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Bacterial colonisation of neonates in two hospitals in Tanzania and the role of the hospital environment

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
an der Medizinischen Fakultät der Universität Hamburg

vorgelegt von:

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Hamburg 2024

Angenommen von der
Medizinischen Fakultät der Universität Hamburg am: 30.04.2025

Veröffentlicht mit Genehmigung der
Medizinischen Fakultät der Universität Hamburg.

Prüfungsausschuss, der/die Vorsitzende: Prof. Dr. Johannes K.-M. Knobloch

Prüfungsausschuss, zweite/r Gutachter/in: Prof. Dr. Jürgen May

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List of abbreviations

A. – Acinetobacter

AMR – Antimicrobial Resistance

API 20E – Analytical Profile Index for Enterobacteriaceae

API 20NE – Analytical Profile Index for Non Enterobacteriaceae

App – application

CI – confidence interval

CRF – case report form

CS – caesarean section

DDD – defined daily doses

E. – Escherichia

e.g. – for example

ESBL – extended-spectrum beta-lactamase

ESBL-PE – extended-spectrum beta-lactamase producing Enterobacteriaceae

GLASS – Global Antimicrobial Resistance and Use Surveillance System

GNB – Gram-negative bacteria

HICs – high income countries

ICF – informed consent form

ID – study identifier

IPC – infection prevention and control

IQR – interquartile range

K. – *Klebsiella*

KTCH – Korogwe Town Council Hospital

LMICs – low- and middle-income countries

MRSA – methicillin resistant *Staphylococcus aureus*

MDR – multidrug resistant

NICU – neonatal intensive care unit

NIMR – National Institute for Medical Research

P. – *Pseudomonas*

PBP – penicillin-binding protein

PCR – polymerase chain reaction

REDCap – Research Electronic Data Capture

S. – *Staphylococcus*

SIM – Sulfur-Indole-Motility

SOP – study specific procedure

SSA – Sub-Sahara Africa

SVD – spontaneous vaginal delivery

TRRH – Tanga Regional Referral Hospital

WHO – World Health Organisation

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1. Introduction

1.1. Antimicrobial Resistance (AMR)

1.1.1 Definition

AMR refers to the capability of microorganisms to resist antimicrobial drugs, whose purpose is to inhibit their growth or kill them. While the development of resistance is a naturally occurring process, it is strongly accelerated by selection pressure exerted through the widespread use of antimicrobials in human medicine and animal husbandry (1). A sub-category of AMR is antibiotic resistance, which specifically concerns bacterial resistance against antibiotics. Resistant bacteria are ubiquitous, present in humans, animals, food and the environment (2–4). They can be transmitted between these different reservoirs and can lead to colonisation or infection of humans with severe health outcomes (2–4).

1.1.2 The global health burden of AMR

AMR is one of the leading public health challenges of our time. Since the first antibiotic was discovered by Alexander Fleming in 1928, the enormous benefits antibiotics have brought to medicine have been comprised by the rapid development of resistance. Worldwide, clinicians are facing difficulties in treating infections which once used to be easy to cure. There is documented resistance to all commonly used antimicrobial drugs and typically resistance occurs shortly after a new drug is introduced (5). When resistant to antibiotic treatment, bacterial infections usually lead to longer hospitalisation, higher medical costs and augmented morbidity and mortality (6). In 2019, the World Health Organisation (WHO) declared AMR as one of the top ten global health threats facing humanity (1).

In order to combat the increase of AMR, it is essential to understand its global burden. This knowledge will help to set research priorities, distribute resources and enforce regulations (7). However, due to the complexity of AMR and the lack of data (especially from low-resource settings) on prevalence, incidence and mortality, it is challenging to quantify its impact (7–9). As part of the Global Research on Antimicrobial Resistance project, Murray *et al.* published the first systematic analysis of its kind in 2022, capturing the global burden of AMR. According to this analysis, 4.95 million people died in the year 2019 associated with a drug-resistant bacterial infection and 1.27 million of these deaths were directly caused by bacterial AMR

(10). When comparing this to the underlying causes of death as defined in the Global Burden of Disease Study of 2019, infections associated with bacterial AMR would have been the third leading cause of death worldwide, following ischemic heart disease and stroke (10, 11). In 2014, the UK government initiated the "Review on Antimicrobial Resistance" describing the global burden of AMR and proposing solutions. According to this review, an estimated ten million people will die as a result of AMR-related infections every year worldwide by 2050 if no action is taken (6, 12). Almost half of these deaths are expected to occur in Sub-Sahara Africa (SSA) (6).

Besides increasing mortality rates, AMR hampers routine clinical procedures. Most surgeries, including caesarean section (CS), and immunosuppressive therapies rely on prophylactic treatment with antibiotics to reduce the risk of infection. If antibiotic resistance rises further, many common interventions will be too dangerous to perform and maternal mortality following CS could rise (6, 12).

As stated in reviews on antibiotic resistance in Europe, higher antibiotic consumption correlates with higher drug resistance (13, 14). According to a systematic analysis conducted by Browne *et al.*, the lowest antibiotic consumption worldwide is observed in SSA (15). In this region an average of 11.8 defined daily doses (DDD) per 1000 population are consumed per day, compared to 20.6 DDD in high-income countries (HICs) (15). Nevertheless, Murray *et al.* found the highest disease burden due to antibiotic resistance to be in SSA (10): mainly affecting western SSA, where in 2019 the researchers estimated a death rate of 114.8 per 100 000 associated with antibiotic resistance, directly followed by eastern SSA (7). In comparison, in HICs, 56 per 100 000 people died (10). These findings highlight that although the use and overuse of antibiotics are drivers of antibiotic resistance, more factors contribute to its vast disease burden in low- and middle-income countries (LMICs).

Firstly, the incidence of critical infections is higher in LMICs compared to HICs (9, 11). In LMICs community-acquired infections, such as respiratory and gastrointestinal infections, are still the leading causes of death (9). High rates of HIV, malnutrition and malaria might render patients more susceptible to bacterial infections (16). The incidence and mortality of sepsis in LMICs are the highest worldwide (17). Further, a large number of people live in crowded and unsanitary conditions, which facilitate the spread of infections. In Tanzania, only 19 % of households use improved, non-shared toilet facilities and 10 % have no toilet at all (18). Poor sanitation and water quality are strongly and positively correlated with antibiotic resistance (19). In addition, household subsistence farming is common, which favours contagion with zoonotic bacteria (20).

Secondly, low governance and poor enforcement of policies in many LMICs are associated with antibiotic resistance (19). Little regulation of the dispensing and use of antibiotics leads to misuse in humans and animals. Antibiotics are sold without prescription over the counter and sub-standard drugs are circulating (21, 22). This non-prescription use is associated with inappropriate drug and dose choice and a higher risk of adverse effects (21). Further, misconceptions about the effects of antibiotics are widespread in the community. In a cross-sectional study conducted in South Africa, 66 % of participants responded that virus infections like cold and flu could be treated with antibiotics (23). In several districts of Tanzania over half of the study population, including farmers frequently administering antibiotics, were not aware of AMR (24). Uncontrolled antibiotic use in animal farming is a common practice, with poor adherence to recommended antibiotic-withdrawal periods and monitoring for antibiotic residues in food products (25–29).

At the same time, the lack of access to essential antibiotics is an enormous challenge in LMICs (30, 31). Presently, 5.7 million people die of treatable infections each year due to the unavailability of antibiotics, whereby children are especially at risk (32). In 2019, one in five deaths caused by AMR occurred in children under the age of five years and 99.6 % of these children lived in LMICs (10). The most vulnerable period for a child's survival are the first 28 days of life, the neonatal period. SSA has the highest neonatal mortality rate in the world, which is ten times higher than in HICs (33). The leading cause of neonatal death in SSA, including Tanzania, are bacterial infections, e.g. sepsis (34). Several multicentred studies and systematic reviews showed that most pathogens isolated from neonates with sepsis in LMICs have become resistant to the first-line empiric treatment (Ampicillin and Gentamicin) recommended by the WHO (35–38). In addition, almost half of Gram-negative bacteria (GNB) isolated are resistant to second-line treatment (third generation Cephalosporins) (35). Reserve antibiotics, which are needed to treat multidrug-resistant (MDR) infections, are inaccessible or unaffordable in most settings in SSA (9, 39).

Further, diagnostic options offering differential diagnosis of disease are scarce and empirical treatment is a common practice (40). Even if microbiological laboratories are available, the barriers for testing are high, due to lack of experienced staff, little cooperation between clinicians and laboratory staff and limited financial means to pay for tests (41). Without bacterial identification and resistance testing, the possibility to adapt antibiotic treatment is limited (16). In addition, essential infection prevention and control (IPC) measures are lacking in hospitals. Substandard cleaning, disinfection and sterilisation practices are common and wards are often overcrowded (35, 42). These factors are making hospitals hot spots for MDR bacteria. In particular

neonates rapidly become colonised with bacteria from their peri- and postnatal surroundings (35, 43–45). Colonisation serves as a risk factor for infection (46, 47), whereby neonates are especially vulnerable, due to their immature immune system (48). In LMICs neonatal infections reported for hospital-born babies are three to 20 times more common than in HICs (35). However, microbial sampling of sources of transmission is rarely performed nor reported from hospitals in LMICs (42, 49).

All of these circumstances are fostering the emergence of antibiotic resistance and leading to difficult-to-treat infections with increased morbidity and mortality. However, the lack of surveillance of antibiotic resistance patterns might have the biggest impact. In 2015, the WHO initiated the Global Antimicrobial Resistance and Use Surveillance System (GLASS) in order to standardize AMR surveillance worldwide (50). Despite the improvement of AMR surveillance systems in some countries, the WHO states that there are still serious data gaps from many LMICs (50). In 2021, the United Republic of Tanzania, among other countries, had not yet reported any data on AMR to GLASS (50). With little data available, it is challenging to react to and develop strategies against emerging resistances, as well as to adapt treatment or prevention measures. In addition, the sparsity of data from LMICs might even lead to underestimating the AMR burden in these regions (10). This highlights the need to expand microbiology laboratory capacity and data collection in low-resource settings.

1.2. Aims of the presented work

MDR bacteria are common causes of neonatal sepsis in SSA. However, data from SSA, including Tanzania, on carriage of MDR bacteria during the neonatal period is insufficient. Transmission reservoirs and in particular the role of the hospital environment are not well defined. This information is essential for successful patient management and implementation of IPC measures, in order to reduce the disease burden.

Therefore, this study aimed

a. to determine the frequency of colonisation with MDR bacteria for neonates and mothers, as well as hospital staff and surfaces in two hospitals in the Tanga Region of Tanzania
b. and to investigate the role of the hospital environment as a potential transmission reservoir of bacteria to neonates.

2. Background

2.1. Origins of Antibiotic Resistance

2.1.1 Intrinsic resistance

Intrinsic antibiotic resistance is a naturally occurring phenomenon that predates antibiotic chemotherapy and is present in all bacterial species (51). It is independent of previous antibiotic use or horizontal gene transfer. The most common bacterial mechanisms involved in intrinsic resistance are reduced permeability of the outer membrane of the bacterium and efflux pumps (51). For example, *Pseudomonas* and *Klebsiella* species are intrinsically resistant to Ampicillin (5), and therefore survive treatment with this drug.

2.1.2 Acquired resistance

Acquired resistance occurs when a particular bacterium obtains the ability to resist an antibiotic to which it was previously susceptible. This process is driven by selection pressure exerted by the use of antibiotics. Acquisition of genetic material that confers resistance is possible through mutations of the bacteria's DNA or through horizontal transfer of mobile genetic elements, e.g. plasmids containing resistance genes, between bacteria (5). The latter includes conjugation (transfer of plasmids through direct cell contact), transformation (DNA uptake from the external environment) and transposition (transfer of DNA by bacteriophages) (52). Gene transfer can take place within the same bacterial species, but also between different species. Further, warm and humid climates facilitate the development and transfer of resistance genes (53). Unlike intrinsic resistance, traits associated with acquired resistance are found only in some strains or subpopulations of a bacterial species (54).

2.2. Resistance mechanisms

There are four main mechanisms of antibiotic resistance (55):

a) Enzymatic degradation of antibacterial drugs

A group of bacterial enzymes called beta-lactamases can induce the hydrolysis of beta-lactam antibiotics by destroying their beta-lactam ring. The production of beta-lactamases is among the most common resistance mechanisms used by GNB.

The clinically most important beta-lactamases are extended-spectrum beta-lactamases (ESBLs). These enzymes are mainly produced by members of the bacterial order of Enterobacterales (5). ESBL genes are located on plasmids and can be transferred between different strains and even bacterial species (56). ESBLs cause resistance against penicillins, extended-spectrum cephalosporins and monobactams. Plasmids responsible for ESBL resistance often carry additional genes which confer resistance to further antibiotics (57).

Another large group of beta-lactamases are Carbapenemases, which induce resistance to beta-lactam antibiotics, including carbapenems. Along with ESBLs, Carbapenemases have spread globally among GNB (58). Further, Carbapenem resistance can be acquired through ESBL expression coupled with changes in membrane permeability (59).

b) Alteration of antibiotic targets

The alteration of a bacterial protein, which acts as an antibiotic target, occurs in the case of Methicillin resistance in *Staphylococcus aureus* (*S. aureus*). This resistance is obtained through the integration of the *mecA* gene into the bacterial chromosome (5). The *mecA* gene is a part of a mobile genetic element termed SCC*mec*. It is not a gene native to *S. aureus* but rather acquired from an unknown source (60). Its gene product results in the alteration of the antibiotic target protein called penicillin-binding protein (PBP) 2A. This penicillin-binding protein possesses a decreased affinity for beta-lactam antibiotics, compared to native PBPs (60).

c) Limiting drug uptake

Though changes in their membrane permeability bacteria can limit the uptake of antibiotics. Members of the order Enterobacterales are known to become resistant against antibiotics through the reduction of porins in their outer membranes (61). Other bacteria, such as *Pseudomonas aeruginosa* (*P. aeruginosa*) are able to form biofilms, which are difficult to penetrate for antimicrobial drugs (5).

d) Active drug efflux

Most bacteria possess many different types of efflux pumps, which enable the bacterial cells to dispose of toxic substances. For example, *Acinetobacter baumannii* (*A. baumannii*) carries efflux pumps of the major facilitator super family, which can export erythromycin and chloramphenicol. *Escherichia coli* (*E. coli*) also possesses pumps of this family for the export of macrolides, fluoroquinolones and trimethoprim (62, 63). In addition *E. coli* can carry efflux pumps of the Resistance-Nodulation-Division transporter family, which confers resistance to penicillin, fluoroquinolones and tetracycline (64).

2.3. WHO priority pathogens list for research and development

The bacteria of concern in this study were chosen in consideration of the "WHO priority pathogens list for research and development of new antibiotics" (65). This list was published by the WHO in 2017 and includes bacteria which can be pathogenic and for which new antibiotics are urgently needed, due to high levels of resistance. It is divided into three categories: critical, high and medium priority. The critical group includes MDR bacteria, which pose a particular threat in hospitals. These are the following: *A. baumannii* (carbapenem-resistant), *P. aeruginosa* (carbapenem-resistant) and bacteria of the Enterobacteriaceae family, including *Klebsiella pneumoniae* (*K. pneumoniae*) and *E. coli* (both carbapenem-resistant and ESBL producing). The group of high risk contains Methicillin-resistant *S. aureus* (MRSA) (65).

2.4. Bacteria of concern in this study

2.4.1 *A. baumannii*

A. baumannii is a non-fermentative GNB and a typical nosocomial pathogen. It is strictly aerobic, oxidase negative and catalase positive. The pathogen is capable of biofilm formation and very resistant to desiccation. These factors enable it to persist on inanimate surfaces for a long time (66). It has been isolated for example from door handles, floors and mattresses of hospital wards (67). In addition, *A. baumannii* has the capacity for great genetic plasticity, including the integration of mobile genetic elements into its DNA (66). This allows the rapid development of resistance.
MDR *A. baumannii* is defined as being resistant to three classes of antibiotics including cephalosporins, fluoroquinolones and aminoglycosides (68). Globally, approximately 45 % of *A. baumannii* isolates are MDR bacteria (69). MDR *A. baumannii* typically causes opportunistic infections, which can be treated with carbapenems.

However, with the development of extensively-drug resistant (resistance to carbapenems) and pan-drug resistant (resistance also to polymyxins) *A. baumannii* strains, the treatment options become limited to polymyxins and tigecycline or no possible treatment at all (68). The mentioned treatments are accompanied by severe side effects, such as nephron, neuro and pulmonary toxicity (68).

A. baumannii infections in neonates are rising and outbreaks in neonatal intensive care units have occurred (70-73). The pathogen is responsible for sepsis and respiratory tract infections in neonates. In a study conducted in a tertiary hospital in Mwanza, Tanzania, *A. baumannii* was found in 16% of neonates with sepsis (74). Risk factors for acquisition are reported to be low birth weight, length of hospitalisation and prior antibiotic use (70).

2.4.2 *P. aeruginosa*

P. aeruginosa is a non-fermentative, encapsulated, Gram-negative and aerobic bacterium. It is citrate, catalase and oxidase positive. In addition, it produces the blue-green pigment pyocyanin and has a distinct sweet odour when grown in culture. The pathogen thrives in a wide range of environments, ranging from water, soil and plants to human skin and hospital surfaces (75). *P. aeruginosa* prefers moist surfaces and is therefore a frequent colonizer of medical devices such as humidifiers, ventilators and nebulizers (75). It is responsible for airway infections (often ventilator-associated), burn injury infections and sepsis, among other nosocomial infections (75). Due to its high intrinsic resistance to many antibiotics (e.g., ampicillin, first and second generation cephalosporins), the acquisition of new resistances (e.g., through obtaining ESBLs) and its ability to create biofilms, it causes severe therapeutic challenges (76, 77).

In neonates, *P. aeruginosa* can lead to various infections, such as sepsis, pneumonia, meningitis and diarrhoea. Outbreaks of *P. aeruginosa* in neonatal intensive care units (NICUs) are not as commonly reported, compared to outbreaks caused by other GNB (78). Nevertheless, in the reported events, a contaminated sink, water bath and hospital staff members may have been sources of transmission (78–81). During a *P. aeruginosa* outbreak in a NICU in Italy, active surveillance and improvement of hand hygiene were able to control colonisations and infections (78).

2.4.3 *E. coli* and *K. pneumoniae*

E. coli and *K. pneumoniae* both belong to the family of Enterobacteriaceae (order Enterobacterales) and are lactose fermenting, facultative anaerobic GNB. *K. pneumoniae* is ubiquitous in the environment and colonises both plants and animals (82). Variants of both species are part of the physiological intestinal flora of humans. At the same time, *E. coli* consists of a wide range of pathogenic variants and *K. pneumoniae* is well known as a nosocomial pathogen (83, 84). *E. coli* is responsible for causing gastroenteritis, cholecystitis, urinary tract infections and sepsis. While *K. pneumoniae* causes a similar spectrum of diseases, it is also known for highly complicated pneumonia. Transmission usually occurs faecal-oral, either directly or indirectly through the hands of healthcare workers or contaminated surfaces.

Both pathogens are known for their great phenotypic and genetic diversity (82, 85), which has allowed them to develop a variety of resistance mechanisms. Over the last decade, a considerable number of strains have acquired ESBLs (56). These enzymes enable the bacteria to resist most beta-lactam antibiotics, except carbapenems. This development has led to an enormous public health threat, with up to 60 % of *E. coli* and *K. pneumoniae* reported to be ESBL-positive worldwide (56, 86).

Data on ESBL producers from SSA is scarce (56). However, Murray *et al*. estimated *K. pneumoniae* to be the leading cause of AMR burden in this region (10). A study conducted in a NICU in Mwanza found that 54.6 % of neonates were colonised with ESBL-producing Enterobacteriaceae (ESBL-PE) (74). Similar colonisation frequencies were reported for children in Ghana (87). This is of great concern, as gastrointestinal carriage of ESBL-PE serves as a reservoir for resistance and is highly associated with severe infections (46, 47, 88, 89). ESBL-positive *K. pneumoniae* and *E. coli* are common causes of neonatal sepsis in SSA, including Tanzania (90). According to a systematic review conducted in 2017 by Flokas *et al.*, the mortality in neonates with sepsis caused by ESBL-PE was double that of all other neonates with sepsis (91).

