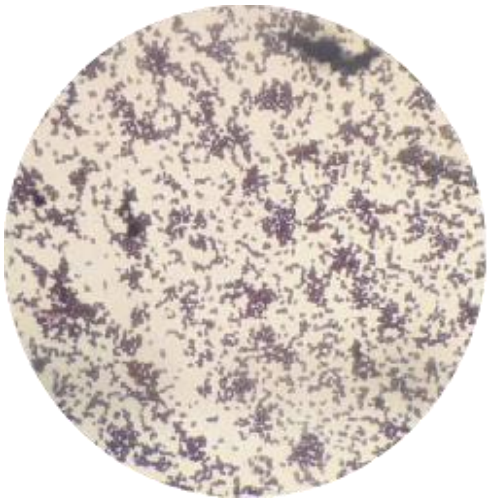

Figure 4: Gram-positive cocci arranged in short chains depicting *Streptococcus pneumoniae*

For Gram-negative, TSI (Figure:5), urea (Figure:7), Citrate (Figure:6) and indole test were done for identification and confirmation. For Gram-positive, cocci, catalase test, coagulase, tests, optochin, Novobiocin and bacitracin sensitivity test were performed for identification and confirmation. Stick picking of some of the colonies also as confirmation.

Culturing of air, items surfaces, skin samples of nurses, mothers and neonates' sample, isolation of causative microbe, identification testing for bacterial, all of them happened within forty-eight hours and the results recorded.

3.10.2 Biochemical Analysis

Triple Sugar Iron Agar Test: This is used to extricate microorganisms based on glucose and lactose fermentation, gas and hydrogen sulphide production. Using a sterile straight inoculating wire, some of the colonies of the test organism were picked and stabbed into the centre of the medium through to the button of the medium. It was then redrawn and the needle streaked on the facet of the slant. Incubation was done at a temperature of 37 ^{O}C for 18 – 24 hours. Alkaline (red) slant and acid (yellow) butt suggest fermentation of glucose only. Acid slant (yellow- acid (yellowed) butt indicates fermentation of glucose and lactose. Alkaline (red) slant- alkaline butt (red) indicates that test organism is a non- fermenter. Splits, cracks or bubbles in medium will indicate production of gas. Any amount of black precipitate in medium indicates hydrogen sulphide (H_2S) production as shown in Figure 5 below. (Cheesbrough,2000).

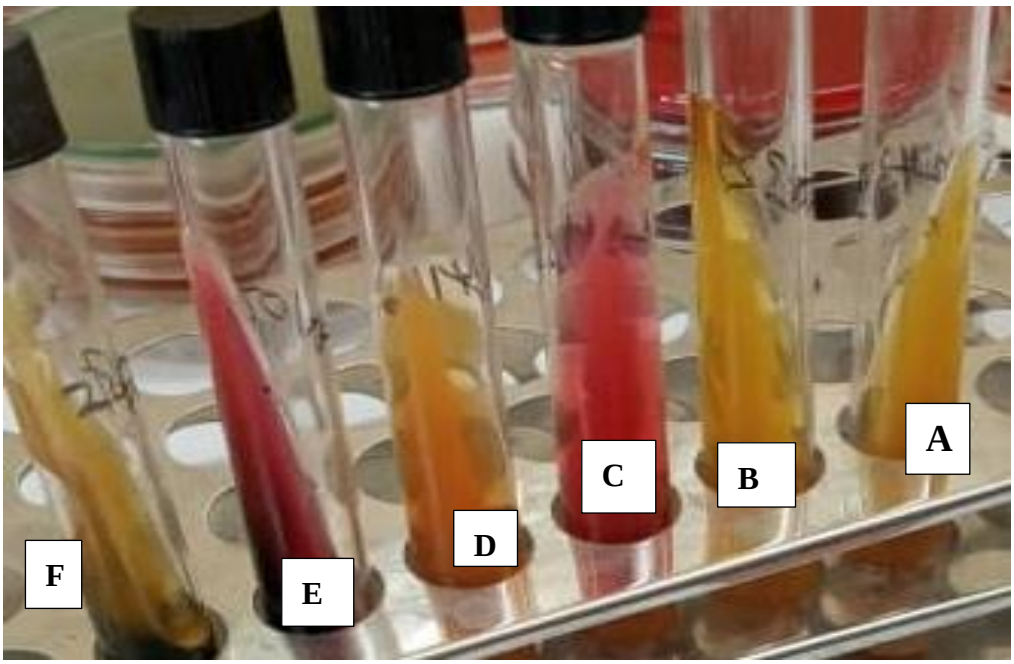

Figure 5: A TSI test (A&B: showing both lactose and glucose fermentation. C: showing no fermentation for lactose D: showing a partial fermentation for glucose. E: showing non- lactose fermentation with hydrogen sulphide production and F: showing both glucose and lactose fermentation with hydrogen sulphide production and gas production as a results of glucose fermentation.

Citrate Test: used for distinguishing between Gram-negative bacteria in the Enterobacteriaceae family. Mechanism of Citrate Utilization Test:

Citrate agar is used to determine an organism's ability to use citrate as an energy source. The medium's sole carbon source is citrate, while the only nitrogen source is inorganic ammonium salts ($NH_4H_2PO_4$). Bacteria that can thrive in this medium create an enzyme called citrate-permease, which converts citrate to pyruvate. Pyruvate can then join the organism's metabolic cycle, producing

energy. Growth indicates the consumption for citrate, an intermediate molecule in the Krebs cycle. When bacteria utilize citrate, the ammonium salts are converted into ammonia, increasing alkalinity. The pH change turns the bromothymol blue indicator in the medium.

Above pH 7.6, the isolate is aseptically picked from the medium and stabbed through to the butt of the Citrate containing tube. It was then streaked back and forth with an inoculate obtained from the center of a well-isolated colony.

Incubation was done aerobically at 37°C for 18-24 hours, and observed for a color change from green to blue for positive results or remain green for negative findings, as shown in Figure 6.

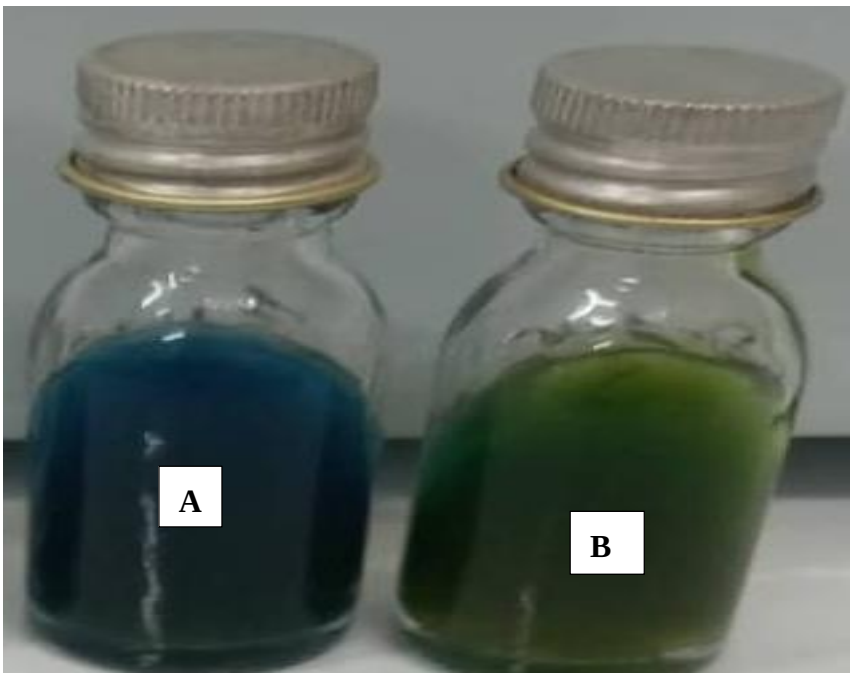

Figure 6: Citrate test (A: Inoculated and positive, B: Inoculated and negative)

Urea Test: The urease test discovers organisms that can hydrolyze urea, producing ammonia and carbon dioxide. It largely distinguishes urease-positive bacteria from other Enterobacteriaceae. The isolate was carefully removed from the agar plate and aseptically transferred to the tube holding the semi broth agar by stabbing through to the butt and withdrawing to streak the surface; thus, the slant back and forth and closed to incubate aerobically at 37°C for 24 hours. And was observed for color change. As shown in Figure:7.

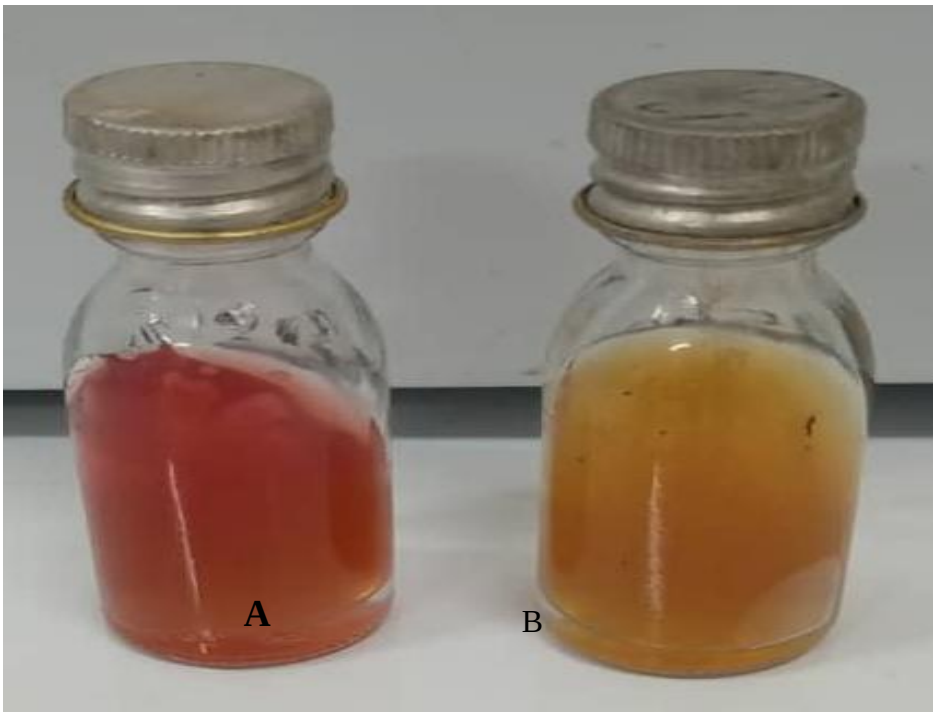

Figure 7: Urea test (A: Inoculated and positive, B: Inoculated and negative)

Indole Test*:* Indole test is used to establish the ability of an organism to split amino acid tryptophan to form the compound indole. A tube containing peptone

water was inoculated with the test organism. It was then incubated at a temperature 37 ^{O}C for 24 hours. 0.5ml of Kovac's reagent was added to broth culture. Positive test was identified by a red ring on the surface of the broth. (Cheesbrough, 2000).

Coagulase Test: Coagulase test is used to extricate *Staphylococcus aureus* from *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. The Plasma (human) was brought to room temperature and three small test tubes were labelled as follows; T = test organism P = positive control N = negative control The following steps were used:

- 200µl of plasma were pipetted into each tube.
- 800µl of the test broth culture was adding on to tube T.
- 800µl of known *staphylococcus aureus* isolate was then added to the tube; labelled P.
- 800µl of sterile peptone water was then added to the tube labelled N.
- the three tubes were mixed gently and incubated at 37 ℃ it was examined for clot after 24hr. It was observed that Fibrin clot in tube T indicates that the test organism was *Staphylococcus aureus.* No clot or fibrin clot in tubes T and N confirms the presence of the other staph

Species. (*Staphylococcus epidermidis* and *Staphylococcus saprophyticus*) other than *staphylococcus aureus* (Cheesbrough, 2000).

