Vaginal Colonization and Neonatal Infections in Central Uganda

Etiology, Antimicrobial Resistance and Associated Factors

Josephine Tumuhamye

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2023

UNIVERSITY OF BERGEN

Vaginal Colonization and Neonatal Infections in Central Uganda

Etiology, Antimicrobial Resistance and Associated Factors

Josephine Tumuhamye

Thesis for the degree of Philosophiae Doctor (PhD)
at the University of Bergen

Date of defense: 26.10.2023

Year: 2023

Title: Vaginal Colonization and Neonatal Infections in Central Uganda

Name: Josephine Tumuhamye

Print: Skipnes Kommunikasjon / University of Bergen

I dedicate this work to my parents; the late Major Joseph Byarugaba and the late Mrs. Juliet Tumushabe Byarugaba.

The thoughtless person playing with penicillin treatment is morally responsible for the death of the one who succumbs to infection with the penicillin-resistant organism. I hope this evil can be averted

Sir Alexander Fleming

Scientific Environment

This research was done in collaboration between the Centre for International Health, University of Bergen, Norway, and Makerere University Schools of Public Health and Medicine, Uganda. The Ph.D. candidate was supported by a grant from the Norwegian State Education Loan Fund under the Quota scheme (Lånekassen). The research work was conducted with financial support from the Centre for Intervention Science in Maternal and Child Health (https://www.uib.no/en/cismac), through the Chlorhexidine trial, which was funded by the Research Council of Norway (Young investigator grant to Dr. Victoria Nankabirwa, project number 234500) through its centers of excellence scheme (project number 223269) and the University of Bergen, Norway. Three of the four studies in this thesis were nested within the Chlorhexidine (CHX) trial: The trial aimed to estimate the efficacy of umbilical cord cleansing with a single application of 4% chlorhexidine to prevent omphalitis and severe illness in HIV unexposed newborns. The Ph.D. candidate was also part of the Survival Pluss project (Project Number; UGA-13-0030), under the Norwegian Programme for Capacity Development in Higher Education and Research for Development (NORHED), which provided additional financial support for laboratory work, the travels and progress meetings. The funders did not play any role in design of the study, data collection process and data analysis process.

Table of Contents

List of figures

List of tables

Abbreviations

AMR: Antimicrobial resistant bacteria

CI:	Confidence interval
CHX:	Chlorhexidine
CISMAC:	Centre for Intervention Science of Maternal and Child health
CLSI:	Clinical and Laboratory Standard Institute
DNA:	Deoxyribonucleic acid
EOS:	Early-Onset Sepsis
ESBL:	Extended-spectrum beta-lactamase
EUCAST:	European Committee on Antimicrobial Susceptibility Testing
FDA:	Food and Drug Administration
HIV:	Human Immunodeficiency Virus
LOS:	Late-Onset Sepsis
MDG:	Millennium Development Goal
MDR:	Multidrug-resistance
mecA:	Methicillin resistance structural gene
MIC:	Minimum Inhibition Concentration
MLSB:	Macrolide-lincosamide-streptomycin b resistance
MRSA:	Methicillin-resistant *Staphylococcus aureus*
NMR:	Neonatal Mortality Rate
NORHED:	Norwegian Programme for Capacity Development in Higher Education and Research for Development
PCR:	Polymerase chain reaction
PROM:	Premature rupture of membranes
SDG:	Sustainable Development Goal
VRE:	Vancomycin-resistant *Enterococcus*
VRSA:	Vancomycin-resistant *Staphylococcus aureus*
WHO:	World Health Organization

Definition of Terms

Vaginal colonization. Vaginal colonization with potentially pathogenic bacteria was defined as isolation of at least one of the following types of bacteria from a vaginal swab: *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae, Klebsiella oxytoca,* group A *Streptococcus*

(GAS), group B *Streptococcus* (GBS), *Enterococcus* species, *Pseudomonas* species, *Enterobacter* species, *Citrobacter* species, *Proteus* species and/or *Acinetobacter* species.

Vaginal MDR Colonization. Vaginal colonization with bacterial isolates that are non-susceptible to at least one antibiotic in at least three of the antibiotic classes. The classes of antibiotics include beta-lactam, aminoglycosides, fluoroquinolones, glycopeptides, macrolides, sulfonamides, oxazolidinones.

Umbilical Cord Infection (omphalitis). Omphalitis was defined by presence of pus on the umbilical cord stump.

Clinical sepsis. An illness among neonates (up to 28 day old infants) associated i) with any of the following danger signs observed or verified by a study clinician: inability to feed or vomiting of everything and unable to keep anything down, a history of convulsions, lethargy or unconsciousness, severe lower chest in-drawing, axillary temperature of ≥37.5ºC or <35.5ºC, grunting, or cyanosis and/or ii) results in hospitalization and/or iii) results in death.

Culture-positive sepsis. Is a clinical syndrome characterized by systemic signs and symptoms of infection in the first 28 days of life and isolation of a pathogen in blood.