Treatment options for ESBL-PE are limited, with Carbapenems being currently the most favourable (56). Consequently, the numbers of Carbapenem-resistant Enterobacteriaceae, especially *K. pneumoniae*, are rising. On the African continent resistance against Carbapenems is estimated to lie between 1-10 % (92). In a neonatal unit in South Africa the proportion of Carbapenem-resistant Enterobacteriaceae causing neonatal sepsis, increased from 2.6 % to 8.9 % within only two years (93). As carbapenem resistance is often accompanied by further resistance, treatment options are extremely limited (94).

2.4.4 *S. aureus*

S. aureus is commonly found on human skin and can cause skin and other infections, including sepsis. It is Gram-positive and coagulase-positive and expresses several virulence factors which help it to avoid the hosts' immune response (5). One of these toxins, Panton-Valentine Leucocidin (PVL), causes pore formation in macrophages and is associated with invasive soft tissue infections and necrotising pneumonia (95). Shortly after the drug Methicillin was introduced in 1959, *S. aureus* acquired resistance against it (96). This resistance is caused by the acquisition of the *mecA* gene. This gene encodes for the mentioned PBP 2A, which has a lower affinity to beta-lactam antibiotics and therefore inhibits them from disrupting the cell wall synthesis of the bacteria (97).

Today MRSA poses an enormous challenge to health care settings worldwide (98-100). It is a major cause of nosocomial infections, with immunocompromised patients being especially at risk. At the same time, community-acquired MRSA infections are of growing concern and are known for their rapid transmission (101, 102). Due to the asymptomatic colonisation of skin and nose, MRSA can easily spread from person to person through direct or indirect contact (103). Most of the isolates are multiply resistant, only susceptible to few antibiotics such as glycopeptides (99). MRSA infections have a two to three times-higher mortality rate than infections with Methicillin-susceptible *S. aureus* (5). According to the systematic review conducted by Murray et al, MRSA is the most burdensome pathogen-drug combination worldwide (10).

According to a study conducted by Eibach *et al.* on admission to a hospital in Ghana, 22.1 % of children below 15 years were nasal carriers of *S. aureus* and 2 % of MRSA (104). Similar and higher prevalence have been reported in Europe (105–107). In neonatal intensive care units, MRSA causes prolonged infection outbreaks (108–110). According to a study conducted during an outbreak in a NICU in Germany, very low birth weight infants (< 1500 g) were especially susceptible to MRSA infection and "unknown MRSA positive" neonates posed an increased risk of transmission to others (111). However, in general, the reservoirs of *S. aureus* and MRSA transmission to neonates are only poorly understood (111, 112).

2.5. Resistance reservoirs

2.5.1 Community

Formerly MDR bacteria were mainly associated with hospital settings. An alarming development is their spread within the community, which leads to an enlargement of their reservoir and a greater population at risk of infection (102). In SSA, a significant amount of the healthy population, especially children, are colonised with MDR bacteria. In Mwanza 33.1% of healthy children under the age of five years were colonised with ESBL-PE (113). Okeke *et al.* showed a rapid increase in the proportion of multidrug resistance in commensal *E. coli* from 30.2 to 70.5% within twelve years, isolated from healthy university students in Nigeria (88). In eastern Uganda, 5.7% of healthy children in the community were nasal carriers of MRSA (114). This is alarming, as colonisation may serve as a precursor to infection (115, 116).

In addition to humans, animals act as one of the most important reservoirs for MDR pathogens. In animal farming, antibiotics are used on a large scale to stimulate growth and to prevent sickness (12). In the US over 70% of antibiotics, known as medically important for humans, are used in animal farming (12). Among these are third and fourth-generation cephalosporins, which are on the WHO list for "Critically important antimicrobials for human use" and are used to treat severe infections (2, 4, 117). The transmission of resistant bacteria from animals to humans may occur in various ways, with the oral uptake of contaminated meat and water being the most common. For MRSA direct contact with animals seems to be the major route of transmission (4). Studies from Kenya reported antibiotic residues in cattle meat higher than the recommended maximum levels (27, 118). In Morogoro, Tanzania, 100% of tested commercial chicken eggs were positive for antibiotic residues (18). From the wounds of Nigerian outpatients, resistant staphylococci were isolated, which are typically associated with animal infections (20).

Furthermore, the environment can serve as a reservoir for resistance. Bacteria can persist in different environments (e.g., soil, rivers, lakes) for prolonged periods. Wastewater from pharmaceutical factories and hospitals is often contaminated with antibiotic components (119), which exerts extensive selective pressure on bacteria in water and soil. A study conducted in India found higher concentrations of Ciprofloxacin in a wastewater treatment plant than would be recommended for intravenous treatment of patients (120). Eighty-six percent of bacterial strains isolated from the treatment plant were resistant to at least 20 different antibiotics (120). In the Republic of Congo, hospital effluent waters were highly contaminated with human pathogenic bacteria leading to deterioration of the water quality in several rivers (119).

2.5.2 Hospital

Worldwide healthcare facilities present to be high-risk environments for the transmission of bacterial infections (121). In hospitals the selection pressure for bacteria is high and patients are vulnerable, which allows resistance to develop and spread easily. Common hospital-acquired bacteria include *E. coli, K. pneumoniae*, *P. aeruginosa, A. baumannii* and *S. aureus*, among others.

In LMICs, where hospitals are often overcrowded, necessary diagnostics are scarce and adequate treatment is costly, nosocomial infections cause a major problem (22). Hospital-born babies in LMICs have an increased risk for neonatal infections due to poor intrapartum and postnatal infection control practices (35). Further, they have a greater risk of colonisation with MDR-GNB, than neonates born at home or in a health centre, according to a study conducted in Cambodia (122). In Kilifi, Kenya, 55% of admitted neonates acquired carriage of ESBL-producing bacteria during their hospital stay (123). Colonisation with MDR bacteria was identified as an independent risk factor for neonatal sepsis in several studies (74, 124). Therefore, the understanding of transmission reservoirs and pathways within hospitals in LMICs is of great interest.

Firstly, hospital surfaces are frequently colonised with pathogens and may act as reservoirs for the transmission of bacteria to patients (125). A study sampling 559 high-touch surfaces across five hospitals in Kenya found 95.9% of surfaces to be contaminated, mostly with extremely high bacterial loads (126). In Muhimbili National Hospital of Tanzania 19.5% of surfaces sampled were contaminated with MRSA (127). Also, in two hospitals in Mwanza high frequencies of MDR-GNB, including *A. baumannii,* were detected on various hospital surfaces (128). The mentioned pathogens, as well as *E. coli*, *K. pneumoniae* and *P. aeruginosa* can survive for months on inanimate surfaces (129). Alarmingly, the leading pathogens causing neonatal sepsis were all found in the close environment of neonates e.g., in incubators in a NICU in South Africa (130). Further, in six out of seven sampled delivery facilities on Zanzibar maternity beds were highly contaminated with pathogenic bacteria (42). However, the role of the hospital environment in the transmission of MDR bacteria is controversial, as bacterial strains from patients and surfaces do not always match (131). Nevertheless, contamination of the environment can certainly lead to contamination of the hands and clothes of hospital staff, who can transmit the bacteria to patients (132).

Secondly, IPC measures, such as cleaning and disinfection, are limited in hospitals across SSA. Material resources (e.g., alcohol hand rub) and essential infrastructure are often lacking (42, 133). In Tanzania, only 24 % of delivery rooms have basic improved water and sanitation standards (including piped water, availability of soap and functioning latrine) (134). Further, there is a lack of training of hospital staff on decontamination of high-risk surfaces (42, 135). If cleaning is not performed correctly, pathogens might persist on surfaces for long periods and thus endanger susceptible patients or healthcare workers (129). In the mentioned delivery facilities on Zanzibar, 60 % of cleaning mops were highly contaminated (42). In addition, adherence to hand hygiene by hospital staff is often not coninuously practised (42, 133, 136). A cross-sectional study including 18 healthcare facilities and 212 health staff in Mwanza found that approximately a quarter of the healthcare worker's hands were contaminated with GNB and MRSA (133). Medical or personal devices of health staff may also act as unrecognised sources of transmission. Studies from Nigerian hospitals showed that 94.6 % and 79 % of the mobile phones and stethoscopes respectively were contaminated with bacteria associated with nosocomial infections (137, 138).

Lastly, the hospitalized patients themselves can shed MDR bacteria when infected or colonised. Therefore, they can pose a risk of transmitting these bacteria to other patients, either directly or indirectly through hospital staff, leading to cross-contamination. In SSA this risk is especially high, as sharing of beds and equipment is common. In Kilifi, Kenya the rate of acquisition of ESBL-PE in neonates was positively associated with the number of known ESBL carriers and the total number of neonates in the same ward (123). In the case of neonates, the colonisation of the mother and other caretakers is also of relevance. Pregnant women colonised with ESBL in the gastrointestinal tract or MRSA in the nose may transmit these bacteria to their respective neonates. In several studies, the colonisation of mothers with MDR bacteria has been identified as a risk factor for the colonisation of neonates (45, 139). High frequencies of ESBL and MRSA carriage in mothers and their neonates have been found in Tanzania and Gabon, suggesting other important sources for transmission such as the hospital environment and hospital staff (44, 45). As screening for contamination with MDR bacteria is rarely performed in hospitals in SSA, the transmission reservoirs often remain unknown.

3. Materials and Methods

3.1. Study design

The work described in this chapter was a cross-sectional and hospital-based study conducted from the 27th of April until the 2nd of November 2022 in two hospitals in the Tanga region of Tanzania. The main objective was to analyse the colonisation of neonates, their mothers, hospital staff and surfaces with five different bacterial species: ESBL *E. coli*, ESBL *K. pneumoniae*, MDR *A. baumannii, P. aeruginosa* and *S. aureus*. Concerning neonates, the acquisition of these bacteria during their hospital stay was also studied. The secondary objective was to investigate the role of the hospital environment as a potential transmission reservoir of bacteria to neonates. For these purposes, swab samples were collected from study participants and surfaces and investigated at the local laboratories.

The study was an ancillary study of a larger project called TRINEO (Transmission Reservoirs and Acquisition of Multidrug-Resistant Bacteria in Neonates admitted to two hospitals within the Tanga region of Tanzania), which is being conducted in cooperation between the Bernhard-Nocht-Institut für Tropenmedizin in Hamburg and the National Institute for Medical Research (NIMR) in Tanzania. As part of this cooperation, laboratory capacities are built, staff training is facilitated and an AMR surveillance program is being established.

3.2. Study setting

3.2.1 Tanga Region, Tanzania

The study was conducted in the Tanga Region of Tanzania. The Tanga Region, with Tanga town as its capital, is one of Tanzania's 31 administrative regions. It is situated in north-eastern Tanzania, between four and six degrees below the Equator, bordering Kenya to the north and the Indian Ocean to the east. The region occupies an area of 27.348 square kilometres and accommodates a population of approximately two million people. Most of Tanga features tropical savannah climate with two rainy seasons from March to May and from October to November. The temperature is relatively stable around the year, averaging between 23 and 28 degrees Celsius. The coastal area is dominated by palm gardens and sisal estates. In contrast, the upland plateaus are marked by bushland and forest, interrupted by village cultivations. Agriculture is the largest source of employment and the predominant crops

grown are citrus fruits, sisal, coconuts, bananas, maize and rice. However, the declining productivity of the agricultural sector, resulting from less reliable rainfall and increasing land infertility, causes unemployment to be a problem (140).

3.3. Study sites

3.3.1 Tanga Regional Referral Hospital (TRRH)

The TRRH is a governmental hospital located in Tanga town. It functions as a referral hospital for the whole of Tanga region serving a population of two million people. The hospital's services include female, male and paediatric wards with a capacity of 600 beds. Further, there is an outpatient clinic attending 400 patients a day, as well as surgical and x-ray facilities. In addition, the hospital offers maternity care including a labour ward, a theatre specialized on CS, a post-surgery ward and an ante- and postnatal ward. Approximately 500 neonates are born in the hospital per month. The hospital is equipped with a neonatal ward including 20 baby beds, eleven incubators as well as 30 mother and baby beds. This neonatal unit cares for referral cases, as well as neonates born within the hospital. It is divided into a NICU, a Kangaroo Mother Care section and a general neonatal section. Equipment includes continuous positive airway pressure machines, baby warmers, cardiac monitors and phototherapy machines among others.

Figure 1: The maternity building of TRRH.[1]

1 © Author

3.3.2 Korogwe Town Council Hospital (KTCH)

KTCH is situated in Korogwe town, the second largest town in the Tanga region with approximately 60 thousand inhabitants and a semi-urban character. The hospital has a capacity of 142 patient beds and receives approximately 6 000 paediatric admissions per year. Based on hospital data, the leading clinical diagnoses among inpatient children involve gastroenteritis, malaria, pneumonia, septicaemia and anaemia. Further, the hospital accommodates a maternity ward with a labour, ante- and postnatal section. The labour section has a capacity of eight delivery beds and the surgical theatre performs CS. Approximately 150 neonates are born in this hospital per month. Recently, the hospital opened a NICU with an incubator and four mother-neonate beds. However, medical care is very limited, due to a lack of equipment such as suctions, cardiac monitors and oxygen devices. In case of medical complications neonates are referred to the TRRH. Further, the hospital experiences frequent shortages of water and at the time of study, there were no lavatories present in the maternity ward.

Figure 2: The labour ward of KTCH [2]

2 © Author

3.3.3 NIMR

The NIMR is a parastatal research organization of the Tanzanian Ministry of Health. It hosts national as well as several international research projects. In recent years, the institute has achieved an excellent reputation in the area of clinical trials for malaria vaccines and antimalarial drugs. One of NIMR's well-established research laboratories is located in Korogwe next to the KTCH. The laboratory, built in 2007, includes a clinical chemistry section, parasitology section, real-time polymerase chain reaction (PCR) and microbiology units. The partner laboratory in Tanga, neighbouring the TRRH, is also equipped with a parasitology laboratory and PCR machine. Recently, a new microbiology laboratory has been established in Tanga. Bacterial identification for this study was conducted in the microbiology laboratories of the NIMR in Tanga and Korogwe.

Figure 3: The NIMR in Tanga town [3]

3 © Author

3.4. Study population

Tanzania's population is young with 46 % being under 15 years (18). Life expectancy at birth is 64 years for women and 60 years for men (141). Maternal and neonatal mortality is high with 556 and 20 deaths per 100 000 live births respectively (18). Only 64 % of deliveries are attended by a skilled health worker and nine out of ten women do not have health insurance (18). The total fertility rate amounts to 5.2 children per woman and the median age at first delivery is 19.8 years (18). In the Tanga Region, 20 % of women have experienced genital cutting (142). Over the past years, Tanzania has substantially reduced child mortality. However, this mainly concerns children above the age of one month and neonatal mortality has not been brought down (18). Each year, at least 51 000 Tanzanian neonates die, which accounts for one-third of deaths of children under five years (34). Overall, neonatal disorders are the most common cause of death in the Tanzanian population (141).

3.5. Sample size calculation

The goal of the following sample size calculation was to recruit sufficient numbers of mothers and neonates, who were positive for the studied bacteria, to conduct further analysis of the bacteria. We estimated the sample size based on an example prevalence of colonisation with MDR bacteria in neonates of 10 % and in mothers of 20 % (44, 74, 143–146). An average of 500 neonates are born at TRRH and 150 at KTCH each month and we aimed to recruit a total of 500 to 600 neonates within six months. Based on the outlined assumptions, approximately 50 to 60 neonates were expected to be positive for the bacteria under investigation, as well as 100 to 120 mothers. The prevalence of carriage acquisition by neonates during their hospital stay ranges between 50 to 60 % in literature published from eastern SSA (44, 123, 147).

In addition, we decided to collect similar numbers of samples from hospital surfaces and staff. Thus, 30 (18 surface and twelve staff) samples were planned to be taken each week at TRRH and 15 (nine surface and six staff) at KTCH, as this is a smaller hospital.

3.6. Study procedures

3.6.1 Recruitment of mothers

Adult women admitted for delivery to the study hospitals (TRRH and KTCH) were asked for potential participation in this study. The procedure of informed consent took place in the admission cubicles of the respective labour wards, where the

privacy of the mother was ensured. An information sheet and the informed consent form (ICF) was handed out to each mother. Additionally, the study procedures and aims were explained by a trained study nurse. Further, it was explained to the mother that none-participation had no effect on any care provided by the hospital. After the mother had enough time to ask questions and consider her decision, she was asked to consent herself and her unborn child into the study by signing the ICF. In the case of illiteracy, a thumbprint was accepted. In the latter case, an independent witness had to provide his or her signature. It is important to note, that it was not possible for the mother to consent only herself or only her child. By signing, both were permitted to be included in the study.

3.6.1.1. Inclusion criteria of mothers

- women admitted to the labour ward to give birth
- age ≥ 18 years
- term of pregnancy ≥ 34 weeks
- consent given

3.6.1.2. Exclusion criteria mothers

- participant not willing to provide a nasal/ perineorectal sample
- participant not able to comprehend the purpose, background and risks of the study (e.g., due to severe labour pain)

3.6.2 Sample and data collection of mothers

Once a mother was included in the study, she was assigned an individual Study Identifier (ID) to ensure anonymisation. This Study ID included the letter “M” for mother (T22-MXXXX-XR) to differentiate it from IDs for neonates. All Study IDs were pre-printed on stickers with matching barcodes. Before delivery and, if possible before the first pelvic examination performed in the hospital, one nasal and one perineorectal swab sample were collected from the mother. The samples were collected using sterile cotton swabs in Amies liquid transport medium (eSwab®, Copan, Italy). They were taken in a uniform and aseptic manner according to the study-specific procedure (SOP) “Sampling of mothers and their neonates” (Appendix). Stickers with the mother’s individual ID were used to label the swab samples and corresponding laboratory forms. These were brought to the laboratory within a maximum of two hours.

Before or just after sample collection, personal information was requested from the mother. She was asked about her medical history, current pregnancy and antibiotic intake. In addition, the antenatal card, which includes the gestational age, number of antenatal care visits and HIV status, was a source of information. The data was captured using the data collection software Research Electronic Data Capture (REDCap), whose offline application (App) version was installed on the study tablets. The study's database was opened in the App and the study arm "Mother" was selected. A new record for a specific mother was created by scanning the barcode on the mother's ID. Clicking on the mother's ID, the electronic Case Report Form (CRF) "Mother Recruitment" was offered and data was entered. The CRF included mostly multiple choice and a few free-text questions, to enable both comparative and descriptive analysis (Appendix).

3.6.3 Recruitment of neonates

Neonates, which were born in one of the study hospitals and whose mothers consented to the study, were recruited for sample collection. The recruitment took place as soon as possible after birth. A neonate born during the day was recruited immediately after birth, while a neonate born at night was recruited the next morning (maximum 16 hours after birth). If all of the inclusion and none of the exclusion criteria applied, the neonate was enrolled in the study. In the case of twins or triplets, only the first-born was included. Once a neonate was enrolled, he or she was assigned an individual Study ID including the letter "I" for "Inborn-Neonate" (T22-IXXXX-XX). For each participating neonate there was a sheet of stickers with an individual Study ID and barcode pre-printed. The ID on some of these stickers ended with the letter "R" for recruitment and on others with the letter "D" for discharge to differentiate the time points of swab sampling.

3.6.3.1. Inclusion criteria of neonates

- consent given by the mother

3.6.3.2. Exclusion criteria of neonates

- stillbirth
- severe congenital malformation

3.6.4 Sample and data collection of neonates

3.6.4.1. Sample and data collection at recruitment

One nasal and one perineorectal sample were collected from neonates at the time of recruitment after birth. For each sample, a sterile cotton swab (eSwab®, Copan) was used. Stickers with the neonate's individual Study ID, ending on the letter "R" for recruitment, were used to label the swab samples and laboratory forms. In addition, a physical examination was conducted evaluating the neonates breathing, skin colour, movements and umbilicus, among other features. In the REDCap App on the tablet, a record was created for the neonate in the same way as described for mothers. In this case, the study arm "Neonate" was selected and the electronic CRF called "Recruitment Inborn Neonate" was completed (Appendix).