Figure 8: Fungal isolates from the delivery room on Sabouraud agar

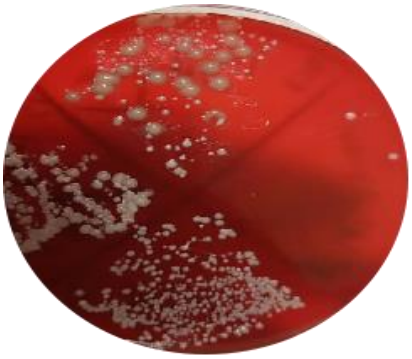

*Figure 9 :*A blood agar with isolates from neonates' skin

Figure 10: Open air sample isolates from lying-in room

3.11 Storage of Fungal and Bacterial Isolates

3.11.1 Bacteria storage

Part of isolates were sub-cultured and kept in the fridge at temperature of 4°C for the biochemical test and identification purposes. For long term reference, a single colony of the isolates were inoculated by stabbing aseptically using straight wire in vials containing compatible media agar loosely covered and incubated at 37°C for twelve hours and then tightly closed and kept in the refrigerator at a temperature of 4°C in dark.

3.11.2 Fungal storage

Sabouraud Dextrose Agar (SDA) tinted with Tetracycline was used for the sub-culturing. A single colony of a pure isolate was sub-cultured on a fresh medium for 7 days. Pieces of filter paper of about one square centimetre was sterilized by autoclaving. They were then placed on the agar surface of the same type of media (SDA) to isolate the fungus. A loop full of the pure culture was aseptically transferred on the filter paper. The petri dishes were sealed and placed in an incubator for 14 days for the fungi to colonize it. The colonized filter papers were transferred into sterilized petri dish without media and incubate again for drying. In 20 days. They were then removed placed in glassing envelope, labelled and stored at 20 °C.

3.12 Data Management

Data obtained were kept anonymously and analyzed using SPSS statistical tool 2013. The stored isolates will be disposed of by the laboratory lay-down rules. The storage of isolate is necessary because there could be a need for replication, confirmation or evidence of a job done by publishers, when published. The investigator, the supervisor and the UCCIRB monitoring team can have access to this anonymous information when the need arises.

3.13 Statistical Analysis

General linear model was used to do the multivariant analysis on SPSS. Multivariate analysis is a sophisticated statistical approach that may assist researchers understand the intricacies of cross-sectional data. Using this technique, it helps to uncover hidden links between variables and obtained an improved comprehension of the data. It was also utilized for analyzing categorical variables, to examine whether variations between observed and expected data were random or owing to a relationship connecting with respect to the p-value of the variables being studied. It was helpful since it applied to data measured on an arbitrary or categorical scale. Furthermore, it was used to determine whether there is a distinction among multiple sets of respondents or

participants. A cross - tabulation and Pearson chi - square analysis was also performed to study the relationship between the categorical variables of the organisms isolated after birth.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Demographic Characteristics

Out of the 59 participants, the youngest mother was 21 years old whilst the oldest mother was 38 years old. The mean age was 28 years (Table 1 and 2). highest percentage of the participants (42.4%) have gained a tertiary level of education, 22% of the participants had senior high school as their highest level of education, 16% had junior high school whilst 8.5% of the participants have no level of formal education (Figure 11).

The participants had various occupations from different fields. Most of them were traders with a few of them being in the cooperate world (Figure 12).

Table 2: Age distribution of neonates' mothers

Age range	Frequency	Percentage (%)
20 - 25	14	24.14
26 - 30	21	36.21
31 - 35	18	31.03
36 - 40	5	8.62

Table 3: Descriptive statistics of the ages of the mothers

	N	Minimum	Maximum	Mean	Std. Deviation
Age	59	21	38	28.71	4.457

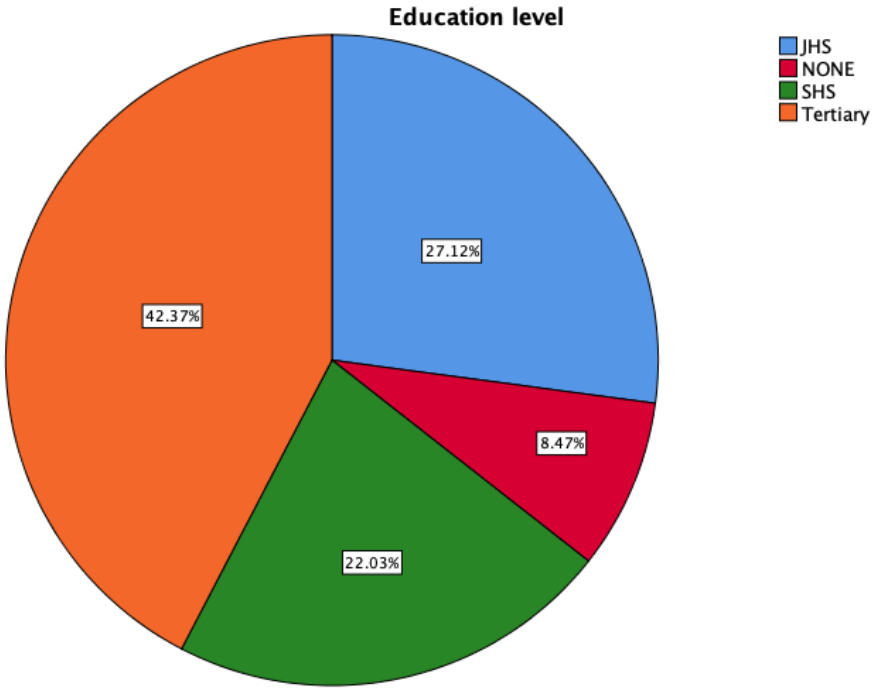

Figure 11: Educational level of mothers screened in this study

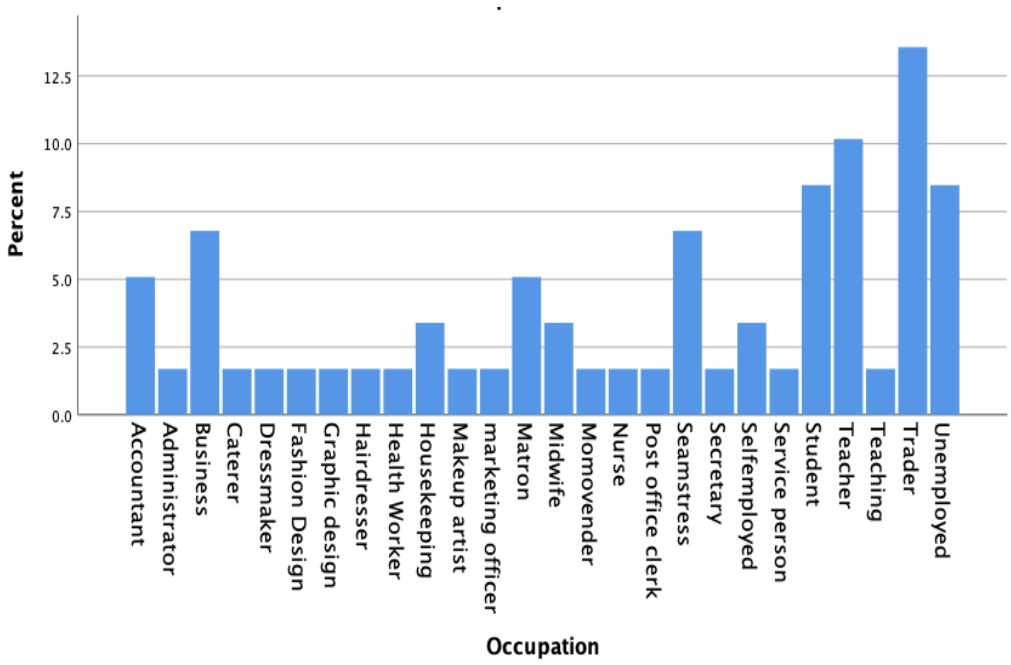

Figure 12: Occupations of mothers

4.1.2 Microbial Load of Nurses' Palms, Mothers' Palm and Babies' Skin

Categorical analysis of isolates from the nurses', mothers' and the babies' palms.

The data was divided into two categories: samples showing bacterial growth and samples with no bacterial growth. Among the neonate isolates, 79.7% showed bacterial growth, while 20.3% had none. For the isolates from nurses' palms, 22% showed bacterial growth, and 78% had no growth. In the case of the isolates from mothers' palms, 68% showed bacterial growth, while 32% had no growth (Table 3).

Table 4: A Categorical analysis of Isolates from the nurses' palms, mothers' palms and the neonates' body.

Isolates from neonate	Frequency	Percentage
Bacteria Growth	47	79.7
No Bacterial Growth	12	20.3
Isolates from nurses' palms		
No Bacteria Growth	46	78.0
Bacterial Growth	13	22.0
Isolates from mothers' palms		
Bacteria Growth	40	67.8
No Bacterial Growth	19	32.2

Microbial isolates from neonates' skin and the number of neonates infected

The highest number of infections among the neonates was caused by *Staphylococcus epidermis*, affecting 29 neonates. *Micrococcus* infected 14 neonates, *Streptococcus pneumoniae* infected 11, *Escherichia coli* infected 8, and *Staphylococcus aureus* infected 6. Additionally, 5 neonates were infected by *Candida albicans*, and 2 by *Bacillus* species. *Proteus vulgaris* infected 3 neonates, while *Klebsiella pneumoniae*, *Aspergillus flavus*, *Providencia species*, and *Morganella morganii* each infected 1 neonate (Table 4).

Table 5: Frequency of microorganisms isolated from the neonates' skin surface

Organism	Number of neonates infected
Staphylococcus epidermidis	29
Micrococcus Sp.	14
Streptococcus pneumoniae	11
Escherichia coli	8
Staphylococcus aureus	6
Candida albicans	5
Pseudomonas aeruginosa	4
Proteus vulgaris	3
Bacillus Sp.	2
Aspergillus flavus	1
Klebsiella pneumoniae	1
Providencia stuartii	1
Morganella morgana	1

Organisms isolated from the nurses' palm

Staphylococcus epidermis, was the commonly isolated organism from the nurse's palms, followed by *Micrococcus* while *Candida albicans, Pseudomonas aeruginosa, Klebsiella pneumoniae., Streptococcus pneumoniae, Staphylococcus aureus* and *Proteus vulgaris* were the least isolated organisms (Table 5).

Table 6: Microorganisms isolated from the nurses' palms

Organism	Number of nurses whose palm were infected
Staphylococcus epidermidis	6
Micrococcus	4
Pseudomonas aeruginosa	1
Staphylococcus aureus	1
Klebsiella pneumoniae	1
Streptococcus pneumoniae	1
Proteus vulgaris	1
Candida albicans	1

Organisms isolated from the mothers' palms

With regards to organisms isolated from mothers' palm, 14 mothers had their palms contaminated with *Staphylococcus epidermis* while 12, 7, 5 and 3 mothers had *Micrococcus, Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae* respectively. *Pseudomonas aeruginosa* was found in the palms of 2 mothers and 1 mother each had *Candida albicans, Aspergillus flavus* and *Proteus vulgaris* isolated from their palms (Table 6).

Table 7: Microorganisms isolated from the mothers' palm

Organism	Number of mothers whose palm were infected
Staphylococcus epidermidis	14
Micrococcus	12
E. coli	7
Staphylococcus aureus	5
Streptococcus pneumoniae	3
Pseudomonas aeruginosa	2
Aspergillus flavus	1
Candida albicans	1
Proteus vulgaris	1
Klebsiella pneumoniae	0

Multivariant analysis of the effect of the organisms isolated from the nurses and mothers' palms on the neonates

Multivariant analysis was performed on the categorical results of the swabbing on the neonates, nurses' palms and the mothers' palms. This was done to see if there is a possibility of the neonates getting infected from organisms from the nurses' and the mothers' palm. The nurses' and the mothers' palms were set as the dependent variable. There was a significant correlation of 0.040 between the nurses' palm and the neonates. This showed that the organisms

isolated from the nurses' palms had an effect on the neonates. There was a significant correlation of 0.144 between the mothers' palm and the neonates. This showed that the organisms isolated from the mothers' palms had no effect on the neonates (Table 7).