3.6.4.2. Sample and data collection at discharge

In order to identify potential hospital acquisition of bacteria, neonates, who stayed for 48 or more hours in the hospital, were sampled again at discharge. One nasal and one perineorectal sample was taken in the same manner as at the time of recruitment. The discharge swab samples were labelled with Study ID stickers ending on "D" to differentiate them from the samples taken at recruitment. The "Discharge Inborn Neonate" CRF was entered under the neonate's Study ID in the REDCap App. This form was completed with data concerning the neonate's hospital stay, antibiotic intake and diagnosis (Appendix). Neonates which were discharged within 48 hours after birth were not sampled again and no discharge CRF was filled.

3.6.4.3. End of study form

For all neonates, who were enrolled in this study, an "End of Study" CRF was completed in the REDCap browser on a computer (Appendix). This CRF had to be filled at the end of the neonate's stay in the hospital, regardless of how long the neonate was hospitalized. This form aimed to summarize the neonate's stay in the hospital and to verify the uploaded forms.

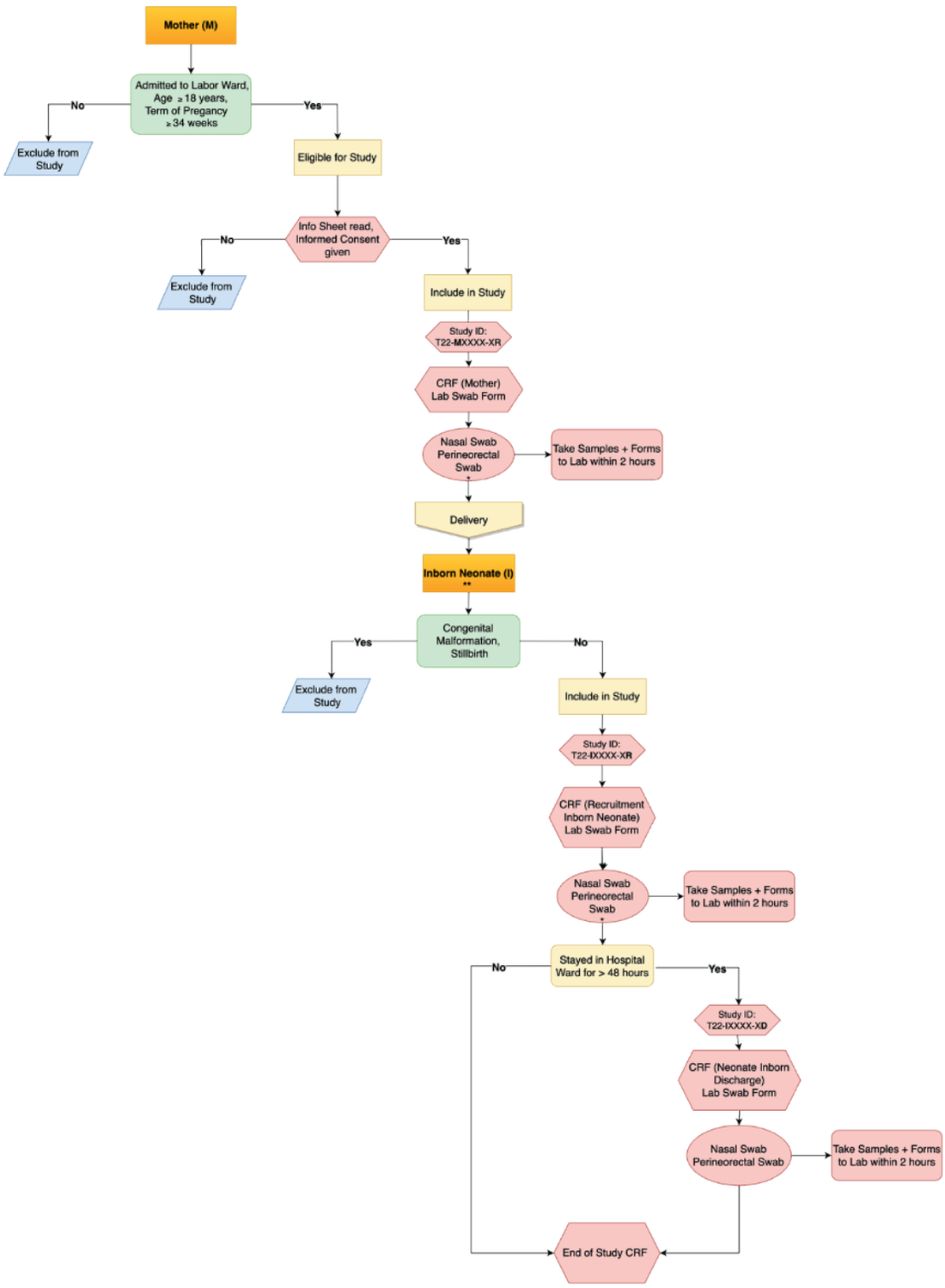

Figure 4: Study flow for the recruitment and sampling of mothers and neonates [4]

4 © Author

3.6.5 Sample collection of hospital staff and surfaces

Surfaces and health staff of the labour and neonatal wards were sampled once per week in TRRH and KTCH, according to the SOP “Sampling of hospital staff and surfaces” (Appendix). The lists of surfaces (e.g., bedside rails) to sample in each ward, as well as lists of health staff roles and localisation of sampling (e.g., Neonatal Ward - Doctor’s hands) were established prior to the start of the study. These lists were randomized using Microsoft Excel. All surfaces and roles of hospital staff had the same chance to be selected for sampling, given a predefined frequency (once a week) and quantity (e.g., eight samples per day). In TRRH 30 different samples of surfaces and staff were taken each week. While in KTCH 15 samples were taken per week, as it is a smaller hospital. Details on all possible sample origins are given in the Appendix.

The hospital staff members were asked to consent to sampling at the start of the recruitment period. Sampling of hospital staff was voluntary and anonymous. Their hands, noses and personal or medical items (e.g., stethoscope, phone or pen) were possible subjects of swab sampling. At each time point, there was only a single sample collected from an individual e.g., nose sample, hand sample or personal item sample. The staff members were not informed before sampling and the same participant could be sampled multiple times during the study period. Sterile cotton swabs in Amies liquid transport medium (eSwab®, Copan) were used for the nose samples. Whereas, for hand, personal and medical item samples sterile cotton swabs containing Amies gel (Amies Swabs, Sarstedt, Germany) were utilized. To ensure anonymisation, the samples were labelled with a Study ID including the letter “S” for staff (T22-SXXXX-XR).

Surfaces for sampling in the labour and neonatal wards of TRRH and KTCH were unknown to the hospital staff. This was done to avoid bias through a change of cleaning procedures. The surfaces included for example bedside rails, incubators and computer keyboards. In the case of surfaces which were multiple (e.g., bedside rails), it was upon the sample collector to choose and change the individual surface during the period of sampling. Amies swabs (Amies Swabs, Sarstedt) were used for the sample collection and the samples were labelled with an ID including the letter “E” for environment (T22-EXXXX-XR).

All samples were taken directly to the laboratories for further analysis.

Figure 5: Examples of surface sampling areas. [5]

5 © Author

3.6.6 Sample processing and identification of bacteria

Processing of swab samples was performed in the microbiology laboratories of the NIMR in Tanga and Korogwe. It involved the usage of standard and chromogenic culture media and biochemical tests for bacteria identification. We screened for GNB in perineorectal samples taken from mothers and neonates; hand and medical or personal item samples taken from hospital staff and in all surface samples. Further, we screened for *S. aureus* in nose samples taken from mothers and neonates; hand, medical or personal item and nose samples of hospital staff and all surface samples.

3.6.6.1. Enrichment of samples (day one)

In the afternoon of the day of sample collection, one millilitre of brain heart infusion broth (OXOID, United Kingdom) was added to each sample. The sample tubes were incubated at 35-37°C for 18-24 hours in normal atmosphere.

3.6.6.2. Inoculation on different culture media (day two)

After incubation, the content of the sample tubes was streaked onto different agar plates, depending on the type of sample. This was done using a one-microlitre inoculation loop (Sarstedt). Nose samples were plated onto Columbia blood agar (OXOID) with a Colistin supplement. Perineorectal samples were plated onto Mac-Conkey Agar, ESBL selective chromogenic Agar (CHROMagar™ Paris, France) and MDR *A. baumannii* selective Agar (CHROMagar™). Environmental, hand and device samples were plated onto all five types of agars used. Hereafter, agar plates were incubated at 35–37 °C for 18–24 hours under aerobic conditions.

3.6.6.3. Identification of bacteria (day three)

3.6.6.3.1. Identification of ESBL *E. coli and* ESBL *K. pneumoniae*

Screening for ESBL *E. coli* and ESBL *K. pneumoniae* was performed using ESBL selective chromogenic Agar (CHROMagar™). In the case of dark pink colonies, ESBL *E. coli* was suspected and confirmed using Indole and Oxidase Tests. If Indole positive and Oxidase negative, the bacterium was considered to be ESBL *E. coli*. In the case of metallic blue colonies, the Sulfur-Indole-Motility (SIM) medium test was conducted to differentiate *Klebsiella* species from *Enterobacter* and *Citrobacter* species. For this test, a colony was injected into the SIM medium by using a straight needle and incubated for 24 hours. Klebsiella species appear non-motile, while *Entero-* and *Citrobacter* species are motile in the mentioned test. If the growth

of bacteria was restricted to the stab line and the surrounding medium was left clear, it was considered non-motile. In this case the analytical profile index for Enterobacteriaceae (API-20 E, bioMérieux SA, Marcy l'Etoile, France) system was used to confirm ESBL *K. pneumoniae*.

Figure 6: SIM medium test [6]

3.6.6.3.2. Identification of P. aeruginosa

MacConkey Agar (OXOID) was used for the screening of *P. aeruginosa*. This pathogen was suspected in case of green-brown and fluorescent growth. The analytical profile index for Non-Enterobacteriaceae (API-20 NE, bioMérieux) system was used to confirm *P. aeruginosa*.

3.6.6.3.3. Identification of MDR A. baumanni

Screening for MDR *A. baumanni* was performed using MDR *A.* selective chromogenic Agar (CHROMagar™) with MDR supplement (CHROMagar™). In the case of pinkish colonies, pure plating was performed on blood agar and conformation was done using the API-20 NE (bioMérieux) system.

3.6.6.3.4. Identification of S. aureus

6 © Author

Screening for *S. aureus* was conducted using Columbia blood agar (OXOID) supplemented with Colistin (to suppress the growth of GNB). In the case of observed ß-haemolysis and typical morphology, purity plating was performed on blood agar (OXIOD) and Coagulase, Catalase and *S. aureus* agglutination tests were carried out. If all were positive, the bacteria were considered to be *S. aureus.* For further confirmation, a Gram stain was conducted.

3.6.6.4. Cryopreservation

For cryopreservation, the bacterial strains were taken from blood agar after purity confirmation. Bacteria were preserved in cryovials and stored at minus 80 °C. In the future, bacterial confirmation, susceptibly testing and genotyping are possible to be performed.

3.6.7 Data entry and management

The clinical, demographic and laboratory data were collected and managed using REDCap electronic data capture tools. This included online data capture via the browser and offline data capture via the REDCap mobile App, as a stable internet connection was not ensured at all times. To collect data offline study tablets (Archos, France), with the REDCap mobile App installed and access to the project CRFs, were used. The offline data was then uploaded to the REDCap server. After all tablets had send their data to the server, the setup of each tablet was refreshed to guarantee each tablet had the latest database structure installed. The process of synchronising the data between the tablet and server took place at the end of each recruitment day.

The CRF called “End of Study Form” for neonates existed only in the REDCap online database. This form included a verification mechanism, for which an internet connection was necessary. Therefore, it had to be completed directly in the RedCap browser on a computer.

The laboratory data was collected using paper-based forms. After finalization of these forms, the data had then to be entered into the REDCap browser. To reduce transferring errors and improve the data quality, the laboratory entry was reviewed by a second person.

At the end of the study, all data collected in REDCap was exported to Stata Statistical Software 14 (StataCorp LP, USA) for analysis.

3.7. Statistical analysis

The data of mothers, neonates, hospital surfaces and staff, who had been enrolled in the study from the 27th of April until the 2nd of November 2022 was analysed. All analyses were conducted using Stata Statistical Software 14. Categorial variables were presented as frequencies with percentages. Continuous variables were described as medians and their corresponding interquartile ranges (IQRs). Statistical tests between dependent and independent variables were performed using Poisson regression. Hereby the Prevalence Ratio (PR) with the 95% confidence interval (CI) was calculated. P-values of less than 0.05 were considered statistically significant. There was only a single observation (birth weight) with a missing value and it was not excluded from the analysis, resulting in a different denominator. For the purpose of clarity, all numbers in the results section are written as digits. All statistical evaluation of the collected data was carried out by the author.

3.8. Ethical considerations

Ethical approval for this study was provided by the responsible ethics committees, namely the Tanzania Medical Research Coordinating Committee (Reference number: NIMR/HQ/R.8a/Vol.III/124) and the Ethikkommission der Ärztekammer Hamburg (Reference number: 2022-100771-BO-ff), Germany. All participating mothers were informed about the study's purpose and procedures. Written informed consent was obtained from the mothers for themselves and their neonates prior to study enrolment. Further, written informed consent was collected from hospital staff members, who participated in this study. All data was treated confidentially.

4. Results

4.1. Study participants

4.1.1 Mothers

A total of 583 women met the inclusion criteria and were enrolled in this study as shown in **Figure 7**. Of these, 334 (57.3%, n/N=334/583) were recruited at TRRH and 249 (42.7%, n/N=249/583) at KTCH. Overall, the median age of participating mothers was 27 (IQR: 22-32) years. At study site level, the median age was 27 (IQR: 22–32) years for TRRH and 26 (IQR: 22–32) years for KTCH. At both study sites, the minimum age was 18 and the maximum 45 years (**Table 1**).

The overall median number of pregnancies, including the current one, was 2 (IQR: 1-3). This was equivalent when considered at study site level. Overall, the minimum number of pregnancies was 2 and the maximum was 9. A total of 212 (36.4%, n/N=212/583) women were primigravidae and 133 (22.8%, n/N=133/583) had their fourth or a higher number of pregnancy (multiparae). At TRRH, 129 (38.6%, n/N=129/334) mothers were primigravidae and 77 (23.1%, n/N=77/334) multiparae. There were slightly less primigravidae and multiparous women at KTCH (33.3%, n/N=83/249 primigravidae and 22.5%, n/N=56/249 multiparae). In total, an ultrasound test had been undertaken by 461 (79.1%, n/N=461/583) women (**Table 1**).

Overall, 38 (6.5%, n/N=38/583) women reported to suffer from an underlying medical condition, whereby HIV was the most frequently reported (2.4%, n/N=14/583). Women recruited at KTCH were 3.4 (95% CI 1.1-10.7, p=0.04) times as likely to have HIV, compared to women recruited at TRRH. Further, 58 (9.9%, n/N=58/583) women stated, that they had been hospitalised during the current pregnancy. A total of 287 (49.2%, n/N=287/583) women had received antibiotic treatment during the pregnancy). In sum, the most frequently administered antibiotic was Amoxicillin, which had been taken by 201 (34.5%, n/N=201/583) women (**Table 1**).

Of the 583 women, who were enrolled in this study, 545 (93.5%, n/N=545/583) delivered a child at TRRH and KTCH during the course of the study. Thirty-eight (6.5%, n/N=38/583) mothers either left the hospital before delivery or delivered after the end of our study period. Regarding the mothers, who delivered during this study, 47 (8.6%, n/N=47/545) experienced premature rupture of membranes (PROM). Seventeen (3.1%, n/N=17/545) reported foul-smelling amniotic fluid and

5 (0.9%, n/N=5/545) had maternal pyrexia (> 38°C) under delivery. In total, 530 mothers (97.2%, n/N=530/545) gave birth to at least 1 live neonate and 15 (2.8%, n/N=15/545) delivered a stillborn baby (**Table 1**).

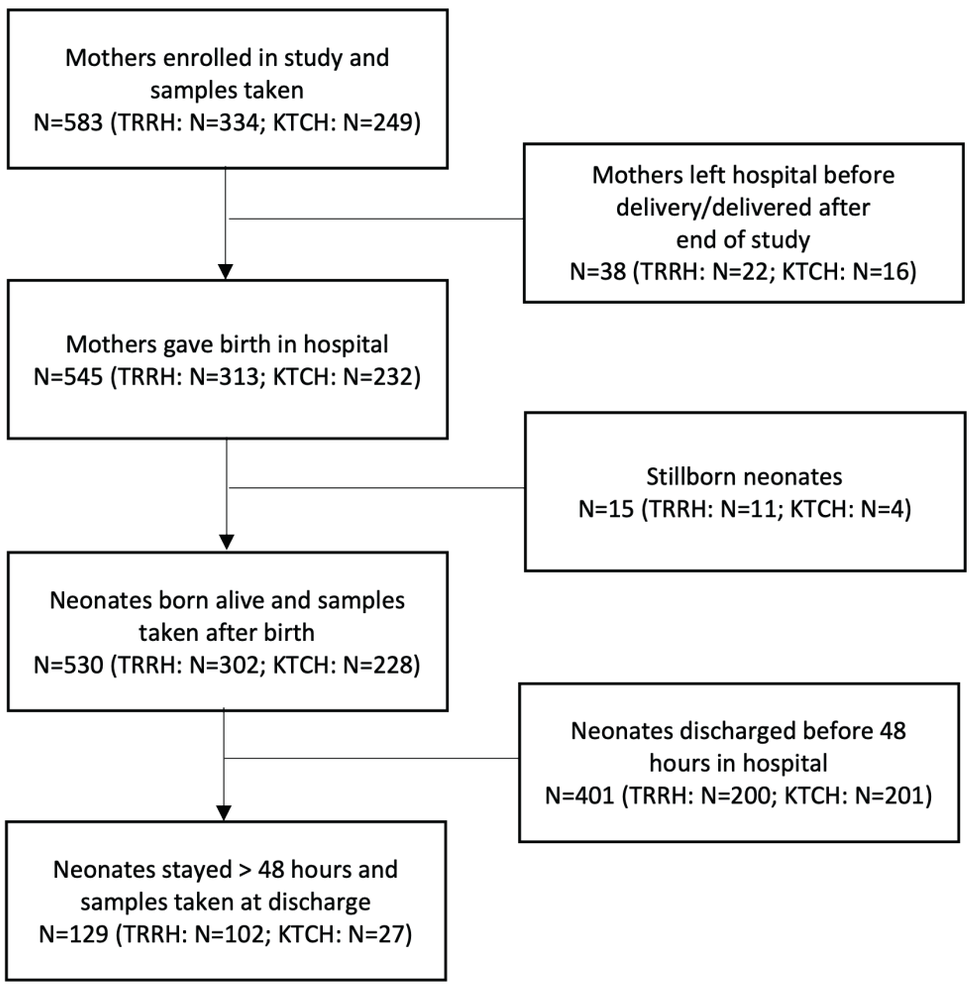

Figure 7: Cohort chart

Table 1: Characteristics of mothers

	Total		TRRH		KTCH	
	Number*	%	Number*	%	Number*	%
Age (years)						
- Median (IQR)	27 (22-32)		27 (22-32)		26 (22-32)	
- Range	18-45		18-45		18-45	
Number of pregnancies						
- Median (IQR)	2 (1-3)		2 (1-3)		2 (1-3)	
- Range	1-9		1-9		1-8	
- Primigravidae	212/583	36.4	129/334	38.6	83/249	33.3
- four or more pregnancies	133/583	22.8	77/334	23.1	56/249	22.5
Underlying medical condition	38/583	6.5	21/334	6.3	17/249	6.8
HIV	14/583	2.4	4/334	1.2	10/249	4.0
Hospital stay during pregnancy	58/583	9.9	38/334	11.4	20/249	8.0
Antibiotics during pregnancy	287/583	49.2	153/334	45.8	134/249	53.8
Amoxicillin	201/583	34.5	104/334	31.1	97/249	39.0
USG performed	461/583	79.1	283/334	84.7	178/249	71.5
Delivered during this stay: no/not yet	38/583	6.5	22/334	6.6	16/249	6.4
Delivered during this stay: yes	545/583	93.5	313/334	93.7	232/249	93.2
- PROM	47/545	8.6	40/313	12.8	7/232	3.0
- Foul amniotic fluid	17/545	3.1	12/313	3.8	5/232	2.2
- Maternal Pyrexia (> 38°C)	5/545	0.9	4/313	1.3	1/232	0.4
- Alive neonate	530/545	97.2	302/313	96.5	228/232	98.3
- Stillborn neonate	15/545	2.8	11/313	3.5	4/232	1.7

* Data are n/N, median or range

4.1.2 Neonates

A total of 530 neonates were enrolled in this study, as shown in **Figure 7**. Of these, 302 (57.0%, n/N=302/530) were born at TRRH and 228 (43.0%, n/N=228/530) at KTCH. Approximately half (50.2%, n/N=266/530) of the enrolled neonates were females. A total of 206 (38.9%, n/N=206/530) neonates were born by CS and 324 (61.1%, n/N=324/530) by spontaneous vaginal delivery (SVD). The relative frequency of CS was slightly higher at TRRH than at KTCH (41.1%, n/N= 124/302 and 36.0%, n/N= 82/228 respectively). Twenty-one (4.0%, n/N=21/530) of the enrolled neonates were the firstborn of twins, whereby the secondborn was not included

in this study. Out of all enrolled neonates, 38 (7.2%, n/N=38/529) were considered to have low-birth-weight (< 2500 grams). Low-birth -weight was more common in TRRH (8.3%, n/N=25/302) than in KTCH (5.7%, n/N=13/227). Overall, only 2 (0.4%, n/N=2/530) neonates had high body temperature (> 37.5°C) after birth and these were enrolled at KTCH. Fifteen neonates (2.8%, n/N=15/530) were suspected to have sepsis during the hospital stay (**Table 2**).