Table 8: Multivariant analysis of organisms isolated from nurses' palms and mothers' palm on the neonates

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Neonates' isolates (categorical)	Nurses' palm isolates (categorical)	.731	1	.731	4.433	.040
	Mothers' palms isolates (categorical)	.477	1	.477	2.192	.144
Total	Nurses' palm isolates (categorical)	98.000	59			
	Mothers' palms isolates (categorical)	179.000	58			

4.1.3 Microbial Result of the Neonate After 3 To 7 Days and their site of Infection

After 3 to 7 days, 18.6% (11 neonates) were susceptible to microbial infections whilst 81.4% (48 neonates) had no infection (Table 8).

Table 9: Analysis on neonates susceptible to microbes after 3 to 7 days of discharge

Outcome	Frequency	Percent (%)
Yes	11	18.6
No	48	81.4
Total	59	100.0

Microorganisms isolated from neonates within 3 – 7 days after birth

Majority of the organisms isolated after 3 to 7 days were *Staphylococcus epidermis* (54.5%), followed by others which were *Candida albicans* (9.1%), *Proteus vulgaris* (9.1%), Both *Candida albicans* and *Staphylococcus epidermis (9.1%), Staphylococcus aureus* (9.1%) and both *Staphylococcus epidermis* and *Proteus vulgaris* (9.1%) (Table 9).

Table 10: Organisms isolated from the Neonates who were susceptible to the infection.

Microbial Isolate	Frequency	Percent
Staphylococcus epidermidis	6	54.5
Candida albicans	1	9.1
Candida albicans, Staphylococcus epidermidis	1	9.1
Proteus vulgaris	1	9.1
Staphylococcus aureus	1	9.1
Staphylococcus epidermidis, Proteus vulgaris	1	9.1

Sites of infection after 3 to 7 days of discharge

The site of infection of majority of those who were infected on their skin (72.7%), followed by ear (9.1%), both skin and tongue (9.1%) and tongue (9.1%).

Table 11: The site of infection of the neonates after 3 to 7 days of discharge

Site of infection	Frequency	Percent
Ear	1	9.1
Skin	8	72.7
Skin and tongue	1	9.1
Tongue	1	9.1

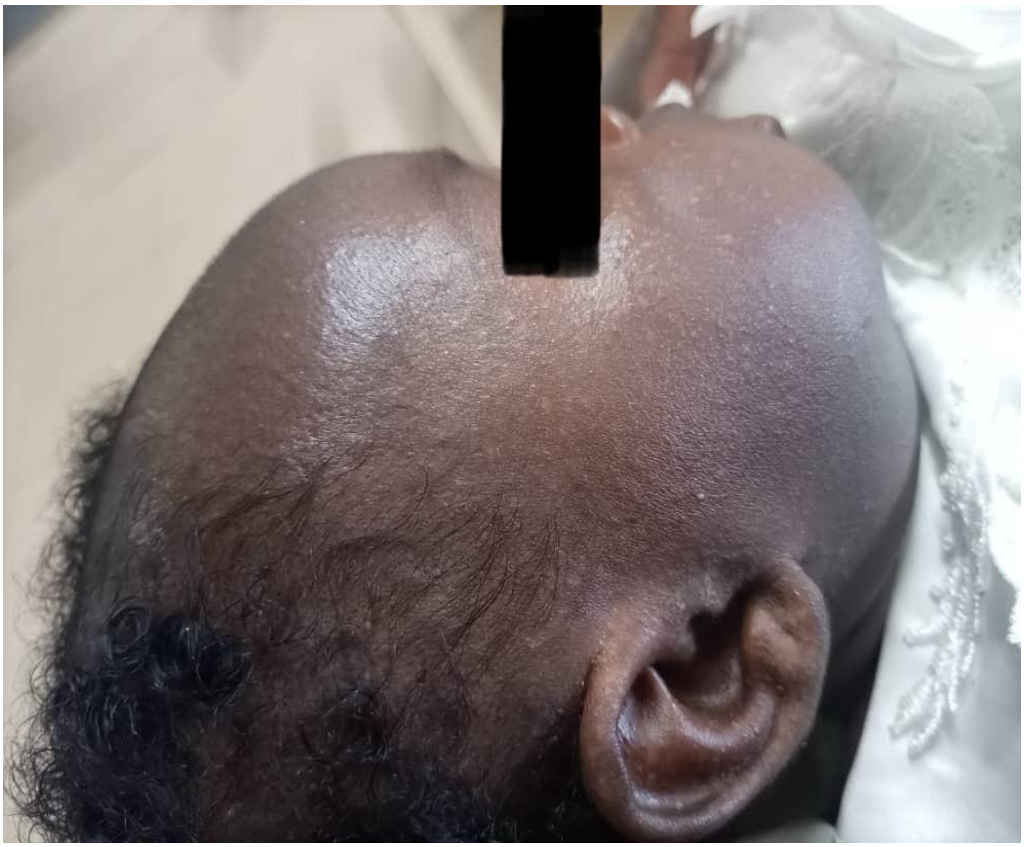

Figure 13: Baby who had a late infection of *Staphylococcus epidermidis* on the skin

Association between organisms isolated from neonates at birth and after 3 to 7 days after discharge

A cross-tabulation and Pearson's chi square analysis were conducted to examine the relationship between organisms isolated immediately after birth and those isolated later. The results indicated that all babies who showed no infection right after birth remained infection-free after 3 to 7 days. Additionally, 23.4% of the babies who had bacterial growth immediately after birth still had infections at 3 to 7 days follow up, while 76.6% no longer had infections. This

relationship was found to be statistically significant, with a p-value of (0.063) (Table 11).

Table 12: A cross-tabulation and Pearson's chi square analysis of neonate bacterial isolates after birth (categorical) * Infection after 3 to 7 days.

Bacterial isolate on neonates after birth (categorical) * Infection after 3 to 7 days Cross-tabulation

			Infection after 3 to 7 days			*df*	*p-value*
			Yes	No	Total		
Neonate isolate after birth (categorical)	No Bacteria Growth	Count	0	12	12	*1*	*0.063*
		% within neonate isolate cat	0.0%	100.0%	100.0%		
		% within Infection	0.0%	25.0%	20.3%		
		% of Total	0.0%	20.3%	20.3%		
	Bacterial Growth	Count	11	36	47		
		% within neonate isolate cat	23.4%	76.6%	100.0%		
		% within Infection	100.0%	75.0%	79.7%		
		% of Total	18.6%	61.0%	79.7%		
Total		Count	11	48	59		
		% within neonate isolate cat	18.6%	81.4%	100.0%		
		% within Infection	100.0%	100.0%	100.0%		
		% of Total	18.6%	81.4%	100.0%		

4.1.4 Analysis of the frequency of microorganisms isolated from the maternity ward during a five week - period sampling

4.1.4.1 Results of week one

At the end of the first week, out of the 45 plates to examine bacterial assessment, 40 plates had bacterial growth (88.9%) whilst 5 plates had no bacterial growth (11.1%). With regards to the fungal examination, 31 plates recorded fungal growth with a percentage of 68.9%, whilst 14 plates had no fungal growth with a percentage of 31.1%. Bacteria such as *Staphylococcus epidermidis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli, Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella aeruginosa, Micrococcus, Bacillus species* and fungi such as *Aspergillus flavus, Aspergillus niger, Candida albicans, Mucor, Aspergillus terreus, Stachybotrys chartarum* were found at various places in the maternity ward such as the, baby warmer and dressing station, Sinks from the unit. Nurses station table, various unit floors, delivery beds, windows, beds in lying- in rooms (Table 12 and 13).

Table 13: Bacteria and Fungi isolated on week one of air sampling

Bacteria isolates (categorical)		
	Frequency	Percentage (%)
No Bacterial Growth	5	11.1
Bacterial Growth	40	88.9
Fungi isolate (categorical)		
	Frequency	Percent
No Fungi Growth	14	31.1
Fungi Growth	31	68.9

Table 14: Microorganisms isolated from different places in the maternity unit during week one.

	Microbial isolates	
Item swabbed	Bacteria	Fungi
Baby warmer and dressing station	*Staphylococcus epidermidis*	*No fungal growth*
Sinks from the unit	*Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli Staphylococcus epidermidis, Streptococcus pneumoniae, Staphylococcus Aureus Klebsiella aeruginosa, Micrococcus*	*Aspergillus flavus, Aspergillus niger, Candida albicans*
Nurses station table	*Staphylococcus epidermidis*	*Aspergillus flavus, Mucor, Aspergillus niger*
Various unit floors	*Staphylococcus epidermidis, Micrococcus, Streptococcus pneumoniae, E. coli, Staphylococcus epidermidis, Klebsiella pneumoniae.*	*Aspergillus flavus, Aspergillus niger, Mucor, Aspergillus terreus, Stachybotrys chartarum*
Delivery bed	*Staphylococcus epidermidis, Streptococcus pneumoniae, E. coli, Micrococcus.*	*Aspergillus flavus, Aspergillus niger Candida albicans*
Windows	*Streptococcus pneumoniae, Micrococcus, Staphylococcus epidermidis, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Bacillus Sp.*	*Aspergillus niger, Candida albicans Aspergillus flavus, Mucor*
Bed in lying-in rooms	*Micrococcus, Staphylococcus epidermidis*	*Aspergillus flavus, Aspergillus niger, Candida albicans Mucor, Aspergillus niger, Aspergillus terreus.*

4.1.4.2 Results of week two

At the end of the second week, majority of the items and air samples (64.7%) had no bacterial growth whilst 35.3% had bacterial growth. 41.2% of the participants had fungal growth and 58.8% had no fungal growth. Bacteria isolates such as Klebsiella aeruginosa, proteus vulgaris, Staphylococcus aureus,

Staphylococcus epidermidis, Micrococcus, Streptococcus pneumoniae, Micrococcus, Pseudomonas aeruginosa, E. coli, Bacillus species and fungi such as *Aspergillus niger, Aspergillus flavus, Mucor, Histoplasma capsulatum, Aspergillus terreus, Stachybotrys, Aspergillus terreus, Penicillium glabrum, Candida albicans* were found at various places in the block; such as the, baby warmer and dressing station, Sinks from the unit. nurses station table, various unit floors, delivery bed, windows, bed in lying rooms (Table 14 and 15).

Table 15: Bacteria and Fungi isolated on week two of air sampling

Bacteria isolate (categorical)		
	Frequency	Percent
No Bacteria Growth	33	64.7
Bacteria Growth	18	35.3
Fungi isolate (categorical)		
	Frequency	Percent
No Fungi Growth	30	58.8
Fungi Growth	21	41.2

Table 16: The various Bacteria and Fungi isolated from different places in the maternity unit in week two.

	Microbial isolates	
Item swabbed	**Bacteria**	**Fungi**
Baby warmer and dressing station	*NBG*	*NFG*
Sinks from the unit	*Klebsiella aeruginosa, Proteus vulgaris, Staphylococcus aureus Staphylococcus epidermidis, Micrococcus, Streptococcus pneumoniae*	*Aspergillus niger*
Nurses station table	*NBG*	*Aspergillus flavus, Mucor*
Various unit floors	*NBG*	*Aspergillus niger, Histoplasma capsulatum*
Delivery bed	*NBG*	*Aspergillus flavus, Aspergillus niger, Mucor, Aspergillus terreus, Stachybotrys chartarum, Aspergillus terreus.*
Windows	*Staphylococcus epidermidis, Micrococcus, Staphylococcus. aureus, Pseudomonas aeruginosa, E. coli, Streptococcus pneumoniae, Micrococcus Sp., Bacillus Sp.*	*Aspergillus niger, Histoplasma capsulatum, Mucor, Penicillium glabrum, Aspergillus niger*
Bed in lying rooms	*Staphylococcus epidermidis*	*Aspergillus terreus, Stachybotrys Aspergillus flavus, Aspergillus niger, Candida albicans. Mucor*

NBG = No bacterial growth; NFG = No fungal growth

4.1.4.3 Results of week three

At the end of the third week, majority of the participants (62.7%) had no bacterial growth whilst 37.3% had bacterial growth. 76.5% of the participants had fungal growth whilst 23.5% had no fungal growth. Bacteria isolates such as *Klebsiella aeruginosa, Proteus vulgaris, Staph. aureus, Staphylococcus epidermidis, Micrococcus, Streptococcus pneumoniae, Bacillus species, P. aeruginosa* and fungi isolate such as *Aspergillus niger, Aspergillus flavus, Mucor, Aspergillus terreus, Stachybotrys, Aspergillus fumugatus, Blastomyces dermatitidis, Histoplasma capsulatum* were found at various places in the facility such as the, baby warmer and dressing station, sinks from the unit, nurses station table, various unit floors, delivery bed, windows, bed in lying rooms.

Table 17: Bacteria and Fungi isolated on week three of air sampling

Bacteria isolate (categorical)		
	Frequency	Percent
No Bacteria Growth	32	62.7
Bacteria Growth	19	37.3
Fungi isolate (categorical)		
	Frequency	Percent
No Fungi Growth	12	23.5
Fungi Growth	39	76.5

Table 18: The various Bacteria and Fungi isolated from different places in the maternity unit in week three

	Microbial isolates	
Item swabbed	Bacteria	Fungi
Baby warmer and dressing station	*NBG*	*NFG*
Sinks from the unit	*Klebsiella pneumoniae Pseudomonas aeruginosa, proteus vulgaris, Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus, Streptococcus pneumoniae*	*Aspergillus niger, Aspergillus flavus, Aspergillus niger*
Nurses station table	*No bacterial growth*	*Aspergillus flavus, Aspergillus niger, Mucor*
Various unit floors	*Micrococcus, Staphylococcus epidermidis, Streptococcus Pneumoniae, Bacillus Sp., Micrococcus.*	*Aspergillus flavus, Aspergillus niger, Mucor, Aspergillus terreus, Stachybotrys*
Delivery bed	*No bacterial growth*	*Aspergillus flavus, Aspergillus niger*
Windows	*Staphylococcus epidermidis, Micrococcus, Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Staphylococcus pneumoniae, Streptococcus pneumoniae, Bacillus Sp.*	*Mucor, Aspergillus fumugatus, Stachybotrys, Mucor, Aspergillus fumugatus, Blastomyces dermatitidis*
Bed in lying rooms	*No bacterial growth*	*Aspergillus niger, Histoplasma capsulatum, Aspergillus terreus, Stachybotrys*

4.1.4.4 Results of week four

At the end of the fourth week, most of the items swabbed (56.6%) had bacterial growth whilst 43.4% had no bacterial growth. 79.2% of the participants had fungal growth whilst 20.8% had no fungal growth. Bacteria

such as *Staphylococcus epidermidis, Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus aureus, Micrococcus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Micrococcus, Bacillus species* and fungi isolates such as *Aspergillus flavus, Aspergillus niger, Mucor, Aspergillus terreus, Stachybotrys, Histoplasma capsulatum, Aspergillus fumugatus, Blastomyces dermatitidis, Penicillium glabrum* were found at various places in the facility such as the, baby warmer and dressing station, sinks from the unit. Nurses station table, various unit floors, delivery bed, windows, beds in lying rooms.

Table 19: Bacteria and Fungi isolated on week four of air sampling

Bacteria isolate (categorical)		
	Frequency	Percent
No Bacteria Growth	23	43.4
Bacteria Growth	30	56.6
Fungi isolate (categorical)		
	Frequency	Percent
No Fungi Growth	11	20.8
Fungi Growth	42	79.2

Table 20: Various bacteria and fungi isolated from different places in the unit in week four.

	Microbial isolates	
Item swabbed	Bacteria	Fungi
Baby warmer and dressing station	*Staphylococcus epidermidis*	*No fungal growth*
Sinks from the unit	*Klebsiella pneumoniae Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus, Streptococcus pneumoniae*	*Aspergillus flavus, Aspergillus niger*
Nurses station table	*Staphylococcus epidermidis*	*Aspergillus flavus, Aspergillus niger, Mucor*
Various unit floors	*Staphylococcus epidermidis*	*Aspergillus flavus, Aspergillus niger, Mucor, Aspergillus terreus, Stachybotrys, Aspergillus niger, Histoplasma capsulatum*
Delivery bed	*No bacterial growth*	*Aspergillus flavus, Aspergillus niger*
Windows	*Streptococcus pneumoniae, Micrococcus species, Pseudomonas aeruginosa, Staphylococcus epidermidis, Bacillus species*	*Mucor, Aspergillus fumugatus, Blastomyces dermatitidis, Penicillium glabrum, Aspergillus niger*
Bed in lying rooms	*Staphylococcus epidermidis*	*Aspergillus niger, Histoplasma capsulatum*

4.1.4.5 Results of week five

At the end of the fifth week, a good number of the items swabbed (94.1%) had bacterial growth whilst only 5.9% had no bacterial growth. 78.4% of the participants had fungal growth whilst 21.6% had no fungal growth. Bacteria such as *Staphylococcus epidermidis, Klebsiella aeruginosa, Proteus vulgaris, Staph. Aureus, Bacillus species, Micrococcus, Pseudomonas aeruginosa, E. coli, S. pneumoniae, Micrococcus Sp., K. pneumoniae and fungi isolates such as Candida albicans, A. flavus, A. niger, Mucor, Histoplasma capsulatum, Penicillium glabrum, A. terreus, Stachybotrys* were found at various places in the facility such as the, baby warmer and dressing station, Sinks from the unit. nurses station table, various unit floors, delivery bed, windows, bed in lying- in rooms.

Table 21: Bacteria and Fungi isolated on week five of air sampling

Bacteria isolate (categorical)		
	Frequency	Percent
No Bacteria Growth	3	5.9
Bacteria Growth	48	94.1
Fungi isolate (categorical)		
	Frequency	Percent
No Fungi Growth	11	21.6
Fungi Growth	40	78.4

Table 22: Bacteria and Fungi isolated from different places in the unit in week five.

	Microbial isolates	
Item swabbed	Bacteria	Fungi
Baby warmer and dressing station	*Staphylococcus epidermidis*	*No fungal growth*
Sinks from the unit	*Klebsiella aeruginosa, Proteus vulgaris, Staphylococcus aureus*	*Candida albicans*
Nurses station table	*Staphylococcus epidermidis, Bacillus Sp.*	*Aspergillus flavus, Aspergillus niger, Mucor*
Various unit floors	*Micrococcus, Staphylococcus aureus Staphylococcus epidermidis*	*Aspergillus niger, Histoplasma capsulatum*
Delivery bed	*Pseudomonas aeruginosa, E. coli, Staphylococcus epidermidis*	*Aspergillus flavus, Aspergillus niger*
Windows	*Staphylococcus epidermidis, Streptococcus pneumoniae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Micrococcus species.*	*Penicillium glabrum, Aspergillus niger, Histoplasma capsulatum, Mucor*
Bed in lying rooms	*Micrococcus species, Staphylococcus epidermidis,*	*Aspergillus terreus, Stachybotrys, A. flavus, Aspergillus niger, Candida albicans*

Comparative analysis of organisms isolated from the maternity ward

As illustrated in Figure 13 and 14, week five had the highest number of bacterial growths followed by week one. The number of bacterial reduced from week one to week two then increased further to week three and week four, then increased massively to week five.

With the fungal growth, the highest number of organisms were isolated in week five. There was a decrease in fungal growth from week one to week two then a continuous increase from week two to week three through to week four then to week five.

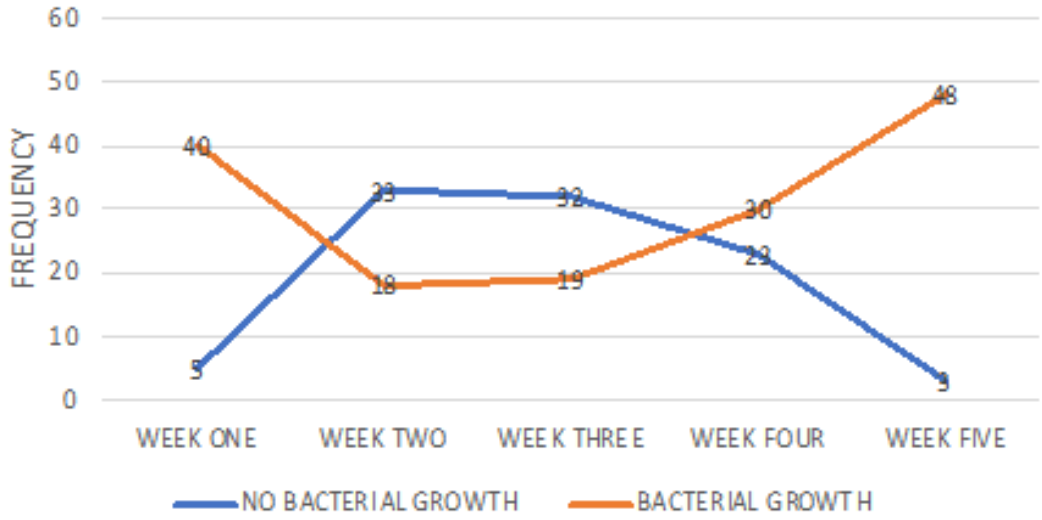

Figure 14: Levels of bacteria growth at the maternity ward over a five week-period

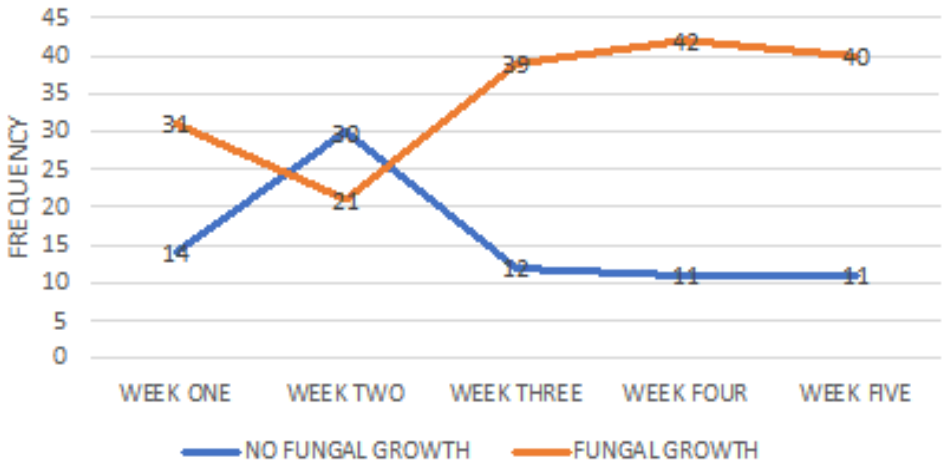

Figure 15: Levels of fungal growth at the maternity ward over a five week-period

4.1.5 Open Plates: The Frequency of Microbial Air Analysis

For bacteria isolates, most of the organisms captured in the air were *Staphylococcus epidermidis* (60), followed by *Bacillus species* (59), *Streptococcus pneumoniae* (58), *Staphylococcus aureus* (48), *Escherichia coli* (42), *Micrococcus species* (31), *Pseudomonas aeruginosa* (13), *Klebsiella pneumoniae* (2) and *Moraxella catarrhalis* (1).

With regards to fungi isolates, majority were *Aspergillus flavus* (53), *Aspergillus fumigatus* (51) *Histoplasma capsulatum (48), Aspergillus niger* (37), *Stachybotrys chartarum* (36), *Mucor (31), Candida albicans* (30), *Blastomyces dermatitidis* (18), *Aspergillus terreus (18), Penicillin glabrum* (4), *Fusarium solani* (1).