In total, 129 (24.3%, n/N=129/530) neonates stayed in the hospital for 48 or more hours. Prolonged stay was more common at TRRH (33.8%, n/N=102/302) than at KTCH (11.8%, n/N=27/228). However, at KTCH more neonates, who stayed for at least 48 hours, received antibiotic treatment (51.9%, n/N=14/27), than at TRRH (8.8%, n/N=9/102). Overall, the main reason for staying 48 or more hours in the hospital was the need for observation of the mother after CS (75.2%, n/N=97/129). Prolonged stay, due to observation of the mother, was predominant at TRRH (87.3%, n/N=89/102) and less frequent at KTCH (29.6%, n/N=8/27). The main neonatal factor for prolonged stay at TRRH was low birth weight (6.9%, n/N=7/102), whereas at KTCH neonatal respiratory distress was common (29.6%, n/N=8/27) (**Table 2**).

Table 2: Characteristics of neonates

	Total		TRRH		KTCH	
	Number*	%	Number*	%	Number*	%
Female	266/530	50.2	151/302	50.0	115/228	50.4
Male	264/530	49.8	151/302	50.0	113/228	49.6
Twins	21/530	4.0	14/302	4.6	7/228	3.1
CS	206/530	38.9	124/302	41.1	82/228	36.0
SVD	324/530	61.1	178/302	58.9	146/228	64.0
Birth Weight > 2500g	492/529	93.0	277/302	91.7	215/227	94.7
Birth Weight < 2500g	38/529	7.2	25/302	8.3	13/227	5.7
Temp > 37.5°C	2/530	0.4	0/302	0.0	2/228	0.9
Temp < 37.5°C	528/530	99.6	302/302	100.0	226/228	99.1
Suspected Sepsis	15/530	2.8	6/302	2.0	9/228	3.9
Stayed > 48h	129/530	24.3	102/302	33.8	27/228	11.8
- received antibiotic treatment	23/129	17.8	9/102	8.8	14/27	51.9
- stayed due to observation of mother	97/129	75.2	89/102	87.3	8/27	29.6
- stayed due to LBW	9/129	7.0	7/102	6.9	2/27	7.4
- stayed due to respiratory distress	8/129	6.2	0/102	0.0	8/27	29.6

***** Data are n/N or median, mean and range
N-values may vary because of missing values

4.1.3 Hospital staff

During the course of the study, 435 samples of hospital staff were taken at the two study sites. Of these, 294 (67.6%, n/N=294/435) were collected at TRRH and 141 (32.4%, n/N=141/435) at KTCH. At TRRH, 150 (51.0%, n/N=150/294) samples were taken from staff of the neonatal ward and 144 (49.0%, n/N=144/294) from staff of the labour ward. This included 94 (32.0%, n/N=94/294) samples of doctors, 105 (35.7%, n/N=105/294) samples of nurses and 95 (32.3%, n/N=95/294) samples of intern doctors. From the staff members, 79 (26.9%, n/N=79/294) nose samples, 106 (36.1%, n/N=106/294) hand samples and 109 (37.1%, n/N=109/294) device samples were taken (**Table 3**).

At KTCH, 70 (49.6%, n/N=70/141) samples were collected from the staff of the neonatal ward and 71 (50.4%, n/N=71/141) from the staff of the labour ward. At this hospital 68 (48.2%, n/N=68/141) samples of doctors and 73 (51.8%, n/N=73/141) samples of nurses were taken. This included 32 (22.7%, n/N=32/141) nose samples, 56 (39.7%, n/N=56/141) hand samples and 53 (37.6%, n/N=53/141) device samples (**Table 3**). There were no intern doctors working in KTCH. All of the sampled staff members were in regular and close contact with mothers and neonates. The same staff member could be sampled multiple times during the study period. As the sampling was anonymous, we cannot say how many staff members were tested more than once.

Table 3: Characteristics of hospital staff

	Total		TRRH		KTCH	
	Number*	%	Number*	%	Number*	%
Neonatal ward	220/435	50.6	150/294	51.0	70/141	49.6
Labour ward	215/435	49.4	144/294	49.0	71/141	50.4
Doctors	162/435	37.2	94/294	32.0	68/141	48.2
Nurses	178/435	40.9	105/294	35.7	73/141	51.8
Intern doctors	95/435	21.8	95/294	32.3	0/141	0.0
Nose samples	111/435	25.5	79/294	26.9	32/141	22.7
Hand samples	162/435	37.2	106/294	36.1	56/141	39.7
Device samples**	162/435	37.2	109/294	37.1	53/141	37.6

***** Data are n/N
** personal or medical device

4.1.4 Hospital surfaces

Overall, 647 samples of hospital surfaces were collected during this study. Details on all sampling origins are given in the Appendix.

In TRRH, 432 (66.8%, n/N=432/647) samples were taken. Approximately half (50.2%, n/N=217/432) of samples were taken from surfaces of the neonatal ward and the other half (49.8%, n/N=215/432) from surfaces of the labour ward. In each ward, there were 12 possible origins for sampling. Generally, all of the sampling origins were chosen because of their proximity to mothers and neonates in the wards.

At KTCH, 215 (33.2%, n/N=215/647) samples were taken. Of these, 33.5% (n/N=72/215) were collected from surfaces of the neonatal ward and 66.5%

(n/N=143/215) from surfaces of the labour ward. In this hospital, there were only 5 possible sampling origins in the neonatal ward, as this ward is very small (only 4 beds). In the labour ward, there were 9 possible sampling origins (Appendix).

4.2. Microbiological culture results

4.2.1 Mothers

Of 583 mothers sampled during this study, 369 (63.3%, n/N=369/583) were tested positive for ESBL *E. coli*, 140 (24.0%, n/N=140/583) for *S. aureus,* 121 (20.8%, n/N=121/583) for ESBL *K. pneumoniae*, 2 (0.3%, n/N=2/583) for *P. aeruginosa* and 1 (0.2%, n/N=1/583) for MDR *A. baumannii.* It must be considered, that the same mother could be colonised with multiple bacteria. In fact, 332 (56.9%, n/N=332/583) mothers were positive for 1 of the tested bacteria, 127 (21.8%, n/N=127/583) for 2 bacteria and 15 (2.6%, n/N=15/583) for 3 bacteria. Only 109 (18.7%, n/N=109/583) mothers were not colonised with any of the studied bacteria.

Figure 8 shows the colonisation frequencies for mothers on a study site level. When comparing the study sites, the colonisation of mothers was similar for GNB. At both sites ESBL *E. coli* was the most frequently collected GNB, followed by ESBL *K. pneumoniae*. In TRRH very few and in KTCH no isolates of MDR *A. baumannii* and *P. aeruginosa* were found. Concerning *S. aureus*, mothers at TRRH were 1.7 (95% CI 1.3-2.4, p<0.001) times as likely to be colonised, compared to mothers at KTCH.

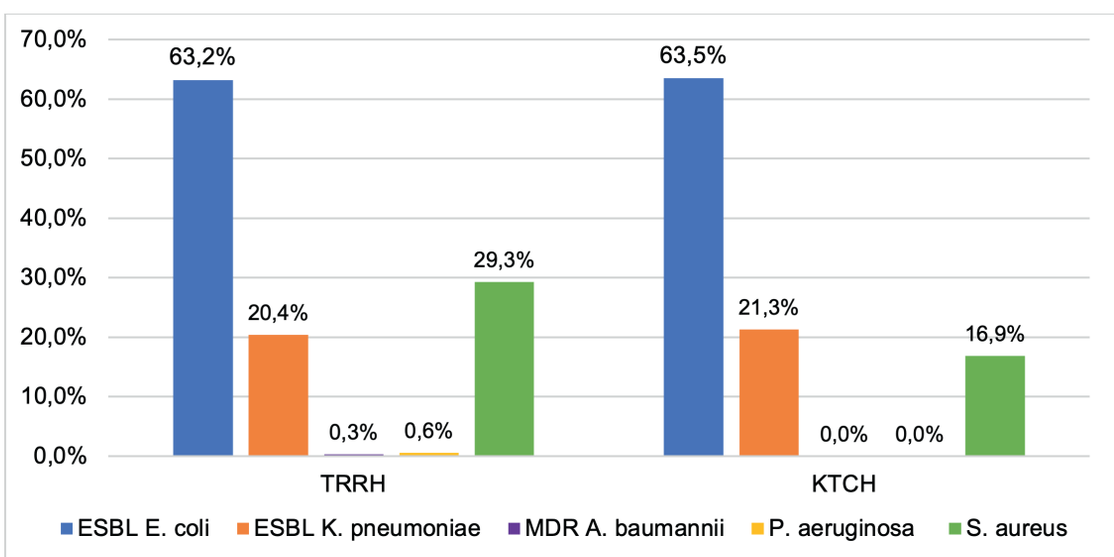

Figure 8: Colonisation frequencies of mothers at TRRH and KTCH

4.2.2 Neonates

4.2.2.1. Colonisation frequencies at recruitment

Of 530 neonates sampled at recruitment, 76 (14.3%, n/N=76/530) were tested positive for *S. aureus*, 54 (10.2%, n/N=54/530) for ESBL *E. coli*, 18 (3.4%, n/N=18/530) for ESBL *K. pneumoniae,* 13 (2.5%, n/N=13/530) for MDR *A. baumannii* and 3 (0.6%, n/N=3/530) for *P. aeruginosa.* Single colonisation occurred in 139 (26.2%, n/N=139/530) cases, 11 (2.1%, n/N=11/530) neonates were colonised with 2 bacteria and 1 (0.2%, n/N=1/530) neonate with 3 bacteria. The majority of neonates were not colonised with the tested bacteria at recruitment (71.5%, n/N=379/530).

Colonisation frequencies on a study site level are shown in **Figure 9**. As for mothers, we found similar colonisation rates for GNB at the hospitals. However, the colonisation with *S. aureus* differed between the study sites. It was 1.6 (95% CI 1.0-2.6, p=0.03) times as likely to be colonised with *S. aureus* at TRRH compared to KTCH.

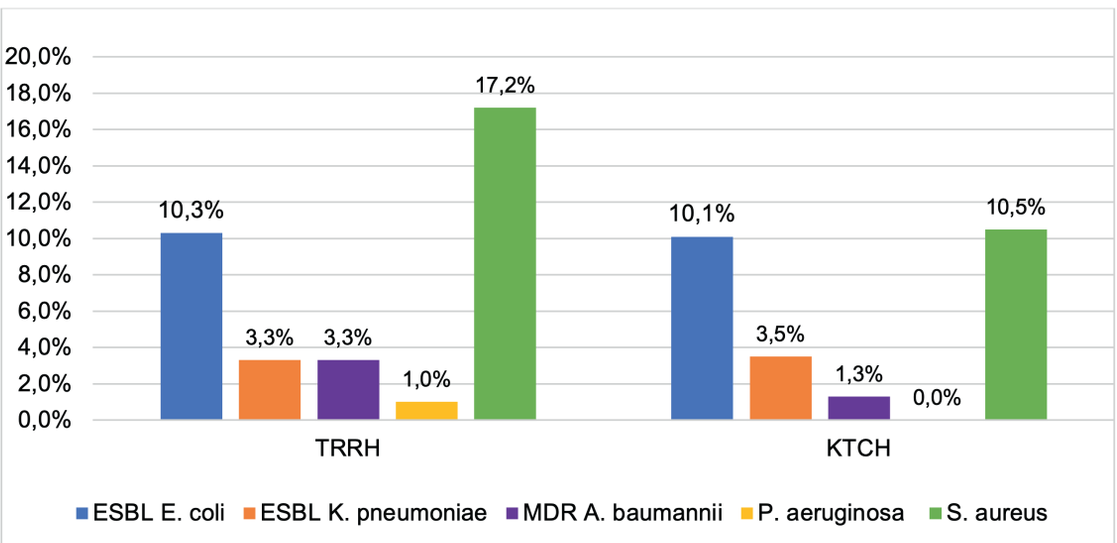

Figure 9: Colonisation frequencies at recruitment in TRRH and KTCH

4.2.2.2. Colonisation frequencies at discharge

Out of the 530 neonates sampled at recruitment, 129 (24.3%, n/N=129/530) stayed for at least 48 hours in the hospital and were therefore sampled again at discharge. The colonisation frequencies at recruitment and discharge of these 129 neonates are displayed in **Table 4**.

Overall, colonisation was highly increased at discharge for all bacteria, except *P. aeruginosa*. The colonisation frequencies were 14.5 (95% CI 3.5-59.5, p<0.001), 3.0 (95% CI 1.2-7.3, p=0.01), 1.9 (95% CI 1.2-2.9, p=0.003) and 2.1 (95% CI 1.1–4.1, p=0.02) times higher at discharge, compared to recruitment for ESBL *K. pneumoniae,* MDR *A. baumannii*, *S. aureus* and ESBL *E. coli* respectively. No *P. aeruginosa* was isolated at discharge. Details on a study site level are displayed in **Table 4**.

Further, dual and multiple carriage was increased. Single colonisation occurred in 47 (36.4%, n/N=47/129) cases, dual colonisation in 28 (21.7%, n/N=28/129) and 4 (3.1%, n/N=4/129) neonates were colonised with 3 bacteria. Unlike after birth, only 50 (38.8%, n/N=50/129) neonates were not colonised with any of the tested bacteria (**Figure 10**).

Of those neonates, who were negative at recruitment, 31.4% (n/N=33/105) acquired *S. aureus*, 22.0% (n/N=28/127) ESBL *K. pneumoniae,* 12.7% (n/N=15/118) ESBL *E. coli* and 9.8% (n/N=12/123) MDR *A. baumannii* and were thus positive at discharge. None of these neonates acquired *P. aeruginosa.* Details on a study site level are displayed in **Figure 11.**

Table 4: Colonisation frequencies at recruitment and discharge

	Recruitment		**Discharge**		
	Number*	%	Number*	%	PR (95% CI)
Total					
ESBL *E. coli*	11/129	8.5	23/129	17.8	2.1 (1.1-4.1)
ESBL *K. pneumoniae*	2/129	1.6	29/129	22.5	14.5 (3.5-59.5)
MDR *A. baumannii*	6/129	4.7	18/129	14.0	3 (1.2-7.3)
P. aeruginosa	0/129	0.0	0/129	0.0	-
S. aureus	24/129	18.6	45/129	34.9	1.9 (1.2-2.9)
TRRH					
ESBL *E. coli*	8/102	7.8	17/102	16.7	2.1 (1.0-4.7)
ESBL *K. pneumoniae*	2/102	2.0	17/102	16.7	8.5 (2.0-35.8)
MDR *A. baumannii*	6/102	5.9	18/102	17.6	3 (1.2-7.2)
P. aeruginosa	0/102	0.0	0/102	0.0	-
S. aureus	21/102	20.6	36/102	35.3	1.7 (1.1-2.7)
KTCH					
ESBL *E. coli*	3/27	11.1	6/27	22.2	2.0 (0.6-7.1)
ESBL *K. pneumoniae*	0/27	0.0	12/27	44.4	-
MDR *A. baumannii*	0/27	0.0	0/27	0.0	-
P. aeruginosa	0/27	0.0	0/27	0.0	-
S. aureus	3/27	11.1	9/27	33.3	3.0 (0.9-9.9)

* Data are n/N

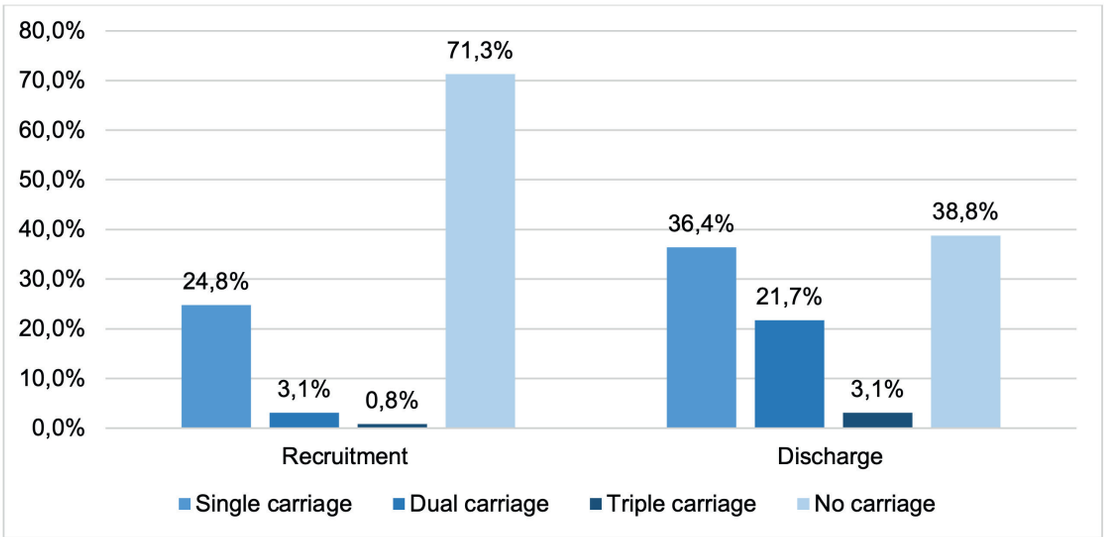

Figure 10: Bacterial carriage of the 129 neonates present at recruitment and discharge

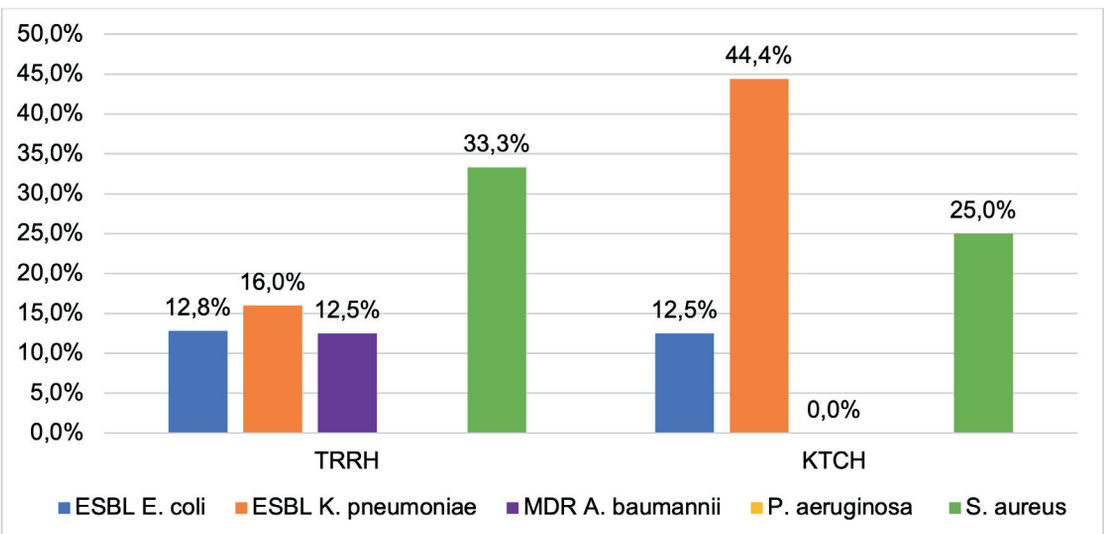

Figure 11: Bacterial acquisition of neonates negative at recruitment

4.2.2.3. Colonisation frequencies of neonates born by CS and SVD

Considering the sum of all tested bacteria, colonisation rates were similar for neonates born by CS and neonates born by SVD (33.0%, n/N=68/206 for CS and 29.6%, n/N=96/324 for SVD). However, this observation differs when analysing at species level. Neonates born by CS were 5.2 (95% CI 1.4-19.0, p=0.01) times as likely to have colonisation with MDR *A. baumannii* than neonates born by SVD. Further, neonates born by CS were 1.9 (95% CI 1.2-3.0, p=0.004) times as likely to be colonised with *S. aureus* than neonates born by SVD. On the other hand, neonates,

who were born by SVD were 3.2 (95% CI 1.6-6.5, p=0.002) times as likely to have colonisation with ESBL *E. coli* than neonates born by CS (**Table 5**).

Neonates born at TRRH by CS were 5.7 (95% CI 1.2-27.0, p=0.03) times as likely to have colonisation with MDR *A. baumannii* than neonates born by SVD. On the contrary, neonates born by SVD were 2.9 (95% CI 1.2-7.0, p=0.02) times as likely to be colonised with ESBL *E. coli* compared to neonates born by CS. For ESBL *K. pneumoniae* and *S. aureus* no statistical difference in colonisation could be observed between the groups. Concerning *P. aeruginosa,* negligible numbers were isolated in this hospital (**Table 5**).

At KTCH, neonate colonisation with *S. aureus* was 3.6 (95% CI 1.5-8.3, p=0.003) times as likely if the neonate was born by CS compared to SVD. Similar to TRRH, ESBL *E. coli* colonisation was 3.7 (95% CI 1.1-12.6, p=0.03) times as likely in neonates born by SVD compared to neonates born by CS. For ESBL *K. pneumoniae* and MDR *A. baumannii* no statistically significant difference between the two groups could be found, as colonisation rates were very low. There was no *P. aeruginosa* found on neonates in this hospital (**Table 5**).

Table 5: Colonisation frequencies of neonates born by CS and SVD

	CS (exposed cases)		**SVD (unexposed cases)**		
	Number*	%	Number*	%	PR (95% CI)
Total					
ESBL *E. coli*	9/206	4.4	45/324	13.9	0.3 (0.2-0.6)
ESBL *K. pneumoniae*	7/206	3.4	11/324	3.4	1 (0.4-2.6)
MDR *A. baumannii*	10/206	4.9	3/324	0.9	5.2 (1.4-19.0)
P. aeruginosa	0/206	0.0	3/324	0.9	-
S. aureus	42/206	20.4	34/324	10.5	1.9 (1.2-3.0)
TRRH					
ESBL *E. coli*	6/124	4.8	25/178	14.0	0.3 (0.1-0.8)
ESBL *K. pneumoniae*	2/124	1.6	8/178	4.5	0.4 (0.1-1.7)
MDR *A. baumannii*	8/124	6.5	2/178	1.1	5.7 (1.2-27.0)
P. aeruginosa	0/124	0.0	3/178	1.7	-
S. aureus	26/124	21.0	26/178	14.6	1.4 (0.8-2.5)
KTCH					
ESBL *E. coli*	3/82	3.7	20/146	13.7	0.3 (0.1-0.9)
ESBL *K. pneumoniae*	5/82	6.1	3/146	2.1	3.0 (0.7-12.4)
MDR *A. baumannii*	2/82	2.4	1/146	0.7	3.6 (0.3-39.3)
P. aeruginosa	0/82	0.0	0/146	0.0	-
S. aureus	16/82	19.5	8/146	5.5	3.6 (1.5-8.3)

* Data are n/N

4.2.2.4. Colonisation frequencies of neonates born by carrier and non-carrier mothers

Overall, neonate colonisation with *S. aureus* at recruitment was 3.3 (95% CI 2.1–5.0, p<0.001) times as likely if the mother carried *S. aureus* in the nose. For ESBL *E. coli* and ESBL *K. pneumoniae,* the difference between the groups was not statistically significant. Concerning MDR *A. baumannii* and *P. aeruginosa,* there was only 1 positive mother each and their children were not positive for the bacteria. Out of the neonates born by non-positive mothers, 13 (2.5%, n/N=13/529) were positive for MDR *A. baumannii* and 3 (0.6%, n/N=3/529) for *P. aeruginosa* (**Table 6**).

At TRRH, neonate colonisation with *S. aureus* after birth was 2.5 (95% CI 1.5–4.4, p=0.001) times as likely if the mother carried *S. aureus* in the nose herself. A similar observation could be made for ESBL *K. pneumoniae*, which was found 3.9 (95% CI 1.1–13.4, p=0.03) times more often in neonates, whose mothers carried the bacterium in her perianal area. For ESBL *E. coli* the difference between the two groups was not statistically significant. Further, for MDR *A. baumannii* and *P. aeruginosa* there was only 1 positive mother each and their children were not positive for the bacteria (**Table 6**).

At KTCH, neonate colonisation with *S. aureus* after birth was 4.5 (95% CI 2.3–8.8, p<0.001) times increased if the mother carried *S. aureus* in the nose. For ESBL *E. coli* and ESBL *K. pneumoniae* the difference between the groups was not statistically significant. Three (1.3%, n/N=3/228) neonates were positive for MDR *A. baumannii,* who were all born by MDR *A. baumannii* negative mothers. There was no *P. aeruginosa* found on neonates in this hospital (**Table 6**).

Table 6: Colonisation frequencies of neonates born by carrier and non-carrier mothers

	Mother + (exposed cases)		**Mother – (unexposed cases)**		
	Number*	%	Number*	%	PR (95% CI)
Total					
ESBL *E. coli*	40/336	11.9	14/194	7.2	1.6 (0.9-3.0)
ESBL *K. pneumoniae*	6/108	5.6	12/422	2.8	1.9 (0.7-5.2)
MDR *A. baumannii*	0/1	0.0	13/529	2.5	-
P. aeruginosa	0/1	0.0	3/529	0.6	-
S. aureus	40/126	31.7	36/404	8.9	3.3 (2.1-5.0)
TRRH					
ESBL *E. coli*	23/192	12.0	8/110	7.3	1.6 (0.7-3.7)
ESBL *K. pneumoniae*	5/62	8.1	5/240	2.1	3.9 (1.1-13.4)
MDR *A. baumannii*	0/1	0.0	10/301	3.3	-
P. aeruginosa	0/1	0.0	3/301	1.0	-
S. aureus	27/90	30.0	25/212	11.8	2.5 (1.5-4.4)
KTCH					
ESBL *E. coli*	17/144	11.8	6/84	7.1	1.6 (0.6-4.2)
ESBL *K. pneumoniae*	1/46	2.2	7/182	3.8	0.6 (0.1-4.6)
MDR *A. baumannii*	0/0	0.0	3/228	1.3	-
P. aeruginosa	0/0	0.0	0/228	0.0	-
S. aureus	13/36	36.1	11/192	5.7	4.5 (2.3-8.8)

* Data are n/N

4.2.3 Hospital staff

The cumulative frequency of the tested bacteria was high among samples taken from hospital staff (22.5%, n/N=98/435). The bacterium most frequently isolated was *S. aureus*, followed by MDR *A. baumannii*, ESBL *K. pneumoniae*, ESBL *E. coli* and *P. aeruginosa* (**Table 7**). In both hospitals, *S. aureus* was found in noses (27.0%, n/N=30/111), on hands (14.2%, n/N=23/162) and on personal devices (10.5%, n/N=17/162). GNB were found on hands (6.8%, n/N=11/162) and personal devices (10.5%, n/N=17/162) and were not screened for in the nose samples.

At TRRH, we found mainly *S. aureus* isolates (17.7%, n/N=52/294), followed by MDR *A. baumannii* (1.7%, n/N=5/294) and *K. pneumoniae* (1.4%, n/N=4/294). There were no ESBL *E. coli* and *P. aeruginosa* isolated from hospital staff in this hospital. The cumulative frequency of positive samples among all sampled staff members was 20.7% (n/N=61/294). The intern doctors were the group of hospital staff with the highest frequency of positive samples (25.3%, n/N=24/95). This was followed by doctors (20.2%, n/N=19/94) and nurses (17.1%, n/N=18/105).

At KTCH, the predominant isolate from hospital staff was also *S. aureus* (12.8%, n/N=18/141), followed by MDR *A. baumannii* (6.4%, n/N=9/141), ESBL *K. pneumoniae* (4.3%, n/N=6/141), ESBL *E. coli* (2.1%, n/N=3/141) and *P. aeruginosa* (0.7%, n/N=1/141). In this hospital, the cumulative frequency of positive samples among all sampled staff members was 26.2% (n/N=37/141). Here nurses had higher frequencies of culture confirmed bacteria (28.8%, n/N=21/73), compared to doctors (23.5%, n/N=16/68). See **Table 9** in the Appendix for further detailed information on a study site level.

Table 7: Colonisation frequencies of hospital staff

	ESBL *E. coli*		ESBL *K. pneumoniae*		MDR *A. baumannii*		*P. aeruginosa*		*S. aureus*	
	Number*	%	Number*	%	Number*	%	Number*	%	Number*	%
Total	3/435	0.7	10/435	2.3	14/435	3.2	1/435	0.2	70/435	16.1
Doctors	2/162	1.2	5/162	3.1	2/162	1.2	0/162	0.0	26/162	16.0
- Nose	-	-	-	-	-	-	-	-	10/37	38.5
- Hand	1/64	1.6	2/64	3.1	1/64	1.6	0/64	0.0	9/64	34.6
- Device**	1/61	1.6	3/61	4.9	1/61	1.6	0/61	0.0	7/61	26.9
Nurses	1/178	0.6	4/178	2.2	9/178	5.1	1/178	0.6	24/178	13.5
- Nose	-	-	-	-	-	-	-	-	11/47	6.2
- Hand	0/64	0.0	2/64	3.1	3/64	4.7	0/64	0.0	6/64	9.4
- Device**	1/67		2/67	3.0	6/67	9.0	1/67		7/67	10.4
Intern Doctors	0/95	0.0	1/95	1.1	3/95	3.2	0/95	0.0	20/95	21.1
- Nose	-	-	-	-	-	-	-	-	9/27	33.3
- Hand	0/34	0.0	0/34	0.0	2/34	5.9	0/34	0.0	8/34	23.5
- Device**	0/34	0.0	1/34	2.9	1/34	2.9	0/34	0.0	3/34	8.8

* Data are n/N
** personal or medical device

4.2.4 Hospital surfaces

In total, 647 surface samples were collected during the study period. Hereby 276 (42.7%, n/N=276/647) bacterial isolates were identified. The most frequently isolated bacterium was MDR *A. baumannii* (14.7%, n/N=95/647). This was followed by ESBL *K. pneumoniae* (10.2%, n/N=66/647), *S. aureus* (10.0%, n/N=65/647), ESBL *E. coli* (7.4%, n/N=48/647) and *P. aeruginosa* (0.3%, n/N=2/647) (**Table 8**). All of the 38 possible sampling surfaces at TRRH and KTCH were contaminated.

At TRRH, the most frequently isolated bacterium was MDR *A. baumannii* (12.3%, n/N=53/432). This was followed by *S. aureus* (10.4%, n/N=45/432), ESBL *K. pneumoniae* (6.7%, n/N=29/432) and ESBL *E. coli* (5.6%, n/N=24/432). There was no *P. aeruginosa* isolated from hospital surfaces at TRRH (**Table 8**).

At KTCH, the most frequently isolated bacterium was also MDR *A. baumannii* (19.5%, n/N=42/215). However, this was followed by ESBL *K. pneumoniae* (17.2%, n/N=37/215), ESBL *E. coli* (11.2%, n/N=24/215), *S. aureus* (9.3%, n/N=20/215) and *P. aeruginosa* (0.9%, n/N=2/215) (**Table 8**).

Comparing the two study sites, the relative frequency of bacterial isolates was 1.7 (95% CI 1.4-2.0, p<0.001) times higher in KTCH than in TRRH. The ward in which relatively the most bacterial isolates were found was the neonatal ward at KTCH (61.1%, n/N=44/72). From the labour ward at KTCH, slightly fewer samples were positive (56.6%, n/N=81/143). At TRRH, half of the samples taken from the neonatal ward were positive (50.2%, n/N=109/217). Noticeably less isolates were collected from the labour ward at TRRH (19.5%, n/N=42/215).

Table 8: Colonisation frequencies of hospital surfaces

	ESBL *E. coli*		**ESBL *K. pneumoniae***		**MDR *A. baumannii***		***P. aeruginosa***		***S. aureus***	
	Number*	%	Number*	%	Number*	%	Number*	%	Number*	%
Total	48/647	7.4	66/647	10.2	95/647	14.7	2/647	0.3	65/647	10.0
TRRH	24/432	5.6	29/432	6.7	53/432	12.3	0/432	0.0	45/432	10.4
Neonatal Ward	19/217	8.8	21/217	9.7	37/217	17.1	0/217	0.0	32/217	14.7
Labour Ward	5/215	2.3	8/215	3.7	16/215	7.4	0/215	0.0	13/215	6.0
KTCH	24/215	11.2	37/215	17.2	42/215	19.5	2/215	0.9	32/217	14.7
Neonatal Ward	4/72	5.6	17/72	23.6	12/72	16.7	1/72	1.4	10/72	13.9
Labour Ward	20/143	14.0	20/143	14.0	30/143	21.0	1/143	0.7	10/143	7.0

* Data are n/N

5. Discussion

In two hospitals in the Tanga Region, we analysed the colonisation of neonates, their mothers, hospital staff and surfaces with five different bacterial species (ESBL *E. coli,* ESBL *K. pneumoniae*, MDR *A. baumannii, P. aeruginosa* and *S. aureus*). Of 583 mothers sampled during this study, 81.3% were tested positive for at least one of the mentioned bacteria. Concerning neonates, 530 were sampled after birth and 129 again at discharge. Their colonisation increased from 28.7% to 61.2% during the hospital stay. Further, their colonisation was influenced by the mode of birth and the colonisation status of the mother. In addition, 42.7% of samples taken from hospital surfaces and 22.5% of samples taken from hospital staff were positive.

5.1. Interpretation of results

5.1.1 Colonisation of mothers

We found high colonisation rates in mothers with ESBL *E. coli* (63.3%) and ESBL *K. pneumoniae* (20.8%). A hospital-based study conducted in the Tanzanian city of Dar es Salaam in 2019, reported a similar colonisation rate (57.1%) for ESBL *E. coli* and a lower one (6.0%) for ESBL *K. pneumoniae* in a similar population (148). Other studies from the region reported cumulative results for ESBL-PE carriage and unlike our study did not differentiate between bacterial species. However, many reported *E. coli* to be the most commonly isolated pathogen from pregnant women (44, 144, 149). Two studies at a hospital in the Tanzanian city of Mwanza from 2013 and 2016 reported prevalence rates of 15% and 28,3% for maternal rectal ESBL-PE carriage (44, 74). These findings may indicate an increase in maternal ESBL-PE carriage over the past years in Tanzania. This could be explained by improved access to antibiotics, more frequent antibiotic treatment during pregnancy and more deliveries in hospitals.

However, ESBL-PE carriage in perinatal women varies between African countries. In South Africa, Nigeria and Madagascar, frequencies of 4.4%, 9.7% and 19.6% respectively were reported between 2015 and 2017 (144–146). A study from Cameroon reported a high ESBL *E. coli* prevalence (57.7%), similar to our study, however, these women had initially presented with suspected urinary tract infection, which could have influenced the results (150). Findings from a systematic review, including ten studies from SSA, showed an average colonisation frequency with ESBL-PE of 15% for perinatal women, being the highest worldwide (143, 151). The findings from our study sites are again high above this average. Differences may be due to local

variations in level of education, hygiene, and misuse of antibiotics (148). In addition, the method of sample collection (rectal vs. stool sample) and different time points of sampling (before vs. after delivery) could influence the results. The high colonisation frequency of mothers in our setting is alarming. Perineorectal colonisation can lead to faecal-vaginal transmission, which can result in urinary tract infection of the mother and colonisation of the neonate during SVD (44, 145, 150, 152, 153).

Further, the nasal colonisation frequency with *S. aureus* (24.0%) was high among mothers in our study. It was higher than the nasal colonisation frequency among general adult patients admitted to a hospital in Mwanza (154). Comparable information on pregnant women from Tanzania is lacking. In Gabon, a slightly higher colonisation rate (29.6%) was found among 311 mothers who were sampled after delivery (45). Whereby, a similar colonisation frequency was reported in a representative study from China, where 25.8% of pregnant women were colonised with *S. aureus* (155). Unlike our study, others included rectal or vaginal sampling of pregnant women for *S. aureus*, which tends to be lower than nasal carriage (149, 156). We did not perform resistance testing for *S. aureus* and can therefore not provide any information on frequencies of MRSA. However, according to a systematic review from 2022, the frequency of maternal MRSA colonisation in Africa ranges between 4.4% and 15.3% (157).

Overall, very few mothers were colonised with *P. aeruginosa* (0.3%) and MDR *A. baumannii* (0.2%). The few colonised mothers were sampled at TRRH, while at KTCH all mothers were negative. Comparable information on perineorectal colonisation of pregnant women is lacking. Slightly higher colonisation frequencies among vaginal samples from pregnant women for MDR *A. baumannii* were reported from Ethiopia (149). *P. aeruginosa* has been reported to cause urinary tract infections among pregnant women, however, results on gut colonisation are lacking (158, 159).

5.1.2 Colonisation of neonates

5.1.2.1. Colonisation at recruitment

Neonates sampled at recruitment were mainly colonised with *S. aureus* (14.3%). There is no comparable data on neonatal *S. aureus* colonisation from Tanzania. However, in Gabon, Schaumburg et al. also sampled neonates shortly after birth and reported a similar colonisation rate (16.4%) (45). Interestingly, baseline data generated within a six-month study at TRRH indicated that *S. aureus* was the most frequent pathogen isolated from blood cultures of neonates with suspected sepsis (unpublished data). Likewise, studies from Mwanza and Dar es Salaam reported

S. aureus to be among the most common pathogens to cause neonatal sepsis (160, 161). In a German study, neonatal *S. aureus* colonisation was significantly associated with *S. aureus* infection, suggesting that eradication of colonisation might be useful to prevent infection (105). As we did not perform resistance testing for *S. aureus,* we cannot report on frequencies of MRSA among neonates in our study. In a study in Ghana, 2% of children had nasal MRSA carriage (104). For neonates, MRSA carriage below 1% was reported from China, Brazil and Canada (155, 162, 163).

Further, neonates were colonised with ESBL *E. coli* (10.2%) and ESBL *K. pneumoniae* (3.4%). In a hospital in Mwanza, Nelson et al. found lower colonisation rates for ESBL *E. coli* (4.0%), however higher colonisation rates for ESBL *K. pneumoniae* (21.4%) among neonates on their first day of life (44). Lower cumulative ESBL-PE carriage shortly after delivery was reported for neonates from Nigeria (145). Marando et al. reported colonisation rates of 19.7% for ESBL *E. coli* and 36.8% for ESBL *K. pneumoniae* for neonates aged between zero and 28 days from Mwanza (74). Even higher colonisation rates were reported from Dar es Salaam, where 68.4% of infants between zero and three months had positive stool samples for ESBL-PE (164). The reported colonisation rates may differ due to different time points of sampling. We took samples as soon as possible after birth (maximum 16 hours), while most other studies sampled neonates admitted to hospitals in the first days or weeks after birth, finding higher colonisation frequencies (74, 164, 165).