Table 23: Open plates: Number of the various bacteria and fungi isolated over 5 weeks

	Bacteria isolates										
	Mc	*Ec*	*Sa*	*Kp*	*BS*	*Se*	*Pa*	*Sp*	*MS*		
Number of times organisms were isolated	1	42	48	2	59	60	13	58	31		
	Fungi isolates										
	Af	*Sc*	*Ca*	*MS*	*Afl*	*An*	*Bd*	*At*	*Pg*	*Hc*	*Fs*
Number of organisms isolated	53	36	30	31	51	37	18	18	4	48	1

Ec= E coli; Sa= Staphylococcus aureus; Bs= Bacillus species; Ms= Micrococcus species; Sp= Streptococcus pneumoniae; Kp= Klebsiella pneumoniae; Se=Staphylococcus epidermidis; Mc= Moraxella catarrhalis; Pa=Pseudomonas aeruginosa; Sc=Stachybotrys chartarum; Afl=Aspergillus flavus; An=Aspergillus niger; Hc=Histoplasma capsulatum; Ms=Mucor species; Bd=Blastomyces dermatitidis; At=Aspergillus terreus; Ca=Candida albicans; Af=Aspergillus fumigatus; Pg= Penicillin glabrum; Fs= Fusarium solani

4.2 Discussion

4.2.1 Organisms Isolated from the Mothers' and the Nurses' Palms and How They Affect the Neonates

The study showed that there may be a possibility of the child getting infected from the organism on the palms of the nurses during child delivery. This showed a significant statistical value of 0.040 as all the organisms that were isolated

from the nurses' palms were also isolated from the body of the neonates. These findings were similar to (Buxton et al., 2019)who discovered that nurses had low hygiene compliance rates and that their hand hygiene remained unsatisfactory despite earlier facility-level education concerning hand hygiene guidelines and the availability to hand washing equipment in handy locations across delivery units. This was also reminiscent of earlier research that found an inadequate degree of compliance with hand hygiene requirements in both low and high-income health care settings (World Health Organization, 2009; Alex-Hart and Opara, 2011). Even though there was no significant link (p-value = 0.144). (Some of the organisms discovered on the mother's palms have been identified on the neonates).

Some of the organisms isolated from the neonates after delivery included *Bacillus species., Candida albicans, Staphylococcus epidermidis, E. coli, Micrococcus, Pseudomonas aeruginosa., staphylococcus aureus, Klebsiella pneumoniae, Aspergillus flavus, Streptococcus pneumoniae, Proteus vulgaris, Providencia species, Morganella morgana*. An article by Younge et al. (2018) stated in their research paper that the skin of the neonates was enriched in typical skin-associated bacteria such as Streptococcus and Staphylococcus but also in many taxa that are typically

associated with the gut microbiome, including Escherichia, Enterobacter, and Enterococcus. Many of these members of the skin microbiota are common causes of late-onset sepsis in preterm infants (Younge et al., 2018).

4.2.2 Neonates' Health Status and Organisms Isolated After 3 To 7 Days of Delivery

The health status of neonates during the first week of life is a matter of significant concern in the field of perinatal care. This crucial period is marked by adaptations to extra uterine life, and the health and well-being of the neonate are of utmost importance. A substantial aspect of neonatal health during this time is the susceptibility to infections. After 3 to 7 days, 18.6% (11 neonates) were infected whilst 81.4% (48 neonates) had no infection (Huynh et. al., 2018). Majority of the organisms isolated after 3 to 7 days were *Staphylococcus epidermis* (54.5%), followed by others were *Candida albicans* (9.1%), *Proteus vulgaris* (9.1%), Both *Candida albicans*. and *Staphylococcus epidermis* (9.1%), *Staphylococcus aureus* (9.1%) and both *Staphylococcus epidermis* and *Proteus vulgaris* (9.1%). Neonates' immune systems are not yet fully developed, making them more susceptible to bacterial, viral, and fungal infections (Maródi, 2006). The organisms commonly isolated during these periods include a wide spectrum of pathogens. Endogenous pathogens, often originating from the maternal

genital tract, can lead to early-onset neonatal sepsis. Gram-negative bacteria are frequently implicated. These pathogens can colonize the neonate's skin, mucous membranes, or even the respiratory and gastrointestinal tracts, potentially leading to severe infections if not promptly diagnosed and treated (Simonsen et al., 2014). Exogenous pathogens, on the other hand, may be acquired from the hospital environment or healthcare personnel. *Staphylococcus aureus*, coagulase-negative *Staphylococcus epidermidis* are among the microorganisms that were isolated.

Proper infection control measures and aseptic techniques are paramount in minimizing the risk of exogenous infections (Simonsen et al., 2014). Among the two types of infections, what make the hospital acquired; thus, exogenous one more serious is that it is difficult to treat. Sometimes demands combined therapy and prolonged hospitalization due to the difficulty in eradicating the infection (Horan, et. al., 2008). Understanding the health status of neonates during the 3 to 7 days after delivery and the organisms isolated during this time is pivotal for healthcare providers. Timely detection, appropriate treatment, and preventive strategies are essential to safeguard the health of these vulnerable newborns. This period represents a critical phase in neonatal care, where

vigilance, infection control, and meticulous monitoring play a significant role in ensuring the well-being and survival of neonates.

4.2.3 Bacterial Growth after Swabbing the Maternity Ward Items

The study revealed that bacterial contamination in the maternity ward was widespread. Various surfaces, including baby warmer and dressing station, sinks from the unit nurses station table, various unit floors, delivery bed, windows, bed in lying-in rooms. The floor which was expected to have more microorganism was having scanty growth. The highest positive microbiological samples were obtained from the sinks in the units and the windows. The microbial contamination of these areas can be due to various reasons, from the considerable presence of amniotic fluid, blood samples, tissues, air condition leakages and other biological fluids that serve to be the breeding ground for microorganisms' source and spread (Suleyman et al., 2018).Patients admitted to these wards, including staff, are much more susceptible to infections, particularly when they are severely immunocompromised and thus exacerbated microbial pathogens could pose a significant risk to the mother's and newborn's health, with potentially fatal implications (Pfitscher et al., 2016). This study found that Gram-negative pathogens including *Pseudomonas aeruginosa,*

Proteus vulgaris, Acinetobacter species, E. coli, and *Klebsiella species,* as well as Gram-positive pathogens such *Staphylococcus species,* had a significant impact.

The World Health Organization classifies infection, prevention, and control (IPC) being a scientific method and practical solution to reduce infection-related harm to clients and health workers (UNICEF, 2020). IPC plays an important role in delivering safe, effective, top-notch health care and health coverage to everyone. Effective IPC is essential for reducing HAIs and could decrease the incidence of HAI approximately 30% (Bardossy et al., 2016). The connection of IPC and Water, Sanitation, and Hygiene (WASH), which includes waste management including environmental cleansing, cannot be overlooked. Effective infection prevention and control (IPC) is impossible without adequate WASH services. Poor infrastructure, particularly the lack of proper WASH facilities, forms the basis for inadequate IPC in healthcare settings. Additionally, the increasing number of patients in healthcare facilities can be linked to the shortage of specialized healthcare staff, inadequate IPC training, and limited microbiological surveillance, all of which hinder the delivery of quality care (Manchanda et al., 2018). Poor WASH and IPC lead to HAIs and

disease transmission, while increased antibiotic use exacerbates outbreaks and infection dissemination.

4.2.4 Fungal Growth on the Maternity Ward Items

The current study identified the presence of various fungal species in the delivery ward of the UCC hospital, highlighting the need for vigilant monitoring and infection control measures. The results are in alignment with previous studies conducted elsewhere that have emphasized the proneness of hospital environments to fungal colonization (Hassan et. al., 2017; Chaberny et. al., 2010); Perlroth et al., 2007; Neely et. al., 2000). While fungal growth was observed in multiple areas within the delivery ward, it was most prevalent at poorly ventilated spaces, air condition leakages and due to poor cleaning habit on the various surfaces. This underscores the significance of environmental factors, such as humidity and air circulation, in facilitating fungal colonization (Onmek et al., 2020). The implications of these findings for maternal and neonatal health are of paramount concern. Fungal contamination can pose health risks to both mothers and newborns, potentially leading to respiratory issues, sepsis, skin infections, and other complications (Aghokeng et. al., 2020; Richardson, M., 2005). Therefore, it is essential to establish stringent infection

control measures and environmental management protocols to mitigate these risks.

The comparison of these findings with existing literature reaffirms the need for proactive measures to prevent fungal growth in healthcare facilities. Hospital-acquired fungal infections can have severe consequences, particularly for vulnerable populations such as neonates and postpartum mothers. The study aligns with the broader body of research that emphasizes the role of hospital environments in fungal colonization and calls for increased awareness and preventive efforts (McFee, 2009, Wohrley and Bartlett, 2019).

4.2.5 Microbial Air Quality

The focus of this research was to investigate microbial air quality in the delivery ward. The study aimed to assess the presence of microorganisms in the air of the delivery ward and to analyze the implications of such microbial contamination on neonatal health. The study revealed that the delivery ward, which is typically considered a critical space for maintaining high levels of cleanliness and sterility, is not immune to microbial contamination. Microorganisms were detected in the air, signifying that the maternity ward environment can be a potential reservoir for bacteria and other microorganisms.

The current research is a qualitative as far as air sampling is concerned, although the air samples were collected not by air sampler equipment, rather by gravitation using Koch's sedimentation method (Ali et. al., 2015). The minimum mixed growth was 96 colonies and the maximum mixed growth was 165 colonies.

where “a” is number of colonies per dish, “b” is dish square centimeter, “t” is time of exposure in minutes and “N” is microbial CFU/m^3 of indoor air which is far above the recommended allowable microbes in the indoor air. bacteria such as *Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Micrococcus species, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Moraxella catarrhalis* were isolated and fungi such as *Aspergillus flavus, Aspergillus fumugatus, Histoplasma capsulatum, Aspergillus niger, Stachybotrys chartarum, Mucor species, Candida albicans, Blastomyces dermatitidis, Aspergillus terreus, Penicillin glabrum, Fusarium solani. Moraxella catarrhalis* was isolated once in week five (Table: 22) in the delivery room. This bacterial though a commensal but can have opportunistic effect on the new born. It could be attributed to non-compliance of the use of nose-mask on the side of health professionals. These findings have significant implications for the healthcare setting, particularly concerning the health and

well-being of mothers and newborns. Airborne microorganisms can pose a substantial risk, potentially leading to healthcare-associated infections in postpartum mothers and neonates (Knibbs et al., 2011). The presence of these microorganisms can be attributed to multiple factors, including the ventilation system, the movement of healthcare personnel, air condition leakages and visitors, as well as the physical environment of the ward. This highlights the need for stringent infection control measures, including the regular maintenance of ventilation systems, the use of appropriate personal protective equipment, and adherence to aseptic techniques. This is highly possible due to the fact that the orderlies taught the work was going to be used to sabotage them so they were trying to clean the surfaces where air samples were taken the previous week. This accounted for the reduction in the isolates in week two to week four. On week five, they seemed to be used to the situation and then stopped crossing or interrupting the sample taken. None of the deliveries reported infected amniotic fluid. They were all having their normal light-yellow colour and smell.

4.2.6 The Effect of *Vernix Caseosa* with HAIs on the New Born

Isolates from neonates indicated bacteria growth in 79.7% of neonates whilst 20.3% had no bacteria growth. Narendran et al. (2000) identified surfactant-type

proteins associated with the collectin family within vernix. Collectin surfactant proteins have carbohydrate recognition sites which bind with bacteria, viruses, and fungi. Further study reveals these collectin proteins within vernix may play an important antibacterial role, such as protecting against infection from intrauterine and postnatal bacterial colonization within the gut (Tollin et. al., 2005; Marchini et. al., 2002; Darlow et. al., 2017; Kitzmiller and Lucas, 1974). Preterm newborns that are known to lack vernix due to their gestational immaturity have a greater chance of nosocomial and community-acquired illness (Haubrich, 2003).