The colonisation frequency with MDR *A. baumannii* (2.5%) was low, similar to the results from Mwanza (74). Nevertheless, this is an interesting finding, as it indicates that neonates may acquire bacteria from sources other than their mothers within the first hours of life. The only mother colonised with MDR *A. baumannii* delivered at TRRH and her child was negative for MDR *A. baumannii.* At the same time, there were neonates at both hospitals colonised with MDR *A. baumannii.* As MDR *A. baumannii* was the most common pathogen isolated from surfaces and the second most common from hospital staff, these might be possible sources of transmission to neonates. Further, a study from Morocco found an even higher gut colonisation frequency of 6.5% among hospitalised neonates (166). Several outbreaks of *A. baumannii* in neonatal units have been described (71, 72, 167), highlighting the importance of *A. baumannii* surveillance in neonatal units.

Negligible numbers of *P. aeruginosa* (0.6%) were isolated. However, *P. aeruginosa* is a known cause for outbreaks of infections in neonatal care units (168), therefore continued surveillance of this pathogen is equally important.

5.1.2.2. Colonisation at discharge

Overall, nearly a quarter of neonates stayed for at least 48 hours in the hospital and were sampled a second time at discharge. Prolonged stay was more common at TRRH than at KTCH, with the main reason being the need to observe the mother. This could be explained by the fact that at TRRH it was obligatory to observe mothers who delivered by CS for at least 48 hours. This was not the case at KTCH. Interestingly, at KTCH the frequency of prolonged stay due to neonatal complications was higher than at TRRH. Nearly a third of the neonates at KTCH stayed due to respiratory distress. This could be explained by poorer delivery practices in smaller hospitals, leading to an increase in postnatal complications for neonates (169).

Overall, colonisation of neonates was highly augmented at discharge for all bacteria, except *P. aeruginosa.* While at recruitment only 28.7 % of neonates were colonised, colonisation frequency more than doubled to 61.2 % at discharge. This coincides with findings from Madagascar, where infant ESBL *Enterobacteriaceae* carriage frequency doubled between admission and discharge after more than 48 hours of hospitalisation (147). High acquisition of ESBL colonisation among hospitalised neonates was also reported from Mwanza and Kenya (44, 123). Nelson et al., who sampled neonates on day one, three and seven, found that 60 % of positively tested neonates had acquired ESBL carriage on their first day of life (44). In a hospital in Morocco, the acquisition rate of MDR *A. baumannii* among neonates was 13.7 %, higher than in our study (166). Comparable data for *S. aureus* from SSA is lacking. In a German hospital, 22.9 % of neonates acquired *S. aureus* colonisation at some point during their hospitalisation (105). This was less than in our setting, where 31.4 % of initially negative neonates acquired *S. aureus*.

At our study hospitals, the increase in colonisation frequencies between recruitment and discharge was strongest for ESBL *K. pneumoniae* and *MDR A. baumannii.* This finding matches with the high frequencies of these bacteria isolated from hospital surfaces and supports the assumption that the hospital surfaces may act as a transmission reservoir to neonates. On a study site level, this was equivalent at TRRH and for ESBL *K. pneumoniae* at KTCH. Unexpectedly, there was no MDR *A. baumannii* isolated from neonates at discharge at KTCH. We fail to give a sufficient explanation for this inconsistency and suggest, that there might have been contamination of MDR *A. baumannii* isolates from neonates. The fact that no *P. aeruginosa* was acquired between recruitment and discharge matches with the low *P. aeruginosa* presence on surfaces and staff at both hospitals.

It is important to mention that we did not follow up on neonates who were discharged before 48 hours of hospitalisation. Neither did we sample neonates born at home. Therefore, we cannot rule out that neonates in the community might be equally colonised. There are very few community-based studies on the acquisition of MDR bacteria from SSA. A study from Madagascar reported that neonates, not particularly exposed to a hospital environment, also frequently acquired ESBL-producing bacteria (144). Schaumburg et al. showed that the colonisation with *S. aureus* increased 2.5-fold between birth and one month of life (45). However, studies have shown that neonates born in hospitals have a greater risk of colonisation with MDR bacteria, than neonates born at home and that the length of hospital stay correlates with bacterial acquisition (122, 166). Further, infants discharged from hospitals have been shown to spread MDR bacteria within their family units, acting as transmission reservoirs in the community (170).

5.1.2.3. Neonates born by CS and SVD

In our study, neonates born by SVD had three times higher colonisation with ESBL *E. coli* than neonates born by CS. This could be explained by the high perineorectal colonisation frequency of mothers with ESBL *E. coli*. Because of the proximity to the birth canal, the maternal bacteria can easily be transmitted to the neonate during vaginal birth. This can result in colonisation and infection of the neonate, increasing the risk of neonatal sepsis and mortality (44, 152). Our result matches with the findings of a large cross-sectional study from Nigeria, where 1161 mothers and neonates were sampled. Vaginal delivery was identified as a risk factor for positive neonatal ESBL-PE cultures and further neonatal ESBL-PE carriage was associated with higher neonatal mortality (145). In contrast, other studies from South Africa, Madagascar and Kenya reported birth by CS to be a risk factor for neonatal ESBL-PE carriage (123, 144, 146).

On the other hand, neonates born by CS were colonised with *S. aureus* twice as often as neonates born by SVD. Neonates born by CS are taken care of by multiple hospital staff and usually have longer hospital stays, which might increase their risk of acquiring *S. aureus.* Further, neonates born by CS were five times as likely to be colonised with MDR *A. baumannii.* This could be explained by the high environmental contamination with MDR *A. baumannii.* Neonates born by CS might be placed more frequently on different surfaces (e.g., baby warmers) during and after the procedure than neonates who are born by SVD, who tend to stay with their mothers directly after birth. Further, studies have shown that the intestinal microbiome of neonates born by CS is altered (171, 172), which might make them more prone to colonisation with MDR bacteria.

5.1.2.4. Neonates born by carrier and non-carrier mothers

We can report that at both hospitals neonate colonisation with *S. aureus* after birth was increased if the mother carried *S. aureus* in the nose herself. This observation resembles the findings of Schaumburg et al. in Gabon and multiple studies from HICs (45, 173-175). However, the association between maternal and neonatal colonisation with S. aureus may be explained by other factors than direct transmission from mother to child. Schaumburg et al. reported a direct mother-to-neonate transmission in only 5.6 % of cases, suggesting further sources of transmission (45). A study from Germany found direct mother-to-neonate transmission in approximately half of cases (175). In our study hospitals, there was a high colonisation of hospital staff with *S. aureus* (16.1 %). As these staff members were in close contact to mothers and neonates, they could be a possible source of transmission to both (**Figure 12**).

Likewise, ESBL *E. coli* and ESBL *K. pneumoniae* neonate colonisation after birth was increased if the mother was positive for the bacteria. However, this observation was only statistically significant for ESBL *K. pneumoniae* at TRRH. Several studies did identify ESBL-PE colonisation of the mother as an independent risk factor for colonisation of neonates (74, 145, 152, 175). In contrast, Nelson et al. did not find any phenotypic similarity between ESBL strains from mothers and their neonates, suggesting further sources of transmission (44). A systematic review, including eight studies from HICs and LMICs, found a pooled proportion of 27 % direct mother-to-child transmission of MDR-GNB (176).

5.1.3 Colonisation of hospital staff

S. aureus was by far the most frequently isolated bacterium from hospital staff, with 16.1 % of samples being positive for this pathogen. However, we cannot conclude that 16.1 % of the hospital staff were carriers of *S. aureus*, as a staff member could have been sampled several times. As sampling was anonymous, we do not know how many were tested more than once. *S. aureus* was mainly isolated from noses (27 %), followed by hands (14.2 %) and personal or medical devices (10.5 %). It was the most commonly isolated pathogen from all the mentioned sample origins. Matching our results, *S. aureus* was frequently isolated from hospital staff's hands and devices (stethoscopes) in studies from Uganda and Nigeria (114, 177). Further, the frequency of nasal *S. aureus* carriage ranged between 7.7 % and 18.3 % in Tanzania, Madagascar and Kenya (154, 178, 179). Whereby MRSA nasal carriage frequency was reported to be 1.5 % and 2.5 % in Madagascar and Dar es Salaam, respectively (178, 180).

MDR *A. baumannii* (3.2%) and ESBL *K. pneumoniae* (2.3%) were the second and third most prevalent bacteria found on hospital staff. ESBL *E. coli* and *P. aeruginosa* isolates were negligible few. There were no perineorectal samples taken from hospital staff, which could explain the lower colonisation frequencies with GNB than for mothers. Overall, the mentioned GNB were primarily isolated from personal or medical devices (10.5%), followed by hands (6.8%). A similar isolation frequency of GNB from the hands of hospital staff was found in Ghana (181). During an outbreak in a hospital in Nigeria, phenotypically similar ESBL *K. pneumoniae* strains were simultaneously found on the hands of hospital staff and neonates, suggesting transmission (182). In contrast to our results, a study from Botswana identified *P. aeruginosa* as the most frequently isolated bacterium from hospital staff's hands (183).

Our results suggest high nasal *S. aureus* carriage and limited or inconsistent hand hygiene compliance by hospital staff in the study hospitals. Poor hand hygiene compliance coupled with high surface contamination might increase the risk of cross-contamination to mothers and neonates. We did not study the training, knowledge and adherence to IPC measures among hospital staff. However, in view of results on IPC performance and quality of maternal care from other hospitals in East Africa, we consider it to be of a similar standard (42, 133, 134, 184). We learned that at KTCH hospital staff were performing deliveries without adequate materials, such as sterile gloves or running water. At both hospitals soap and alcohol-based hand sanitisers were not provided at all times. The hospitals´ IPC measures and their implementation require further investigation.

5.1.4 Colonisation of hospital surfaces

Surface contamination was high at both hospitals. Overall, the most frequently isolated bacterium was MDR *A. baumannii* (14.7%). This was also the case in several other studies from the region (128, 165, 185). In these studies, contamination frequency with MDR *A. baumannii* ranged between 3.7% in hospitals across Kenya (185) and 17.5% in a tertiary hospital in Mwanza (165). The common contamination of surfaces with MDR *A. baumannii* can be explained by its resistance against climate extremes and ability to survive for long periods on inanimate surfaces (66). Further, contamination of hospital surfaces with ESBL *K. pneumoniae* (10.2%) and ESBL *E. coli* (7.4%) was common. A recent, systematic review from Ethiopia, reported similar pooled surface contamination for both pathogens (186). Lower contamination frequencies were reported from Kenya and other hospitals in Tanzania (128, 185, 187). Lastly, contamination of surfaces with *S. aureus* (10.0%) was also frequent. Similar *S. aureus* contamination was found in a study conducted in four

public hospitals in South Africa (188). However, higher *S. aureus* contamination was reported from Dar es Salaam, Uganda and Ethiopia (127, 177, 186).

In contrast to the mentioned bacteria, surface contamination with *P. aeruginosa* (0.3%) was low at our study sites. This may be due to the low presence of *P. aeruginosa* in our study hospitals. However, it could also be explained by our sampling origins. We took samples only from dry inanimate surfaces in the hospitals. P. *aeruginosa* prefers moist surfaces such as sinks and colonizes medical devices such as humidifiers, ventilators and nebulizers (75). Therefore, we might have underestimated the frequency of *P. aeruginosa* isolates. The same low colonisation frequency was reported from hospitals in Kenya (185), whereas the systematic review from Ethiopia found a pooled surface prevalence of 7% (186). A study conducted in a tertiary hospital in Dar es Salaam included sampling of sinks and wash basins and found *P. aeruginosa* to be the most frequently isolated pathogen (187). Differences in colonisation frequencies might further result from variations in local bacteria reservoirs, IPC measures and study design. Moist surfaces in our study hospitals are to be investigated in future studies.

Comparing the study sites, contamination frequency was higher at KTCH (58.1%) than at TRRH (35.0%). This could be explained by the fact that KTCH is a smaller hospital with less developed hygiene standards and less well-trained staff. In addition, local differences in the prevalence of bacteria could play a role. Further, there were also differences on a ward level. The neonatal ward at KTCH was the ward where relatively most isolates were found. This ward was newly opened only shortly before the start of our study in February 2022. Therefore, one might have expected low contamination. The high contamination is alarming and could be due to insufficient cleaning. In addition, the small size of the ward, leading to overcrowding, might further facilitate the transmission of bacteria. At TRRH, the neonatal ward was also more contaminated than the labour ward. This is consistent with findings from other studies, where neonatal wards had the highest contamination (185, 189). By far the lowest frequency of isolates was found in the labour ward of TRRH. It has to be mentioned that the number of possible sampling origins differed between the wards, with most options in the neonatal and labour ward of TRRH and least in the neonatal ward of KTCH. However, the same types of surfaces (e.g., bedside rails) were sampled in all wards. Further, the frequency of swab collections from each origin was randomly generated and was similar. Differences in IPC measures between the hospitals and wards are further to be investigated, to find reasons for differences in contamination rates.

At both hospitals, all of the sampled surfaces were contaminated with at least one of the studied bacteria at some point in the study. The surfaces with the highest frequency of contamination included a round trolley, bedside rails of delivery and postnatal beds and a weighing scale. Further, highly sensitive items such as baby warmers, baby cots and the neonatal examination area were contaminated. This is in line with two systematic reviews on maternity ward hygiene in Malawi and Zanzibar, which found delivery beds to be frequently contaminated (42, 49). In a study from Kenya, patient beddings, incubators and baby cots were most frequently contaminated (185). A study from Mwanza reported the contamination of baby cots to be a risk factor for rectal colonisation and bacteraemia of neonates (165). The bacteria may be transferred directly to the neonates e.g., through direct contact to a contaminated surface. Further, the bacteria may be passed on indirectly through the hands of mothers or hospital staff after touching a contaminated surface and then caring for the neonate, leading to cross-contamination (**Figure 12**). The high frequency of surface contamination in this study indicates an imminent risk of hospital-acquired infections for neonates.

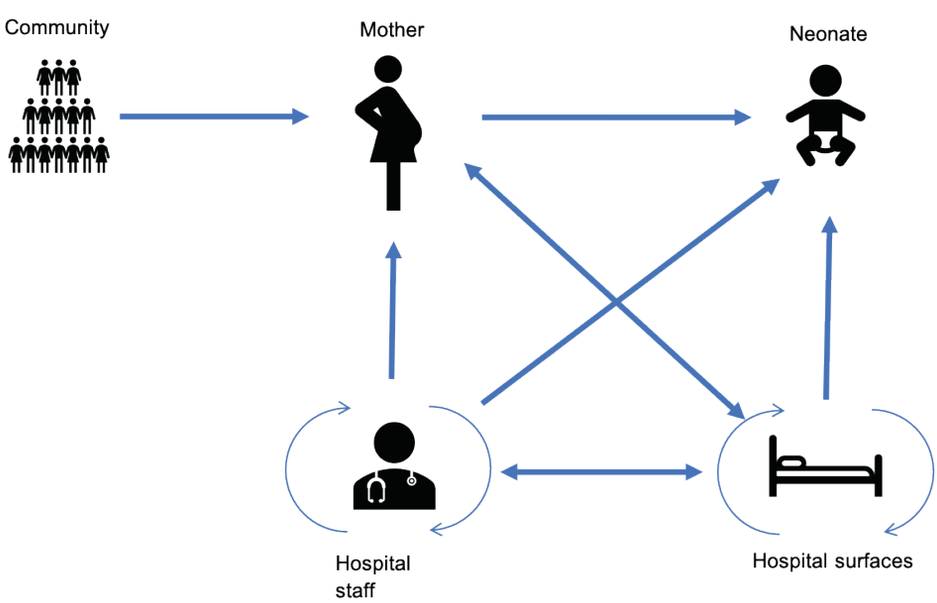

Figure 12: Possible transmission pathways of bacteria to neonates[7]

7 © Author

5.2. Future recommendations

The main strength of our study is its in vivo setting. The study was integrated into the hospital's daily routine. There were no changes to usual procedures. Therefore, we gained a realistic picture of bacterial contamination in the hospitals and can draw practical relevance from our results. Given the high contamination of hospital surfaces, measures should be taken by the study hospitals:

Firstly, hospital staff must be informed about the current situation in their wards. In addition, they need to be sensitized to the issue of transmission reservoirs and their risk to vulnerable patients, such as neonates. As soon as awareness is established, in place IPC measures can be analysed and improved. Evidence-based components of IPC measures include staff education, selection of adequate cleaning products, monitoring of environmental cleaning and performance feedback (190). Observations from similar settings in Malawi indicate that improvement of IPC practices, results in the reduction of microbial contamination (49).

Several studies used cleaning bundles and multimodal strategies to improve cleanness (130, 191, 192). A multicentre study conducted in Kenya, within the national IPC programme, presented the following measures which effectively reduced contamination: cleaning high-touch surfaces five times a day, providing soap at hand wash stations and providing gloves and gowns to hospital workers (126). Another quasi-experimental study from South Africa found that involving mothers in cleaning their babie's incubators to be effective and to reduce the workload of nurses (130). Methods are multiple and they have to be cost-effective to work in low-resource settings. At the time of this study, there were no reliable hand-washing facilities available at KTCH. At both hospitals, soap was not provided at all times. Alcohol-based hand sanitisers could be used as an alternative, low-cost solution to help achieve hand hygiene (126).

Further, the hospitals could benefit from routine biomonitoring to analyse changes in colonisation frequencies and the effects of their IPC measures. For this purpose, we recommend chromogenic-culture-media-based sampling, as used in our and other studies (183). This is a simple and cost-effective method, which can be used to identify transmission reservoirs. Fluorescent gel and ultraviolet markers could be helpful to monitor cleaning adherence (130). Ideally, resistance testing and whole genome sequencing should be performed in the future to determine resistance patterns and clonal similarities of bacteria and uncover their direct transmission routes.

5.3. Limitations of this study

This study has several limitations. Firstly, we relied on self-reported information concerning data on medical and pregnancy-related history of mothers. This may have introduced a recall bias, as some questions required the mothers to recall back several months. Additionally, all interviews were conducted in Swahili, the most widely used language in the Tanga Region. This might have led to misunderstanding and translation bias, as linguistical structures in English and Swahili vary greatly. Also, rigid stigmatisation still exists in Tanzanian society. Therefore, biases to hide medical information, especially being infected with HIV, cannot be ruled out.

Despite strict adherence to laboratory protocols, some possible limitations in sampling and laboratory procedures should be considered. We used perineorectal samples and not stool samples for mothers and neonates, which could have led to an underestimation of colonisation frequencies. Further, specimen storage and transport can influence bacterial culture yield, especially at high temperatures. However, all samples were processed on the day of collection and at least within eight hours after collection. Therefore, we do not consider transport or temperature to have significantly influenced our results. For bacterial identification, we used standard culture media, including chromogenic agar, and straightforward biochemical tests. These methods are easy to establish and cost-effective, which is relevant in our setting. We did not conduct bacterial confirmation, nor resistance testing. This was due to the limited time of this study. Therefore, we cannot present resistance patterns of the studied bacteria.

Further, we did not follow up on the duration of bacterial carriage. Therefore, we can neither make a statement about the duration of colonisation nor its clinical relevance. The colonisation of mothers and neonates might only be transient and have no health effects or increase in risk of bacterial infection. Data from other studies suggests that neonates colonised after birth have an augmented risk for neonatal infection (35, 47). A follow-up study with a longitudinal approach, as conducted by Schaumburg et al. (45), would be needed to assess if in our setting colonisation increases or decreases with time and if there is any health impact for the neonates.

Moreover, this was a purely qualitative analysis of bacterial contamination. We cannot make any statement about the quantity of bacteria on specific surfaces. This could be achieved by including a dilution method and calculation of bacterial colonies. Even though a quantitative analysis was not the intention of this study, it could help to investigate the relevance of specific surfaces as a source of bacterial

transmission. Surfaces with a high quantity of bacteria could be more relevant for transmission than those with a low quantity.

Lastly, we cannot provide any information on direct transmission pathways of bacteria between individuals or between surfaces and individuals. In order to identify transmission pathways, clonal similarities between bacterial strains must be assessed. For this, genotyping would be necessary. Nevertheless, this study was able to fill data gaps in the field of neonatal bacterial contamination and transmission reservoirs of MDR bacteria.

5.4. Conclusions

We observed high frequencies of colonisation for mothers, neonates, hospital staff and surfaces with the studied bacteria in our setting. Further, we were able to show that neonate colonisation with *S. aureus,* ESBL *E. coli* and ESBL *K. pneumoniae* after birth was increased if the mother was colonised with the bacteria herself. However, this result was only significant for *S. aureus* and we cannot derive direct transmission from concomitant colonisation. Nevertheless, mothers are a possible source of transmitting bacteria to their neonates.

In addition, we can report that neonates born by CS had higher colonisation frequencies with MDR *A. baumannii* and *S. aureus*, than neonates born by SVD. These bacteria were the most common bacteria isolated from surfaces and staff respectively. As neonates are handled by multiple hospital staff and are placed on various surfaces during and after CS, they may acquire bacteria from them. In contrast, neonates born by SVD were more likely to be colonised with ESBL *E. coli*. This could be explained by the high perineorectal colonisation rate of mothers with ESBL *E. coli*, which might be transmitted to the neonate during SVD.

Apart from mothers, hospital staff and surfaces may act as possible transmission reservoirs of bacteria to neonates. Colonisation of hospital staff and surfaces was high at our study sites, which makes them likely sources of transmission. Neonates were colonised with all of the bacteria also found on hospital staff and surfaces. Further, neonates were colonised with MDR *A. baumannii,* which was isolated from only a single mother at TRRH, but frequently from hospital surfaces of TRRH and KTCH. Lastly, the colonisation of neonates who stayed for at least 48 hours in the hospitals was increased at discharge.

In conclusion, we identified high colonisation among all mentioned cohorts and found various possible transmission reservoirs in the study hospitals. In most

hospital settings in SSA, the surveillance of MDR bacteria is poor or non-existent. At the same time, SSA has the highest neonatal mortality rate in the world, with the leading cause being bacterial infections. Therefore, filling data gaps in the field of neonatal bacterial colonisation, infection and transmission is essential to reduce the disease burden. Due to our study's in vivo setting, we can draw practical relevance from our results. The transmission reservoirs within the hospitals have to be addressed and IPC measures should be improved, to provide a safe environment for neonates. This is especially important in light of rising antibiotic resistance in SSA.

6. Abstract

Introduction: Antimicrobial resistance poses a significant burden in Sub-Sahara Africa (SSA), particularly to neonates. Data from this region on the colonisation of neonates with multidrug-resistant (MDR) bacteria is scarce and transmission reservoirs often remain unknown. However, its understanding is essential, as colonisation can act as a precursor to infection. Bacterial infections are the main cause of neonatal mortality in SSA. Therefore, this study aimed to provide data on the colonisation of neonates, their mothers, hospital staff and surfaces with five different bacterial species: ESBL *E. coli*, ESBL *K. pneumoniae*, MDR *A. baumannii, P. aeruginosa* and *S. aureus.* In addition, the role of the hospital environment as a potential transmission reservoir of bacteria to neonates was investigated.

Methods: This observational study was conducted in two hospitals in the Tanga region of Tanzania. A nasal and a perineorectal swab sample were taken from adult women admitted for delivery. Their respective neonates were sampled in the same manner after birth and, in case of hospitalisation for at least 48 hours, a second time at discharge. Further, swab samples of noses, hands and personal or medical devices were collected from hospital staff. Lastly, samples were taken from various surfaces in the neonatal and labour wards of the hospitals. Bacterial identification was carried out at the local laboratories. Data on the medical and pregnancy-related history of the mothers and on the status of the neonates was collected using digital case report forms. All analyses were conducted using Stata Statistical Software 14.

Results: In total 583 mothers were sampled during this study. Of these, 63.3 % were tested positive for ESBL *E. coli*, 24.0 % for *S. aureus,* 20.8 % for ESBL *K. pneumoniae*, 0.3 % for *P. aeruginosa* and 0.2 % for MDR *A. baumannii.* Further, 530 neonates were sampled shortly after birth (recruitment) and 14.3 % were tested positive for *S. aureus*, 10.2 % for ESBL *E. coli*, 3.4 % for ESBL *K. pneumoniae,* 2.5 % for MDR *A. baumannii* and 0.6 % for *P. aeruginosa.* Neonates born by caesarean section were more likely to be colonised with MDR *A. baumannii* and *S. aureus* than neonates born by spontaneous vaginal delivery (SVD). On the other hand, neonates born by SVD were more likely to have colonisation with ESBL *E. coli*. In addition, neonate colonisation with *S. aureus* was increased if the mother carried *S. aureus* in the nose herself. Out of all neonates, 129 stayed in the hospital for 48 or more hours and were therefore sampled again at discharge. Bacterial colonisation at discharge was increased for all bacteria, except *P. aeruginosa.* Lastly, there was high colonisation of hospital staff and surfaces in both hospitals. The cumulative frequency of isolated bacteria from samples of hospital staff was 22.5 % and of

surfaces 42.7%. MDR *A. baumannii* was frequently found in the hospital environment and on neonates, however on only a single mother.
Discussion: The study identified high colonisation among all mentioned study participants and surfaces with the mentioned bacteria. Colonisation frequencies of mothers in our study were in line with and higher than in other studies from SSA. Comparable data on colonisation frequencies for neonates, hospital staff and surfaces from the region is scarce. We identified the hospital environment as a potential source of transmission of MDR bacteria to neonates. This highlights the urgent need to improve infection prevention and control measures in both hospitals.

Einleitung: Antibiotika Resistenzen stellen in Subsahara-Afrika (SSA) eine erhebliche Krankheitslast dar, insbesondere für Neugeborene. Jedoch liegen nur wenige Daten zur Besiedlung von Neugeborenen mit multiresistenten Bakterien aus dieser Region vor, und deren Übertragungsreservoire sind häufig unbekannt. Diese Kenntnis ist jedoch von entscheidender Bedeutung, da die Besiedlung mit Bakterien als Vorläufer einer bakteriellen Infektion fungieren kann. Bakterielle Infektionen sind die Hauptursache für die neonatale Sterblichkeit in SSA. Daher war es Ziel dieser Studie, Daten über die Besiedlung von Neugeborenen, ihren Müttern, Krankenhauspersonal und Oberflächen mit fünf verschiedenen Bakterienarten zu gewinnen: ESBL *E. coli*, ESBL *K. pneumoniae*, MDR *A. baumannii*, *P. aeruginosa* und *S. aureus*. Darüber hinaus wurde die Rolle der Krankenhausumgebung als potenzielles Reservoir für die Übertragung von Bakterien auf Neugeborene untersucht.
Methoden: Diese Beobachtungsstudie wurde in zwei Krankenhäusern in der Region Tanga in Tansania durchgeführt. Bei erwachsenen Frauen, die zur Entbindung aufgenommen wurden, wurde ein nasaler und perineorektaler Abstrich genommen. Die entsprechenden Neugeborenen wurden nach der Geburt auf die gleiche Weise abgestrichen, und im Falle eines Krankenhausaufenthalts von mindestens 48 Stunden ein zweites Mal bei der Entlassung. Außerdem wurden Abstrichproben von Nasen, Händen und persönlichen oder medizinischen Gegenständen des Krankenhauspersonals entnommen. Zudem wurden Proben von verschiedenen Oberflächen in den Neugeborenen Stationen und Kreißsälen der Krankenhäuser entnommen. Die bakterielle Identifizierung wurde in den örtlichen Labors durchgeführt. Daten zur medizinischen und schwangerschaftsbezogenen Vorgeschichte der Mütter und zum Zustand der Neugeborenen wurden mit Hilfe von digitalen Fallberichtsformularen erfasst. Die Datenanalyse wurde mit der statistischen Software Stata 14 durchgeführt.
Ergebnisse: Es wurden insgesamt 583 Mütter in diese Studie eingeschlossen. Davon wurden 63,3% positiv auf ESBL *E. coli*, 24,0% auf *S. aureus*, 20,8% auf ESBL *K. pneumoniae*, 0,3% auf *P. aeruginosa* und 0,2% auf MDR *A. baumannii* getestet. Außerdem wurden 530 Neugeborene eingeschlossen und kurz nach der

Geburt abgestrichen. Dabei wurden 14,3 % positiv auf *S. aureus*, 10,2 % auf ESBL *E. coli*, 3,4 % auf ESBL *K. pneumoniae*, 2,5 % auf MDR *A. baumannii* und 0,6 % auf *P. aeruginosa* getestet. Neugeborene, die per Kaiserschnitt geboren wurden, waren häufiger mit MDR *A. baumannii* und *S. aureus* kolonisiert als Neugeborene, die per vaginaler Geburt zur Welt kamen. Andererseits waren Neugeborene, die vaginal geboren wurden, eher mit ESBL *E. coli* kolonisiert. Darüber hinaus war die Besiedlung der Neugeborenen mit *S. aureus* erhöht, wenn die Mutter selbst *S. aureus* in der Nase trug. Von allen Neugeborenen blieben 129 Neugeborene für 48 oder mehr Stunden im Krankenhaus und wurden daher bei der Entlassung erneut abgestrichen. Die bakterielle Besiedlung bei der Entlassung war für allen Bakterien erhöht, mit Ausnahme von *P. aeruginosa*. Zudem war in beiden Krankenhäusern eine hohe Besiedlung des Krankenhauspersonals und der Oberflächen festzustellen. Die kumulative Häufigkeit der isolierten Bakterien aus Proben des Krankenhauspersonals betrug 22,5% und von Oberflächen 42,7%. MDR *A. baumannii* wurde häufig in der Krankenhausumgebung und bei Neugeborenen gefunden, allerdings nur bei einer einzigen Mutter.

Diskussion: Die Studie ergab eine hohe Besiedlung aller Studienteilnehmer und Oberflächen mit den genannten Bakterien. Die Kolonisierungshäufigkeit von Müttern in unserer Studie entsprach den anderen Studien aus SSA oder war teilweise höher als in diesen. Vergleichbare Daten über die Häufigkeit der Besiedlung von Neugeborenen, Krankenhauspersonal und Oberflächen aus der Region sind rar. Wir haben das Krankenhausumfeld als potenzielle Quelle für die Übertragung von multiresistenten Bakterien auf Neugeborene identifiziert. Dies unterstreicht die dringende Notwendigkeit, Maßnahmen zur Infektionsprävention und -kontrolle in beiden Krankenhäusern zu verbessern.

7. References

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8. Appendix

Table 9: Colonisation frequencies of hospital staff at TRRH and KTCH

	ESBL *E. coli*		**ESBL *K. pneumoniae***		**MDR *A. baumannii***		***P. aeruginosa***		***S. aureus***	
	Number*	%	Number*	%	Number*	%	Number*	%	Number*	%
TRRH	0/294	0.0	4/294	1.4	5/294	1.7	0/294	0.0	52/294	17.7
Doctors	0/94	0.0	1/94	1.1	0/94	0.0	0/94	0.0	18/94	19.1
- Nose	-	-	-	-	-	-	-	-	8/21	38.1
- Hand	0/37	0.0	1/37	2.7	0/37	0.0	0/37	0.0	6/37	16.2
- Device**	0/36	0.0	0/36	0.0	0/36	0.0	0/36	0.0	4/36	11.1
Nurses	0/105	0.0	2/105	1.9	2/105	1.9	0/105	0.0	14/105	13.3
- Nose	-	-	-	-	-	-	-	-	7/31	22.6
- Hand	0/35	0.0	1/35	2.9	1/35	2.9	0/35	0.0	6/35	17.1
- Device**	0/39	0.0	1/39	2.6	1/39	2.6	0/39	0.0	1/39	2.6
Intern Doctors	0/95	0.0	1/95	1.1	3/95	3.2	0/95	0.0	20/95	21.1
- Nose	-	-	-	-	-	-	-	-	9/27	33.3
- Hand	0/34	0.0	0/34	0.0	2/34	5.9	0/34	0.0	8/34	23.5
- Device**	0/34	0.0	1/34	2.9	1/34	2.9	0/34	0.0	3/34	8.8
KTCH	3/141	2.1	6/141	4.3	9/141	6.4	1/141	0.7	18/141	12.8
Doctors	2/68	2.9	4/68	5.9	2/68	2.9	0/68	0.0	8/68	11.8
- Nose	-	-	-	-	-	-	-	-	2/16	12.5
- Hand	1/27	3.7	1/27	3.7	1/27	3.7	0/27	0.0	3/27	11.1
- Device**	1/25	4.0	3/25	12.0	1/25	4.0	0/25	0.0	3/25	12.0
Nurses	1/73	1.4	2/73	2.7	7/73	9.6	1/73	1.4	10/73	13.7
- Nose	-	-	-	-	-	-	-	-	4/16	25.0
- Hand	0/29	0.0	0/29	0.0	3/29	10.3	0/29	0.0	0/29	0.0
- Device**	1/28	3.6	2/28	7.1	4/28	14.3	1/28	3.6	6/28	21.4

* Data are n/N
** personal or medical device

Study Title: Transmission Reservoirs and Acquisition of Multidrug Resistant Bacteria in Neonates admitted to two hospitals within the Tanga region of Tanzania

Standard Operating Procedure (SOP) for Sampling of Mothers and their Neonates

Written by: Luisa Berckenhagen	**Reviewed by:** Denise Dekker	**Effective date:** 01.04.2022

This Standard Operating Procedure (SOP) has been read and understood by:

S/N	Name	Signature	Date
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Study Title: Transmission Reservoirs and Acquisition of Multidrug Resistant Bacteria in Neonates admitted to two hospitals within the Tanga region of Tanzania

Aim: To collect nasal and perineorectal swab samples from mothers and their neonates, who have been enrolled in the TRINEO study for swab sampling.

Objectives:

- to investigate the colonisation of the nose and the perineorectal area of mothers and their respective neonates,
- to determine acquisition of bacteria in neonates during hospital stay,
- to determine and compare the carriage of multidrug resistant bacteria in neonates and their mothers, as well as in hospital staff and in the hospital environment,
- to identify reservoirs and possible transmission pathways of multidrug resistant bacteria;
- to generate data on AMR and to offer support in setting up hospital reports on antibiotic resistance data;
- to develop evidence-based prevention and hygiene interventions contributing to reduce the transmission of MDR bacteria from hospital surfaces and health staff to mothers and neonates.

1. General Procedure:

1.1 The study nurse is responsible for the collection of swab samples from mothers and neonates.
1.2 Sampling of mothers: One perineorectal and one nasal swab sample will be collected from those mothers, who have been included in the study, after their admission to the labor ward. The samples should be collected **before** delivery or caesarean section. If possible, the sample collection should take place before the first vaginal examination performed in the hospital.
1.3 Sampling of neonates: One perineorectal and one nasal swab sample will be collected from neonates shortly after birth, who have been born in one of the study hospitals and have been included in the study. A second perineorectal and a second nasal swab sample will be collected at discharge from those neonates, who have stayed for over 48 hours in the hospital.
1.4 It is advised, to first collect the nasal sample and then the perineorectal sample from the same mother or neonate.
1.5 The samples will be taken in a uniform manner in both hospitals (see 3. and 4.).
1.6 Before or just after the sample collection the according CRF has to be filled out in the RedCap App on the tablet. For mothers, fill out the “Mother” CRF. For neonates, fill the “Inborn Neonate Recruitment” CRF or the “Inborn Neonate Discharge” CRF, depending on the timepoint of sampling.
1.7 It is important to approach the mother and her neonate in a friendly and calm manner. Explain the procedure to the mother, and provide for her comfort and privacy as good as possible. The mother can assist by holding and calming her child.

2. Safety Measures:

2.1 Wear disposable gloves and change gloves after each sample collection and each study participant.
2.2 Wash hands with soap before and after putting on gloves.
2.3 Handle all swab samples with care and treat them as potentially infectious material.

3. Collection of the nasal sample:

3.1 Use an “Eswab” for the collection of the nasal sample.
3.2 In case of mothers, label the swab tube with the individual Study ID of the mother (T22-**M**XXXX-XR), using the prepared Study ID stickers.
3.3 In case of neonates, label the swab tube with the individual Study ID of the neonate (T22-**I**XXXX-XX). For the sample collection after birth, use the ID ending on “R” for recruitment. In case of sample collection at discharge, use the ID ending on “D” for discharge.
3.4 Additionally, label the swab tube with the origin of sample (nose), using a waterproof marker.
3.5 Wash your hands and put on disposable gloves.
3.6 Open the tube and take out the swab only touching the handle and not the shaft or tip of the swab. If the tip of the swab does come into contact with anything, discard and use a new swab.
3.7 Sampling of mothers: ask the mother to tilt her head back slightly. Insert the swab into one nostril approximately 1 -2cm parallel with the bridge of the nose. Gently rotate the swab for 3 - 4 seconds to collect sample material. Use the same swab repeating the procedure in the other nostril.
3.8 Sampling of neonates: ask the mother to hold the neonate and tilt the neonate’s head back slightly. Insert the swab very gently into one nostril, parallel with the bridge of the nose, until resistance is met. Gently rotate the swab for 3 - 4 seconds to collect sample material. Use the same swab repeating the procedure in the other nostril.
3.9 After sample collection, replace the swab into the same tube without touching the edges of the opening and close the tube.
3.10 Take off the gloves and wash your hands again.
3.11 A laboratory request form, called “TRINEO Laboratory Swab Form” has to be filled for each sample. Place the swab sample and the according Laboratory Swab Form into a plastic zip bag.
3.12 After sample collection, the samples and forms have to be taken to the laboratory as soon as possible or within two hours after sampling.

4. Collection of the perineorectal sample:

4.1 After the nasal sample has been taken, one perineorectal sample should be collected from the same mother or neonate.
4.2 Use an "Eswab" for the collection of the perineorectal sample.
4.3 For mothers, label the swab tube with the individual Study ID of the mother (T22-**M**XXXX-XR), using the prepared Study ID stickers.
4.4 In case of neonates, label the swab tube with the individual Study ID of the neonate (T22-**I**XXXX-XX). For the sample collection after birth, use the ID ending on "R" for recruitment. In case of sample collection at discharge, use the ID ending on "D" for discharge.
4.5 Additionally, label the swab tube with the origin of sample (perineorectal), using a waterproof marker.
4.6 Wash your hands and put on disposable gloves.
4.7 In case of sampling a mother, ask her to uncover her anal region, while providing for her privacy as well as possible. Explain to her, that the sample collection might be uncomfortable, but is not painful.
4.8 In case of sampling a neonate, ask the mother or a caregiver to hold the neonate so that s/he is lying on her/his side, with hips and knees flexed.
4.9 Open the tube and take out the swab only touching the handle and not the shaft or tip of the swab. If the tip of the swab does come into contact with anything, discard and use a new swab.
4.10 Swab the perineal area of the mother or neonate by rubbing the swab gently up and down around the anus, while rotating the tip of the swab. Then insert the tip of the swab into the anus (approximately 3 cm for mothers and 1 cm for neonates) and gently rotate the swab back and forth for 10 seconds.
4.11 Pull the swab out of the anus, replace it into the tube and close the swab tube.
4.12 Take off your gloves and wash your hands.
4.13 Fill out the "TRINEO Laboratory Swab Form" indicating the date and time of sampling and the origin of sampling. Place the swab sample and the according Laboratory Swab Form into a plastic zip bag.
4.14 After sample collection, the samples and forms have to be taken to the laboratory as soon as possible or within two hours after sampling.

5. Materials required:

i. Eswab for nasal sampling
ii. Eswab for perineorectal sampling
*iii. Study ID Stickers (T22-**M**XXXX-XR or T22-**I**XXXX-XX)*
iv. Waterproof Marker
v. Laboratory Swab Forms
vi. Disposable gloves

Study Title: Transmission Reservoirs and Acquisition of Multidrug Resistant Bacteria in Neonates admitted to two hospitals within the Tanga region of Tanzania

Standard Operating Procedure (SOP) for Sampling Hospital Staff and Surfaces

Written by: **Luisa Berckenhagen**	**Reviewed by:** **Denise Dekker**	**Effective date:** **01.04.2022**

This Standard Operating Procedure (SOP) has been read and understood by:

S/N	Name	Signature	Date
1			
2			
3			
4			
5			
6			
7			
8			
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13			
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Study Title: Transmission Reservoirs and Acquisition of Multidrug Resistant Bacteria in Neonates admitted to two hospitals within the Tanga region of Tanzania

Aim: To sample health staff and surfaces in the neonatal and labour wards, in order to identify reservoirs of multiple drug resistant (MDR) bacteria, which could be a possible source of transmission to mothers and their neonates. Please note: sampling of health staff will be anonymous.