Majority of the neonates (29 neonates) were infected by *Staphylococcus epidermis* isolation. 14 neonates were infected by *Micrococcus*, 11 neonates were infested by *Streptococcus pneumoniae*, 8 neonates were infected by *Escherichia coli*, 6 neonates were infected by *Staphylococcus aureus*, 5 neonates by *Candida albicans* and 2 neonates were infected by *Bacillus species.* Three neonates were infected by *Proteus vulgaris* and 1 neonate each was infected by *Klebsiella pneumoniae, Aspergillus Flavus, Providencia stuartii, Morganella morganii.* It may be said that most of the organisms isolated from the maternity unit are becoming resistant to the antibiotic properties of the vernix and these could be a reason for the high percentage of neonates that were

infected right after birth. Those that were not susceptible could be attribute to the fact that they had proper care at home after discharge. Those who developed thrush due to *Candida species* confessed that they never cleaned the neonates' mouths or tongues. The isolates *Providencia stuartii* and *Morganella morganii* were neither isolated from the air samples nor the nurses palm nor mothers palm nor any other items. This implies they were in the clothing they used on the neonates (Verde et al., 2015). The neonates insusceptible to *Providencia stuartii* and *Morganella morganii* may be attributed to the fact that, the neonates are not resistant to the vernix antibiotic properties which means they were the strains from the community.

This study found that most infections in neonates originated from the head and neck area, likely due to direct exposure of these regions to the air (Fanaroff et al., 2007). The neonates were positioned on their sides in the ward, which may explain why none of them developed respiratory infections or sepsis. One neonate developed a late-onset infection with *Staphylococcus epidermidis*, which was resistant to all the epidermal treatments administered (Figure 13). Another neonate was admitted to the NICU for throat and skin rashes caused by *Candida albicans* and *Staphylococcus epidermidis*. These infections resulted in increased healthcare costs and prolonged hospital stays, highlighting the

significant impact of hospital-acquired infections (HAIs) on the healthcare sector.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

The health status of neonates during their first week of life is a critical focus in perinatal care, as this period involves important adaptations to life outside the womb. The study indicated a possible risk of infection in neonates from organisms present on nurses' palms during child delivery, with a statistically significant association ($p = 0.040$). All organisms isolated from the nurses' palms were also found on the body of the neonates they attended. Additionally, the study revealed widespread bacterial contamination in the maternity ward, with microorganisms detected on various surfaces such as baby warmers, dressing stations, sinks, nurses' station tables, floors, delivery beds, windows, and beds in the lying-in rooms. Surprisingly, the floor, which was expected to harbor more microorganisms, showed only scanty growth. The delivery ward, typically expected to maintain high levels of cleanliness and sterility, was found to have microbial contamination, including airborne microorganisms. These were detected using Koch's sedimentation method, with colony counts ranging from a minimum of 96 to a maximum of 165.

Despite this, none of the neonates developed respiratory infections or sepsis, possibly due to their positioning in the ward. However, one neonate developed a late-onset infection with *Staphylococcus epidermidis*, which was resistant to all epidermal treatments. Another neonate was admitted to the NICU with throat and skin rashes caused by *Candida albicans* and *Staphylococcus epidermidis*. These infections resulted in increased medical costs and prolonged hospital stays, highlighting the critical importance of healthcare-associated infections (HAIs) in the health sector.

5.2 Conclusion

The study identified bacteria and mould such as; *Staphylococcus epidermidis* and *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa* among others. for moulds*; Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Fusarium solani, Candida albicans, Mucor, Penicillin glabrum, Blastomyces dermatttidis, Stachybotrys* and more.

The study showed within the 24hours after delivery 47 neonates representing 79% were infested with pathogens. After seven days of discharge 11 neonates out of the 47 had infections. The pathogens the neonates were

susceptible to were; *Staphylococcus epidermidis, Proteus vulgaris, Staphylococcus aureus* and *Candida albicans.* The most prevalent bacteria and mould were *Staphylococcus epidermidis, Bacillus species. Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Micrococcus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* With regards to fungi isolates, majority were *Aspergillus flavus, Aspergillus fumigatius, Histoplasma capsulatum, Aspergillus niger, Stachybotry chartarum, Mucor species, Candida albicans, Blastomyces dermatitidis, Aspergillus terreus.*

Microorganisms were detected in the air, and was widespread signifying that the maternity ward environment may be a potential reservoir for pathogens. The health status of neonates during their first week of life is a matter of significant concern in the field of perinatal care. This crucial period is marked by adaptations to extra uterine life, and the health and well-being of the neonate are of utmost importance.

The neonates are positioned at their side at the ward; these positions may attribute to the reason why none of the neonates developed respiratory infection and sepsis. One of the neonates had late onset of *Staphylococcus epidermidis* on her skin which has resistance against all the epidermal treatment given to her. Another neonate among those participated was also admitted at the NICU

for throat and skin rushes as a results of *Candida albicans* and *Staphylococcus epidermidis*. All these brought about cost and prolonged hospital stay.

Infection control measures play a pivotal role in preventing HAIs, protecting vulnerable patients and healthcare workers, reducing antimicrobial resistance, and ensuring a safe healthcare environment. Implementing and adhering to these measures are crucial for improving patient outcomes, promoting public health, and maintaining the highest standards of patient care in healthcare settings. Ensuring a high level of indoor air quality in the maternity ward is essential for creating a safe and healthy environment for neonates during their critical post-delivery period. Proper infection control measures, including adequate ventilation, cleaning, and hand hygiene, may significantly reduce the risk of airborne infections and respiratory issues in these vulnerable infants. Healthcare Associated Infections (HAIs) impose a significant burden on healthcare systems, leading to increased hospitalization, extended lengths of stay, additional healthcare costs, and potential legal issues for healthcare facilities. Maintaining proper indoor air quality in the maternity ward requires strict adherence to infection control measures. Implementing ventilation systems that provide adequate air exchange and filtration may help reduce the concentration of airborne pathogens. It is essential for the maternity ward, to

prioritize indoor air quality management to create a safe and healthy environment for neonates during their critical post-delivery period. Proper infection control measures and ventilation systems may significantly reduce the risk of airborne infections and respiratory issues in these vulnerable infants.

Proper hygiene practices and regular cleaning are crucial in mitigating the risks posed by *Aspergillus* in the maternity ward. The priority is to ensure that this special place remains a haven for the celebration of new life and the beginning of beautiful journeys for families. Aseptic techniques, and appropriate antimicrobial stewardship, are crucial for reducing the incidence of HAIs in neonates. Healthcare facilities should prioritize measures to prevent these infections and protect the health of vulnerable neonates in maternity wards and Neonatal Intensive Care Unit (NICUs).

5.3 Recommendations

The following are recommended:

1. Further research should include antibiotic and antifungal susceptibility pattern of the isolates. This may help to obtain antibiotic and antifungal database for the facility.

2. Surveillance research similar to the current study should be conducted every year to ensure that the hospital environment is clean and best hygienic practices are adhered to by health professionals including nurses.
3. There should be frequent general cleaning of the rooms including all surfaces to avoid neonatal infections and reduce healthcare expenses.
4. March and April are the busiest delivery months, during which the majority of newborns can be enrolled in any additional studies, according to the facility's delivery data.
5. When mothers become conscious to breastfeed their children, they should be mandated to wash their hands or do proper alcohol hand-rub with 70% alcohol gel by the midwives before their babies are given to them.

REFERENCES

Agorastos, T., Hollweg, G., Grussendorf, E. I. & Papaloucas, A. (1988). Features of vernix caseosa cells. *American journal of perinatology*, 5**,** 253-259.

Aghokeng, A. F., Atem, P. N., Ndze, V. N., & Mbunkah, H. A. (2020). Fungal infections in neonates: Clinical consequences and approaches to infection prevention and control in resource-limited settings. *Frontiers in Microbiology*, 11, 527303. https://doi.org/10.3389/fmicb.2020.527303

Albuquerque, C. A., Nijland, M. J. & Ross, M. G. (1999). Human and ovine amniotic fluid composition differences: implications for fluid dynamics. *The Journal of Maternal-Fetal Medicine,* 8**,** 123-129.

Alex-Hart, B. A. & Opara, P. I. (2011). Handwashing practices amongst health workers in a teaching hospital. *Am J Infect Dis,* 7**,** 8-15.

Ali, A. M., & Siddiqui, R. (2015). Airborne bacterial contamination in a hospital setting: Comparative study using active and passive air sampling methods. *International Journal of Environmental Health Engineering*, 4(1), 1-6. https://doi.org/10.4103/2277-9183.157708

Allegranzi, B., Nejad, S. B., Combescure, C., Graafmans, W., Attar, H., Donaldson, L. & Pittet, D. (2011). Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *The Lancet*, 377**,** 228-241.

Alone, L. (2014). Mold and Mycotoxins: Often Overlooked Factors in Chronic Lyme Disease.

Baker, S. M., Balo, N. N. & Aziz, F. A. (1995). Is vernix caseosa a protective material to the newborn? A biochemical approach. *The Indian Journal of Pediatrics*, 62**,** 237-239.

Bardossy, A. C., Zervos, J. & Zervos, M. (2016). Preventing hospital-acquired infections in low-income and middle-income countries: impact, gaps, and opportunities. *Infectious Disease Clinics*, 30**,** 805-818.

Bisana, S. (2014). Evolution of mammary gland function: a study using monotreme models. *Deakin University.*

Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., Scheld, M., Spellberg, B. & Bartlett, J. (2009). Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clinical infectious diseases*, 48**,** 1-12.

Brewer, J. H., Thrasher, J. D. & Hooper, D. (2013). Chronic illness associated with mold and mycotoxins: is naso-sinus fungal biofilm the culprit? *Toxins*, 6**,** 66-80.

Buxton, H., Flynn, E., Oluyinka, O., Cumming, O., Esteves Mills, J., Shiras, T., Sara, S. & Dreibelbis, R. (2019). Hygiene during childbirth: an observational study to understand infection risk in healthcare facilities in Kogi and Ebonyi states, Nigeria. *International journal of environmental research and public health*, 16**(**1301).

C.D.C. (2019). Antibiotic Resistant Germs in Hospitals: Information for Patients and their Families [Online]. Available: https://www.cdc.gov/hai/patientsafety/ar-hospitals.html#:~:text=One%20of%20the%20biggest%20risks,hospitals%20than%20in%20the%20community. [Accessed 07/09/23].

Camacho-GonzaleZ, A., Spearman, P. W. & StolL, B. J. (2013). Neonatal infectious diseases: evaluation of neonatal sepsis. *Pediatric Clinics*, 60**,** 367-389.

Cason, C., D'AccoltI, M., Campisciano, G., Soffritti, I., Ponis, G., Mazzacane, S., Maggiore, A., Risso, F. M., Comar, M. & Caselli, E. (2021). Microbial contamination in hospital environment has the potential to colonize preterm newborns' nasal cavities. *Pathogens*, 10**,**(615).

Casadevall, A., Kontoyiannis, D. P., & Robert, V. (2019). On the emergence of Candida auris: climate change, azoles, swamps, and birds. *MBio*, *10*(4), 10-1128.

Chaberny, I. F., Kotterik, S., & Gastmeier, P. (2010). Fungal contamination of air samples in intensive care units: A problem to address. *Journal of Hospital Infection*, 74(1), 90-95. https://doi.org/10.1016/j.jhin.2009.09.010

Chander, J. (2017). *Textbook of medical mycology*. JP Medical Ltd.

Chen, S., Ren, F., Huang, X., Xu, L., Gao, Y., Zhang, X., ... & Liu, M. (2022). Underestimated prevalence of HIV, hepatitis B virus (HBV), and hepatitis D virus (HDV) triple infection globally: systematic review and meta-analysis. *JMIR public health and surveillance*, *8*(11), e37016.

Cheesbrough, J. S., Green, J., Gallimore, C. I., Wright, P. A., & Brown, D. W. G. (2000). Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiology & Infection*, *125*(1), 93-98.

Coffin, S. E. & Zaoutis, T. E. (2011). HealthCare–associated infections in the nursery. *Infectious diseases of the fetus and newborn***,** 1126.

Darlow, B. A., & Vyas, J. R. (2017). Antimicrobial peptides and proteins in vernix caseosa and amniotic fluid: Implications for newborn innate immunity. *Acta Paediatrica*, 106(3), 326-331. https://doi.org/10.1111/apa.13708

Dearborn, D. G., YIKE, I., Sorenson, W., Miller, M. J. & Etzel, R. A. (1999). Overview of investigations into pulmonary hemorrhage among infants in Cleveland, Ohio. *Environmental health perspectives*, 107**,** 495-499.

Elbehiry, A., Marzouk, E., Moussa, I., Mushayt, Y., AlgarnI, A. A., Alrashed, O. A., Alghamdi, K. S., Almutairi, N. A., Anagreyyah, S. A. & Alzahrani, A. (2023). The Prevalence of Multidrug-Resistant Acinetobacter baumannii and Its Vaccination Status among Healthcare Providers. *Vaccines*, 11**,** 1171.

Elsaid, A. M. & Ahmed, M. S. (2021). Indoor air quality strategies for air-conditioning and ventilation systems with the spread of the global coronavirus (COVID-19) epidemic: Improvements and recommendations. *Environmental Research*, 199**(**11), 13-14.

Esposito, S., Galeone, C., Lelii, M., Longhi, B., Ascolese, B., Senatore, L., Prada, E., Montinaro, V., Malerba, S. & Patria, M. F. (2014). Impact of air pollution on respiratory diseases in children with recurrent wheezing or asthma. *BMC pulmonary medicine*, 14**,** 1-9.

Fanaroff, A. A., Stoll, B. J., Wright, L. L., Carlo, W. A., Ehrenkranz, R. A., Stark, A. R., & Poole, W. K. (2007). Trends in neonatal morbidity and mortality for very low birthweight infants. *American Journal of Obstetrics and Gynecology*, 196(2), 147.e1-147.e8. https://doi.org/10.1016/j.ajog.2006.09.014

Gomez-lopez, N., Romero, R., Varrey, A., Leng, Y., Miller, D., Done, B., XU, Y., Bhatti, G., Motomura, K. & Gershater, M. (2021). RNA sequencing reveals diverse functions of amniotic fluid neutrophils and monocytes/macrophages in intra-amniotic infection. J*ournal of Innate Immunity*, 13**,** 63-82.

Guen, G.-L., Fournier, S., Andre-Richet, B., Caillon, J., Chamoux, C., Espaze, E., Richet, H., Roze, J. & Lepelletier, D. (2007). Almond oil implicated in a Staphylococcus capitis outbreak in a neonatal intensive care unit. *Journal of Perinatology*, 27**,** 713-717.

Hardin, B. D., Kelman, B. J. & Saxon, A. (2003). Adverse human health effects associated with molds in the indoor environment. *Journal of Occupational and Environmental medicine*, 45**,** 470-478.

Harun, M. G. D., Anwar, M. M. U., Sumon, S. A., Hassan, M. Z., Haque, T., Mah-e-muneer, S., Rahman, A., Abdullah, S. A. H. M., Islam, M. S. & Styczynski, A. R. (2022). Infection prevention and control in tertiary care hospitals of Bangladesh: results from WHO infection prevention and control assessment framework (IPCAF). *Antimicrobial Resistance & Infection Control*, 11**(**125).

Hassan, M., Kassem, A., & Merzoug, H. (2017). Fungal contamination in hospital environments: A serious health hazard. *International Journal of Environmental Research and Public Health*, 14(2), 215-222. https://doi.org/10.3390/ijerph14020215

Haubrich, K. A. (2003). Role of Vernix caseosa in the neonate: potential application in the adult population. *AACN Advanced Critical Care*, 14**,** 457-464.

Hoath, S., Pickens, W., Scarborough, T.,Kasting, G., Visscher, . & Marks, R. (2004). Characterization of vernix caseosa: relevance to stratum corneum. *Stratum Corneum IV, Paris.*

Hoath, S. B., Pickens, W. & Visscher, M. O. (2006). The biology of vernix caseosa. *International journal of cosmetic science*, 28**,** 319-333.

Hoeger, P., Schreiner, V., Klaassen, I., Enzmann, C., Friedrichs, K. & Bleck, O. (2002). Epidermal barrier lipids in human vernix caseosa: corresponding ceramide pattern in vernix and fetal skin. *British Journal of Dermatology,* 146**,** 194-201.

Horan, T. C., Andrus, M., & Dudeck, M. A. (2008). CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. *American Journal of Infection Control*, 36(5), 309-332.
DOI: 10.1016/j.ajic.2008.03.002

Hornik, C. P., Fort, P., Clark, R. H., Watt, K., Benjamin JR, D. K., Smith, P. B., Manzoni, P., Jacqz-aigrain, E., Kaguelidou, F. & COHEN-Wolkowiez, M. (2012). Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. *Early human development*, 88**,** 69-74.

Huynh, B., Kermorvant-Duchemin, E., Herindrainy, P., Padget, M., Rakotoarimanana, F., Feno, H....Delarocque-Astagneau, E. (2018). Bacterial Infections in Neonates, Madagascar, 2012–2014. *Emerging Infectious Diseases*, 24(4), 710-717. https://doi.org/10.3201/eid2404.161977.

Imam, F. B., Darmstadt, G. L., & Zaidi, A. K. (2025). Neonatal infections: A global perspective. *In Remington and Klein's Infectious Diseases of the Fetus and Newborn Infant* (pp. 21-53). *Elsevier.*

Jernigan, J. A., Hatfield, K. M., Wolford, H., Nelson, R. E., Olubajo, B., Reddy, S. C., Mccarthy, N., Paul, P., Mcdonald, L. C. & Kallen, A. (2020). Multidrug-resistant bacterial infections in US hospitalized patients, 2012–2017. *New England Journal of Medicine,* 382**,** 1309-1319.

Joglekar, H. S., Samant, S. D., & Joshi, J. B. (1991). Kinetics of wet air oxidation of phenol and substituted phenols. *Water Research*, *25*(2), 135-145.

Kai-Larsen, Y. (2009). Antimicrobial peptides and proteins in host-microbe interaction and immediate defense. *Karolinska Institutet (Sweden).*

Keri, V. C., Kumar, A., Singh, G., Xess, I., Khan, M. A., Rastogi, N., SAHU, M. & Wig, N. (2021). Fungal carriage on healthcare workers' hands, clothing, stethoscopes and electronic devices during routine patient care: a study from a tertiary care center. *Journal of Preventive Medicine and Hygiene*, 62**(**170).

Kim, K.-H., Kabir, E. & Jahan, S. A. (2018). Airborne bioaerosols and their impact on human health. *Journal of Environmental sciences,* 67**,** 23-35.

Kitzmiller, J. L. & Lucas, W. E. (1974). Retarded growth of E. coli in amniotic fluid. *Obstetrics & Gynecology,* 43**,** 311-312.

Knibbs, L. D., Morawska, L., Bell, S. C. & GRZYBOWSKI, P. (2011). Room ventilation and the risk of airborne infection transmission in 3 health care settings within a large teaching hospital. *American journal of infection control*, 39**,** 866-872.

Kraft, S., Buchenauer, L. & PoltE, T. (2021). Mold, Mycotoxins and a Dysregulated Immune System: A combination of concern? *International journal of molecular sciences*, 22(12269).

Kucova, P., Kantor, L., Fiserova, K., Lasak, J., Röderova, M. & Kolar, M. (2021). Bacterial pathogens and evaluation of a cut-off for defining early and late neonatal infection. *Antibiotics*, 10**(**278).

Kuhn, D. M. & Ghannoum, M. (2003). Indoor mold, toxigenic fungi, and Stachybotrys chartarum: infectious disease perspective. *Clinical microbiology reviews*, 16**,** 144-172.

Kumar, S., Shankar, B., Arya, S., Deb, M. & Chellani, H. (2018). Healthcare associated infections in neonatal intensive care unit and its correlation with environmental surveillance. *Journal of infection and public health*, 11**,** 275-279.

Kutlubay, Z., Tanakol, A., Engýn, B., Cristina, O., Sýmsek, E., Serdaroglu, S., Tuzun, Y., Yilmaz, E. & Bülent, E. (2017). Newborn skin: common skin problems. *Maedica*, 12**(**42).

Leung, A. K., Wong, A. H., Leung, A. A. & HON, K. L. (2019). Urinary tract infection in children. *Recent patents on inflammation & allergy drug discovery*, 13**,** 2-18.

Levine, A. M. & Whitsett, J. A. (2001). Pulmonary collectins and innate host defense of the lung. *Microbes and infection*, 3**,** 161-166.

Manchanda, V., Suman, U. & Singh, N. (2018). Implementing infection prevention and control programs when resources are limited. *Current Treatment Options in Infectious Diseases*, 10**,** 28-39.

Marchini, G., Lindow, S., Brismar, H., Ståbi, B., Berggren, V., Ulfgren, A. K., Lonne-rahm, S., Agerberth, B. & Gudmundsson, G. (2002). The

newborn infant is protected by an innate antimicrobial barrier: peptide antibiotics are present in the skin and vernix caseosa. *British Journal of Dermatology*, 147**,** 1127-1134.

Maródi, L. (2006). Neonatal innate immunity to infectious agents. *Infection and immunity*, 74**,** 1999-2006.

Mcfee, R. B. (2009). Nosocomial or hospital-acquired infections: an overview. *Disease-a-Month*, 55**,** 422.

Mehler, K., Cornely, O., Seifert, H., Zweigner, J., Janssen, S. & Oberthuer, A. (2022). Molds and more: rare fungal infections in preterm infants< 24 weeks of gestation. *The Pediatric Infectious Disease Journal*, 41**,** 352-357.

Mendell, M. J., MireR, A. G., Cheung, K., Tong, M. & Douwes, J. (2011). Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence. *Environmental health perspectives*, 119**,** 748-756.

Money, N. P., Davis, C. M. & Ravishankar, J. (2004). Biomechanical evidence for convergent evolution of the invasive growth process among fungi and oomycete water molds. *Fungal Genetics and Biology*, 41**,** 872-876.

Moraille, R., Pickens, W., Visscher, M. & Hoath, S. (2005). A novel role for vernix caseosa as a skin cleanser. *Neonatology*, 87**,** 8-14.

Narendran, V., Wickett, R. R., Pickens, W. L. & Hoath, S. B. (2000). Interaction between pulmonary surfactant and vernix: a potential mechanism for induction of amniotic fluid turbidity. *Pediatric research*, 48**,** 120-124.

Neely, A. N., & Maley, M. P. (2000). Survival of enterococci and staphylococci on hospital fabrics and plastic. *Journal of Clinical Microbiology*, 38(2), 724-726. https://doi.org/10.1128/JCM.38.2.724-726.2000

Obeng-Nkrumah, N., Twum-Danso, K., Krogfelt, K. A. & Newman, M. J. (2013). High levels of extended-spectrum beta-lactamases in a major teaching hospital in Ghana: the need for regular monitoring and evaluation of antibiotic resistance. *The American journal of tropical medicine and hygiene*, 89**(**960).

Ocran, I., & Tagoe, D. N. A. (2014). Knowledge and attitude of healthcare workers and patients on healthcare associated infections in a regional hospital in Ghana. *Asian Pacific Journal of Tropical Disease*, *4*(2), 135-139.

Onmek, N., Kongcharoen, J., Singtong, A., Penjumrus, A. & Junnoo, S. (2020). Environmental factors and ventilation affect concentrations of microorganisms in hospital wards of Southern Thailand. *Journal of Environmental and Public Health*, 2020.

Organization, W. H. (2009). WHO guidelines on hand hygiene in health care. *WHO guidelines on hand hygiene in health care*.

Owusu, E., Asane, F. W., Bediako-Bowan, A. A., & Afutu, E. (2022). Bacterial contamination of surgical instruments used at the surgery department of a major teaching hospital in a resource-limited country: an observational study. *Diseases*, *10*(4), 81.

Perlroth, J., ChoI, B. & Spellberg, B. (2007). Nosocomial fungal infections: epidemiology, diagnosis, and treatment. *Medical mycology*, 45**,** 321-346.