Objectives:

- to investigate the colonisation of hands, noses and medical/personal items of health staff in the neonatal and labour ward;
- to investigate the colonisation of hospital surfaces in the neonatal and labour ward;
- to identify reservoirs and possible transmission pathways of multidrug resistant bacteria;
- to generate data on AMR and to offer support in setting up hospital reports on antibiotic resistance data;
- to develop evidence-based prevention and hygiene interventions contributing to reduce the transmission of MDR bacteria from hospital surfaces and health staff to mothers and neonates.

1. General Procedure:

1.1 The laboratory staff will be responsible for the sample collection of hospital staff and surfaces.
1.2 Approximately 720 samples of hospital staff and 720 samples of surfaces will be collected in each hospital (Bombo and Korogwe) during the course of the study.
1.3 Therefore, approximately 30 samples will be obtained in each Hospital per week.
1.4 Mondays and Wednesdays will be the days of sampling.
1.5 The sampling will be done according to a random sampling scheme. The Study Personnel will be informed about which samples to be taken each week.
1.6 Sampling is best done in the morning. There is no need to warn the hospital staff that you will be taking samples.
1.7 The samples will be taken in a uniform manner in both hospitals (see 3. and 4.).

2. Safety Measures:

2.1 Wear disposable gloves and change gloves after each sample collection and each study participant.
2.2 Wash hands with soap before and after putting on gloves.
2.3 Handle all swab samples with care and treat them as potentially infectious material.

3. Collecting Surface Samples:

3.1.1 Verify which samples to take on the specific day according to the random sampling scheme.
3.1.2 Label each swab tube with the Study ID and the origin of sample using a waterproof marker.
3.1.3 Wash your hands and put on disposable gloves.
3.1.4 Open the tube and take out the swab ("Amies Swab") only touching the handle and not the shaft or tip of the swab.
3.1.5 Moist the sterile swab in sterile saline, without touching the swab shaft or tip.
3.1.6 Rub the swab up and down over a selected area (e.g., bedside-rail), rotate it several times in both directions and diagonally to make sure a good sample is obtained.
3.1.7 Replace the swab into the same tube without touching the edges of the opening and close the tube.
3.1.8 Fill out the "TRINEO Laboratory Swab Form" indicating the date and time of sampling and the origin of sampling.
3.1.9 Bring the samples and the forms directly to the Laboratory.

4 Collecting Samples of Hospital Staff

4.1.1 Verify which samples to take on the specific day according to the random sampling scheme.
4.1.2 Approach the Hospital Staff (e.g., doctor), who will be sampled, informing them about the procedure.
4.1.3 Label the swab tube with the Study ID and the origin of sample (e.g., doctor hands) using a waterproof marker.
4.1.4 Wash your hands and put on disposable gloves.
4.1.5 For the collection of hand and medical-/personal item (e.g., smartphone) samples, moist swabs (with sterile saline) will be used in the same way as explained in 3.4 – 3.9.
4.1.6 For the collection of a nasal swab sample an Eswab will be used in the following manner:
4.1.7 Ask the hospital staff member to tilt their head back slightly.
4.1.8 Open the tube and remove the swab. Be careful only to touch the handle of the swab.
4.1.9 Insert the swab into one nostril approximately 1 cm parallel with the bridge of the nose. Gently rotate the swab for 3 - 4 seconds to collect sample material.
4.1.10 Withdraw swab while slowly rotating.
4.1.11 Use the same swab repeating the procedure in the other nostril.
4.1.12 Replace the swab into the same tube without touching the edges of the opening and close the tube.

4.1.13 Fill out the "TRINEO Laboratory Swab Form" indicating the date and time of sampling and the origin of sampling.
4.1.14 Bring the samples and the forms directly to the Laboratory.

5. Materials required:

i. Eswab for nasal sampling
ii. Amies swab with sterile NaCl for surface, hand and device sampling
*iii. Study ID Stickers including "E" for hospital surfaces (T22-**E**XXXX-XR)*
*iv. Study ID Stickers including "S" for hospital staff (T22-**S**XXXX-XR)*
v. Waterproof Marker
vi. Laboratory Swab Forms
vii. Disposable gloves

Mother	☐ multiple choice ○ single choice |__| or __ alphanumeric	BNITM Bernhard Nocht Institute for Tropical Medicine	**TRINEO** Reservoirs and Transmission of Multidrug Resistant Organisms in neonates at Bombo and Korogwe Hospitals in Tanzania	Participant ID (Mother) |__||__||__|- |__||__||__||__||__|-|__||__|

A. Inclusion criteria

A01	Mother admitted to the hospital to give birth?	○ no ○ yes	→ exclusion if no
A02	Maternal age ≥18 years?	○ no ○ yes	→ exclusion if no
A03	Term of pregnancy ≥ 34 weeks?	○ no ○ yes	→ exclusion if no
A04	Informed consent given?	○ no ○ yes	→ exclusion if no

B. Identification

B01	**Hospital ID:**	_____
B02	Interview date:	|_|d|d|/|_|m|m|/|_|y|y|
B03	Age of mother (in years):	|_|y|y|

C. Medical and gynecological history

C01	Number of pregnancies (including current):	|__||__|
C02	Number of previous births (including stillbirths):	|__||__|
C03	Do you have any underlying medical condition? ○ no ○ yes → *if yes*, specify: ☐ Diabetes ☐ Sickle cell disease ☐ Hypertension ☐ Asthma ☐ HIV ☐ Others, specify: _____	

D. Relevant events during current pregnancy

D01	Any hospital stay(s) during current pregnancy?	○ no ○ yes
D02	Was Gestational Diabetes diagnosed during current pregnancy?	○ no ○ yes
D03	Was Pregnancy Induced Hypertension diagnosed during current pregnancy?	○ no ○ yes
D04	Any infections, during current pregnancy, leading to antibiotic treatment? ○ no ○ yes ○ unknown → *if yes*, which antibiotic treatment? ☐ Ampicillin ☐ Amoxcillin ☐ Metronidazole ☐ Erythromycin ☐ Others, specify: _____	
D05	Other problems during pregnancy? ○ no ○ yes →if yes, specify which: _____	

E. Current pregnancy

E01	Any USG performed during this pregnancy? ○ no ○ yes ○ yes, but date unknown → *if yes, please fill out:* Expected date of delivery according to USG? |_|d|d|/|_|m|m|/|_|y|y| Any abnormalities on last obstetric ultrasonography (USG)? ○ no ○ yes ○ unknown → *if yes*, specify: _____	
E02	Date of last menstrual period (LMP) available? ○ no ○ yes → *if yes*, specify date of LMP? |_|d|d|/|_|m|m|/|_|_| → *if yes*, expected date of delivery according to LMP? |_|d|d|/|_|m|m|/|_|y|y|	
E03	Number of antenatal care visits?	|__||__|

F. Investigations

F01	Perineorectal swab taken? (Take before delivery) ○ no ○ yes → *if yes*, prior to vaginal examination? ○ no ○ yes	
F02	Nasal swab taken? (Take before delivery) ○ no ○ yes	

Mother	☐ multiple choice ○ single choice |__| or __ alphanumeric	BNITM Bernhard Nocht Institute for Tropical Medicine	**TRINEO** Reservoirs and Transmission of Multidrug Resistant Organisms in neonates at Bombo and Korogwe Hospitals in Tanzania	Participant ID (Mother) |__||__||__|- |__||__||__||__||__|-|__||__|

G. Delivery

G00	Did the mother deliver during this hospital stay? →*if no*, stop follow up →*if yes*, answer the following questions	○ no ○ yes
G01	PROM > 18 hours?	○ no ○ yes
G02	Was there foul-smelling amniotic fluid?	○ no ○ yes
G03	Was there maternal pyrexia >38.0 °C?	○ no ○ yes
G04	Was the Baby born alive? →*if no*, stop follow up	○ no ○ yes

Y. Remarks

Y01	_____ _____ _____

Z. Responsibilities

	Role in the Study	Signature
Z01	Study Nurse	_____
Z02	Data entry clerk	_____

Recruitment Neonate Inborn	☐ multiple choice ○ single choice |__| or __ alphanumeric	BNITM Bernhard Nocht Institute for Tropical Medicine	**TRINEO** Reservoirs and Transmission of Multidrug Resistant Organisms in neonates at Bombo and Korogwe Hospitals in Tanzania	Participant ID (Neonate Inborn) |__|__|__|- |__|__|__|__|__|-|__|__|

A. Exclusion criteria

A01	Congenital Malformation?	○ no	○ yes	→ exclusion if yes
A02	Twins? /Triplets?	○ no	○ yes	→ if yes, include only first born

B. Link to Mother

B01	**Participant ID (Mother):** |__|__|__|-|__|__|__|__|__|__| - |__|__|
B02	**Hospital ID (Mother):** _____

C. Status of Neonate at birth

C01	Sex:	○ male ○ female
C02	Date and time of birth:	|d|d|/|m|m|/|y|y| |h|h|:|m|m|
C03	Birth Weight:	|__|__|__|__| g
C05	Cesarean section performed?	○ no ○ yes
C06	Temp. °C:	|__|__|. |__|.
C07	Resp. rate (b/min):	|__|__|__|
C08	Difficulty in breathing (grunting, nasal flaring, chest indrawing)?	○ no ○ yes

D. Neonatal Factors

D01	Skin: ☐ Central Cyanosis ☐ Pallor or grey color ☐ Capillary refill time >3 secs ☐ Jaundice ☐ Pustules ☐ Adequate color	D02	Movements: ○ No movements ○ Movements only when stimulated ○ Adequate movements
D03	Feeding: ☐ No or poor sucking ☐ Vomiting after each feed ☐ Other breastfeeding problems ☐ Good feeding	D04	Umbilicus: ☐ Bleeding ☐ Red or draining pus ☐ Clean and dry

D05	Convulsions?	○ no	○ yes
D06	Fontanelle sunken or bulging?	○ no	○ yes
D07	Irritable?	○ no	○ yes
D08	Failure to pass meconium and/or urine?	○ no	○ yes
D09	Eyes swollen and/or pus draining?	○ no	○ yes

E. Sample collection after birth

E01	Perineorectal swab taken?	○ no	○ yes
E02	Nasal swab taken?	○ no	○ yes

Y. Remarks

Y01	_____ _____ _____

Z. Responsibilities

	Role in the Study	Signature
Z01	Study Nurse	_____
Z02	Data entry clerk	_____

Discharge Neonate Inborn	☐ multiple choice ○ single choice |__| or __ alphanumeric	BNITM Bernhard Nocht Institute for Tropical Medicine	**TRINEO** Reservoirs and Transmission of Multidrug Resistant Organisms in neonates at Bombo and Korogwe Hospitals in Tanzania	Participant ID (Neonate Inborn) |__||__||__|- |__||__||__||__||__|-|__||__|

A. Criteria to fill out this Form

A01	Was the neonate enrolled in the TRINEO birth cohort study and stayed in the hospital > 48 hours since birth? ○ no ○ yes	→ *if NO* → DO NOT FILL OUT

B. Identification

B01	**Hospital ID:** _____
B02	Ward of hospitalization: ○ Infectious ○ NICU ○ General Pediatric ○ General Neonates ○ KMCU ○ Labour

C. Care during hospitalization

C01	Antibiotic treatment? ○ no ○ yes →if yes, specify which one(s): ☐ Ampicillin ☐ Ampicillin-Cloxacillin ☐ Gentamicin ☐ Amikacin ☐ Ceftriaxone ☐ Cefotaxime ☐ Metronidazole ☐ Ciprofloxacin ☐ Meropenem ☐ other, specify: _____

D. Sample collection at discharge

D01	Perineorectal swab taken?	○ no	○ yes
D02	Nasal swab taken?	○ no	○ yes

E. Discharge

E01	Final diagnosis? ☐ Respiratory infection ☐ Meningitis ☐ Encephalitis ☐ Urinary tract infection ☐ Malaria ☐ Septicemia ☐ Gastroenteritis ☐ No sickness (stayed due to maternal complication) ☐ Other, please specify: _____
E02	Outcome of diagnosis at discharge: ○ fully recovered without ongoing treatment ○ discharged with ongoing treatment ○ discharged with permanent disability/treatment ○ referred to another hospital ○ died ○ absconded/lost to follow-up

Y. Remarks

Y01	_____ _____ _____

Z. Responsibilities

	Role in the Study	Signature
Z01	Study Nurse	_____
Z02	Data entry clerk	_____

End of Study Form Neonate Inborn	☐ multiple choice ○ single choice |__| or __ alphanumeric	BNITM Bernhard Nocht Institute for Tropical Medicine	**TRINEO** Reservoirs and Transmission of Multidrug Resistant Organisms in neonates at Bombo and Korogwe Hospitals in Tanzania	Participant ID (Neonate Inborn) |__||__||__|- |__||__||__||__||__|-|__||__|

A. Progress of neonate

A00	Date of filling this form: |_d_|_d_|/|_m_|_m_|/|_y_|_y_|
A01	Was the neonate admitted to a Hospital Ward? ○ no ○ yes → *if yes*, please specify ward of hospitalization: ☐ Infectious ☐ NICU ☐ General Pediatric ☐ General Neonates ☐ KMCU ☐ Labour
A02	Did the Neonate present signs of Sepsis during hospital stay? ○ no ○ yes → *if yes*, please make sure blood culture has been taken and blood culture form has been filled
A03	Did the neonate stay in Labor Ward or other Hospital Ward for > 48 hours? ○ no ○ yes → *if yes*, please fill discharge form at discharge → *if no*, do **not** fill discharge form

B. Verification

B01	If all questions in A are answered with no:	→ Only Recruitment and End of Study form should have been filled
B02	If A02 is answered with yes:	→ Blood Culture form should have been filled additionally
B03	If A03 is answered with yes:	→ Discharge form should have been filled additionally
B04	Confirmation: Do you confirm that all of these forms have been entered and uploaded? ○ no ○ yes	

Y. Remarks

Y01	_____ _____

Z. Responsibilities

	Role in the Study	Signature
Z01	Study Nurse	_____
Z02	Data entry clerk	_____

Hospital Sampling Origins

A. TRRH (42 possible origins) -> 30 samples per week

1. Neonatal Ward

1.1. bedside rail (any bed in neonatal ward)
1.2. round trolley (any round trolley in neonatal ward)
1.3. general neonatal ward: baby cot
1.4. infectious neonatal room one: baby cot
1.5. tea room: mouse and keyboard
1.6. examination area: examination bedside rail
1.7. KMCU: weighting scale
1.8. NICU: baby warmer
1.9. NICU: incubator
1.10. NICU: baby cot
1.11. NICU: oximeter
1.12. NICU: thermometer

➔ 9 samples per week

2. Labour Ward

2.1. bedside rail (of any bed in labour ward)
2.2. round trolley (any round trolley in labour ward)
2.3. weighting scale
2.4. resuscitation area: baby warmer
2.5. administration area: mouse and keyboard
2.6. administration area: medication trolley
2.7. postnatal surgery computer room: mouse and keyboard
2.8. ante- and postnatal ward: bedside rail antenatal section
2.9. ante- and postnatal ward: bedside rail postnatal section
2.10. ante- and postnatal ward: mouse and keyboard
2.11. medical device: obstetric stethoscope
2.12. obstetrics theatre (any surface)

➔ 9 samples per week

3. Staff Neonatal Ward

3.1. Doctor Nasal Swab
3.2. Doctor Hand Swab
3.3. Doctor Personal/ Medical Device
3.4. Intern-Doctor Nasal Swab
3.5. Intern-Doctor Hand Swab
3.6. Intern-Doctor Personal/ Medical Device
3.7. Nurse Nasal Swab
3.8. Nurse Hand Swab
3.9. Nurse Personal or Medical Device

➔ 6 samples per week

4. Staff Labour Ward

4.1. Doctor Nasal Swab
4.2. Doctor Hand Swab

4.3. Doctor Personal or Medical Device
4.4. Intern-Doctor Nasal Swab
4.5. Intern-Doctor Hand Swab
4.6. Intern-Doctor Personal/ Medical Device
4.7. Nurse Nasal Swab
4.8. Nurse Hand Swab
4.9. Nurse Personal or Medical Device

➔ 6 samples per week

B. KTCH (25 possible origins) -> 15 samples per week

1. Neonatal Ward

1.1. bedside rail (of any bed in neonatal ward)
1.2. round trolley (any round trolley in neonatal ward)
1.3. NICU: baby warmer
1.4. NICU: oximeter
1.5. NICU: thermometer

➔ 3 samples per week

2. Labour Ward

2.1. bedside rail (of any bed in labour ward)
2.2. round trolley (any round trolley in labour ward)
2.3. weighting scale
2.4. administration area: mouse and keyboard
2.5. ante- and postnatal ward: bedside rail antenatal section
2.6. ante- and postnatal ward: bedside rail postnatal section
2.7. medical device: obstetric stethoscope
2.8. obstetrics theatre (any surface)

➔ 6 samples per week

3. Staff Neonatal Ward

3.1. Doctor Nasal Swab
3.2. Doctor Hand Swab
3.3. Doctor Personal or Medical Device
3.4. Nurse Nasal Swab
3.5. Nurse Hand Swab
3.6. Nurse Personal or Medical Device

➔ 3 samples per week

4. Staff Labour Ward

4.1. Doctor Nasal Swab
4.2. Doctor Hand Swab
4.3. Doctor Personal or Medical Device
4.4. Nurse Nasal Swab
4.5. Nurse Hand Swab
4.6. Nurse Personal or Medical Device

➔ 3 samples per week

9. Danksagung

Ich möchte mich zunächst bei meinem Doktorvater Herrn Professor Jürgen May für die großartige Möglichkeit, am Bernhard-Nocht-Institut promovieren zu dürfen, bedanken. Von unschätzbarem Wert war für mich die hervorragende Betreuung durch Dr. Denise Dekker. Vielen Dank für die Beratung, Hilfestellungen, gute Erreichbarkeit sowie stetige Motivation und Inspiration! Besonderer Dank gilt auch Britta Liedigk für ihre Arbeit im Labor, ihr offenes Ohr und die Gesellschaft in Tanga. Zudem danke ich Anna Jaeger für ihre Hilfe und Geduld bei zahlreichen Telefonaten zur Datenerhebung und Ricardo Strauss für seine Beratung. Weiterhin möchte ich mich bei Dr. Ralf Krumkamp und Daniel Chercos für ihre bedeutende Hilfe bei statistischen Fragestellungen bedanken.

Ganz herzlicher Dank gilt auch den Mitarbeitern und Mitarbeiterinnen des NIMR und der Krankenhäuser in Tanga und Korogwe. Ich danke ihnen für die Herzlichkeit und das Vertrauen, welches sie mir entgegengebracht haben. Zu guter Letzt möchte ich mich bei meinen Kolleginnen Theresia Mtui, Bernadina Ruta, Sarah Ngede, Joyce Kalaghe und Fides Mumbuli, mit denen ich die täglichen Hürden der Arbeit in Tanga bestritten habe, für ihre Gelassenheit und ihren Humor bedanken.

10. Curriculum Vitae

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Geburtsdatum und -ort	20. August 1994, München
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Familienstand	Ledig
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Promotion

2021 – 2024 Promotion am Bernhard-Nocht-Institut für Tropenmedizin in Hamburg

Hochschulausbildung

2015 – 2023 Studium der Humanmedizin, Universität Leipzig
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Stipendium

2018 – 2023 Studienstiftung des deutschen Volkes

Berufsausbildung

2013 – 2015 Ausbildung zur Rettungsassistentin

Schulausbildung

2009 – 2012 Sligo-Grammar-School in Sligo, Irland
2004 – 2009 Günter-Stöhr-Gymnasium, Icking

11. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.
Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....