Pestka, J. J., Yike, I., Dearborn, D. G., Ward, M. D. & Harkema, J. R. (2008). Stachybotrys chartarum, trichothecene mycotoxins, and damp building–related Illness: new insights into a public health enigma. *Toxicological sciences*, 104**,** 4-26.

Pessoa-Silva, C. L., Hugonnet, S., Pfister, R., Touveneau, S., Dharan, S., Posfay-Barbe, K., & Pittet, D. (2007). Reduction of health care–associated infection risk in neonates by successful hand hygiene promotion. *Pediatrics*, *120*(2), e382-e390.

Pfitscher, L., Cecatti, J., Haddad, S., Parpinelli, M., Souza, J., Quintana, S. M., Surita, F., Costa, M., Group, B. N. F. S. O. S. M. M. S. & Pacagnella, R. C. (2016). The role of infection and sepsis in the Brazilian Network for Surveillance of Severe Maternal Morbidity. *Tropical Medicine & International Health*, 21**,** 183-193.

Pickens, W. L., Warner, R. R., Boissy, Y. L., Boissy, R. E. & Hoath, S. B. (2000). Characterization of vernix caseosa: water content, morphology, and elemental analysis. *Journal of investigative dermatology*, 115**,** 875-881.

Pizzorno, J. (2016). Is Mold Toxicity Really a Problem for Our Patients? Part I—Respiratory Conditions. Integrative Medicine: *A Clinician's Journal*, 15**(**6).

Proksch, E., Holleran, W., Menon, G. K., Elias, P. M. & Feingold, K. R. (1993). Barrier function regulates epidermal lipid and DNA synthesis. *British Journal of Dermatology*, 128**,** 473-482.

Randle, J., Clarke, M., & Storr, J. (2006). Hand hygiene compliance in healthcare workers. *Journal of Hospital Infection*, *64*(3), 205-209.

Rawlings, A. V., Scott, I. R., Harding, C. R. & Bowser, P. A. (1994). Stratum corneum moisturization at the molecular level. J*ournal of investigative dermatology*, 103**,** 731-740.

Reuter, S., Moser, C. & Baack, M. (2014). Respiratory distress in the newborn. *Pediatrics in review*, 35**,** 417-429.

Richardson, M. (2005). Environmental fungi and fungal infections in neonates. *Journal of Hospital Infection*, 59(4), 262-268. https://doi.org/10.1016/j.jhin.2004.10.015

Riesenfeld, T., Strömberg, B. & Sedin, G. (1986). The influence of vernix caseosa on water transport through semipermeable membranes and the skin of full-term infants. *Neonatal Physiological Measurements. Elsevier*.

Rissmann, R., Groenink, H. W., Weerheim, A. M., Hoath, S. B., Ponec, M. & Bouwstra, J. A. (2006). New insights into ultrastructure, lipid composition and organization of vernix caseosa. *Journal of investigative dermatology*, 126**,** 1823-1833.

Rissmann, R., Oudshoorn, M. H., Zwier, R., Ponec, M., Bouwstra, J. A. & HENNINK, W. E. (2009). Mimicking vernix caseosa—Preparation and characterization of synthetic biofilms. *International journal of pharmaceutics*, 372**,** 59-65.

Russell, A. R. B. (2011). Neonatal sepsis. *Paediatrics and Child health*, 21**,** 265-269.

Sahaneijo, S. & Tagami, H. (1991). Dry skin of newborn infants: functional analysis of the stratum corneum. *Pediatric dermatology*, 8**,** 155-159.

Saijo, S., & Tagami, H. (1991). Dry skin of newborn infants: functional analysis of the stratum corneum. *Pediatric dermatology*, *8*(2), 155-159.

Saunders, C. (1948). The vernix caseosa and subnormal temperature in premature infants. BJOG: *An International Journal of Obstetrics & Gynaecology*, 55**,** 442-444.

S, A. L., Sánchez, P. J. & Stoll, B. J. (2017). Neonatal sepsis. *The lancet*, 390**,** 1770-1780.

Shane, A. L., Sánchez, P. J., & Stoll, B. J. (2017). Neonatal sepsis. *The lancet*, *390*(10104), 1770-1780.

Sharma, C. M., AgrawaL, R. P., Sharan, H., Kumar, B., Sharma, D. & Bhatia, S. S. (2013). "Neonatal sepsis": bacteria & their susceptibility pattern towards antibiotics in neonatal intensive care unit. *Journal of clinical and diagnostic research: JCDR*, 7(2511).

Sievert, D. M., Ricks, P., Edwards, J. R., Schneider, A., Patel, J., Srinivasan, A., Kallen, A., Limbago, B. & Fridkin, S. (2013). Antimicrobial-resistant pathogens associated with healthcare-associated infections summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. *Infection Control & Hospital Epidemiology*, 34**,** 1-14.

Simonsen, K. A., Anderson-Berry, A. L., Delair, S. F. & Davies, H. D. (2014). Early-onset neonatal sepsis. *Clinical microbiology reviews*, 27**,** 21-47.

Stewart, M. E., Quinn, M. A. & Downing, D. T. (1982). Variability in the fatty acid composition of wax esters from vernix caseosa and its possible relation to sebaceous gland activity. J*ournal of Investigative Dermatology*, 78**,** 291-295.

Suleyman, G., Alangaden, G. & Bardossy, A. C. (2018). The role of environmental contamination in the transmission of nosocomial pathogens and healthcare-associated infections. *Current infectious disease reports*, 20**,** 1-11.

Sumida, Y., Yakumaru, M., Tokitsu, Y., Iwamoto, Y., Ikemoto, T. & Mimura, K. (2001). Studies on the function of Vernix caseosa: The secrecy of Baby's skin. International Federation of the Societies of Cosmetic Chemists 20th International Conference, Cannes, France, 1998. 1-7.

Szabó, S., Feier, B., Capatina, D., Tertis, M., Cristea, C. & Popa, A. (2022). An overview of healthcare associated infections and their detection methods caused by pathogen bacteria in Romania and Europe. *Journal of clinical medicine,* 11**(**3204).

Tetteh, F. K. M., Ablordey, A., Obeng-Nkrumah, N., & Opintan, J. A. (2024). Extended-spectrum beta-lactamases in clinical isolates of Escherichia coli and Klebsiella pneumoniae recovered from patients at the Tamale Teaching Hospital, Ghana. *Plos one*, *19*(4), e0300596.

Thiele, J. J. & Packer, L. (1999). Noninvasive measurement of α-tocopherol gradients in human stratum corneum by high-performance liquid chromatography analysis of sequential tape strippings. *Methods in enzymology. Elsevier.*

Tollin, M., Jägerbrink, T., Haraldsson, A., Agerberth, B. & Jörnvall, H. (2006). Proteome analysis of vernix caseosa. *Pediatric research,* 60**,** 430-434.

Tollin, M., Bergsson, G., Kai-Larsen, Y., Sørensen, O. E., Bjartell, A., Jörnvall, H., & Boman, H. G. (2005). Vernix caseosa as a multi-component defence system based on polypeptides, lipids and their interactions. *Cellular and Molecular Life Sciences*, 62(19-20), 2390-2399. https://doi.org/10.1007/s00018-005-5191-1

UNICEF (2020). COVID-19 Emergency preparedness and response: WASH and infection prevention and control in health care facilities. Guidance note. *United Nations Children's Fund*.

Utturkar, R. S. (2005). Vernix Caseosa: a source of natural moisturizing factors and its possible role in neonatal infant skin hydration, *University of Cincinnati.*

Verde, S. C., Almeida, S. M., Matos, J., Guerreiro, D., MENESES, M., Faria, T., Botelho, D., Santos, M. & Viegas, C. (2015). Microbiological assessment of indoor air quality at different hospital sites. *Research in microbiology*, 166**,** 557-563.

Visscher, M. O., Narendran, V., Pickens, W. L., Laruffa, A. A., Meinzen-Derr, J., Allen, K. & Hoath, S. B. (2005). Vernix caseosa in neonatal adaptation. *Journal of Perinatology*, 25**,** 440-446.

Wakai, R., Lengle, J. & Leuthold, A. (2000). Transmission of electric and magnetic foetal cardiac signals in a case of ectopia cordis: the dominant role of the vernix caseosa. *Physics in Medicine & Biology*, 45**(**1989).

Weinhold, B. (2007). A spreading concern: inhalational health effects of mold. *National Institute of Environmental Health Sciences.*

Wilson, C. B., Nizet, V., Maldonado, Y., Remington, J. S., & Klein, J. O. (2015). Remington and Klein's infectious diseases of the fetus and newborn infant. *Elsevier Health Sciences.*

Wilson, J. (2019). Infection control in clinical practice updated edition. *Elsevier Health Sciences.*

Wohrley, J. D. & Bartlett, A. H. (2019). The role of the environment and colonization in healthcare-associated infections. *Healthcare-Associated Infections in Children: A Guide to Prevention and Management***,** 17-36.

Yoshio, H., Tollin, M., Gudmundsson, G. H., Lagercrantz, H., JörnvalL, H., Marchini, G. & Agerberth, B. (2003). Antimicrobial polypeptides of human vernix caseosa and amniotic fluid: implications for newborn innate defense. *Pediatric research*, 53**,** 211-216.

Y, N. E., Araújo-pérez, F., Brandon, D. & Seed, P. C. (2018). Early-life skin microbiota in hospitalized preterm and full-term infants. *Microbiome*, 6**,** 1-11.

Zhukov, B., Neverova, E., Nikitin, K., Kostiaev, V. & Myshentsev, P. (1992). A comparative evaluation of the use of vernix caseosa and solcoseryl in treating patients with trophic ulcers of the lower extremities. *Vestnik Khirurgii Imeni II Grekova*, 148**,** 339-341.

APPENDIX I

QUESTIONNAIRE FOR PATIENTS

I am a student in Molecular Biology and Biotechnology Department, University of Cape Coast. I am researching into "The effects of microbial air quality and Vernix Caseosa on the health of Neonates during the first seven days after birth". I should be grateful if you could allow your child to participate in this research. Few questions will be asked which will not take much of your time.

I would like to ask these few questions:

Q1. What is your occupation:

Q2. Your Husband occupation:

Q3. Residential address:

Q4. Telephone number:

Observation after seven days of discharge

Name of neonate/ Special code	Chest/ cough	Cord Clean/dry / healed	Eye	Nose Dry/ wet	Skin Rough/ smooth Normal /infected	Mouth Clean/ infected	Ear Dry& clean/ infected

APPENDIX II

ETHICAL CLEARANCE

UNIVERSITY OF CAPE COAST

INSTITUTIONAL REVIEW BOARD SECRETARIAT

TEL:
E-MAIL:
OUR REF: IRB/C.3/Vol.1/0395
YOUR REF:
OMB NO: 0990-0279
IORG #: IORG0011497

26TH OCTOBER, 2023

Ms Mary Fosua
Department of Molecular Biology and Biotechnology
University of Cape Coast

Dear Ms Fosua,

ETHICAL CLEARANCE – ID (UCCIRB/CANS/2023/11)

The University of Cape Coast Institutional Review Board (UCCIRB) has granted Provisional Approval for the implementation of your research **The Effects of Microbial Air Quality and Vernix Caseosa on the Health of Neonates During the First Seven Days After Birth.** This approval is valid from **26th October, 2023** to **25th October, 2024**. You may apply for an extension of ethical approval if the study lasts for more than 12 months.

Please note that any modification to the project must first receive renewal clearance from the UCCIRB before its implementation. You are required to submit a periodic review of the protocol to the Board and a final full review to the UCCIRB on completion of the research. The UCCIRB may observe or cause to be observed procedures and records of the research during and after implementation.

You are also required to report all serious adverse events related to this study to the UCCIRB within seven days verbally and fourteen days in writing.

Always quote the protocol identification number in all future correspondence with us in relation to this protocol.

Yours faithful

Kofi F. Amuquandoh
Ag. Administrator