Abstract

Background: Bacteria colonizing the birth canal of women in labor may be passed to their babies during birth, and some of them thereby develop infections. Umbilical cord stump infection (omphalitis) is an important risk factor for neonatal sepsis. Neonatal sepsis can be difficult to treat with commonly available antibiotics because antimicrobial resistance is widespread. Therefore, there is need to generate updated information about the prevalence and risk factors for infections with resistant bacterial pathogens that may be vaginally transmitted to neonates during birth. The aim of this thesis was to describe the bacteriological profile of potential pathogens colonizing the vagina of women in labor and the umbilical stumps of their newborns as well as the bacteriological profile of pathogens causing neonatal sepsis in Kampala and Mukono districts in central Uganda.

Methods: We conducted two cross-sectional studies (papers I and II) in which we collected vaginal swabs from women in labor and a prospective cohort study (paper III) in which we collected umbilical swabs from their neonates at three primary health care facilities in and near Kampala between July 2016 and July 2018. The neonates in the study were followed-up on days 3, 7, 14 and 28. We also conducted another cross-sectional study (paper IV) in which we studied neonates with symptoms and signs of sepsis at the pediatric emergency care unit of Mulago hospital, a tertiary referral hospital in Kampala. We performed culture and antimicrobial susceptibility testing on all study specimens, including vaginal swabs, umbilical swabs and blood. In addition, we performed polymerase chain reaction to identify methicillin resistant Staphylococcus aureus (MRSA) isolates. Multidrug resistant bacteria were defined as bacteria resistant to antibiotics from ≥ 3 antibiotic classes. We estimated the factors associated with i) vaginal colonization with potentially pathogenic bacteria, ii) vaginal colonization with antimicrobial resistant bacteria, iii) omphalitis and iv) culture-positive neonatal sepsis.

Results: We recruited 1,472 women in labor with a mean age of 25 years (paper I and II). Nine hundred and fifty five of the women (65%; 95% confidence interval [CI] 62% to 67%) were vaginally colonized with potentially pathogenic bacteria. The most commonly isolated potentially pathogenic bacteria from women in labor were *Escherichia coli* (35%), *Klebsiella pneumoniae* (10%) and *Staphylococcus aureus* (8%). Of the 1,472 women, 57 (4%) were colonized with extended-spectrum beta-lactamase (ESBL)-producing bacteria, 27 (7%) were colonized with carbapenem-resistant *Enterobacteriaceae* and 45 (3%) were colonized with MRSA (paper II). A total of 750 of the 1472 women (51%) had multidrug resistant potentially pathogenic bacteria. We followed up 769 of the children born at the clinics in the 2-year period from July 2016, and almost 10% of them developed omphalitis in the first month of life (Article III). The predominant potentially pathogenic bacteria isolated from the 65 babies with omphalitis were *E. coli* (28%), *K. pneumoniae* (11%) and *Citrobacter freundii*, (8%). In the hospital study, we found that 46/359 (13%; 95 % CI 10%, 17%) of the babies with clinical signs of sepsis had a positive blood culture (paper IV). The predominate pathogens among the 46 neonates with clinical signs of sepsis and a positive blood culture were *S. aureus* (63%), *E. coli* (15%) and *K. pneumoniae* (11%). Many of these bacteria were resistant to ampicillin, gentamicin, ceftriaxone and/or methicillin.

Conclusion: In the three study clinics, *E. coli* and *K. pneumoniae* were the two most common bacterial species isolated from the birth canal of women in labor. These were also the two most frequently isolated potential pathogens among study babies who developed omphalitis. Further studies to explore the link between colonization of the birth canal and subsequent neonatal infections are necessary. Neonatal infections were common with almost 10% developing omphalitis in the first month of life. A substantial proportion of the potential pathogens isolated from the birth canal of women and the umbilical cord stumps of

their babies as well as those grown in blood from hospitalized babies with sepsis were resistant to ampicillin, gentamicin and ceftriaxone; common antibiotics used in treating serious neonatal infections. The observed resistance profiles indicate a problem of antimicrobial resistance, which complicates treatment of neonatal infections.

Sammendrag

Bakgrunn: Bakterier som koloniserer fødselskanalen til fødende kvinner kan overføres til deres nyfødte, og noen av dem kan dermed utvikle tidlige infeksjoner. Infeksjon i navlestrengstumpen (omfalitt) er en viktig risikofaktor for neonatal sepsis. Pga. utbredt antimikrobiell resistens kan behandling av nyfødtsepsis være utfordrende. Vi trenger oppdatert informasjon om forekomst av infeksjoner med resistente bakterielle patogener som kan overføres fra fødende kvinner til deres nyfødte babyer. Målet med denne avhandlingen er å beskrive potensielt patogene bakterier som koloniserer skjeden til fødende kvinner og navlestrengstumpene til de av deres nyfødte som får omfalitt, samt å karakterisere bakteriene som forårsaker neonatal sepsis i og i nærheten av Kampala i Uganda.

Metoder: Mellom juli 2016 og juli 2018 gjennomførte vi to tverrsnittstudier (artikkel I og II), der vi samlet inn vaginale prøver fra fødende kvinner ved tre helseklinikker i og nær Kampala, og en prospektiv kohortstudie (artikkel III) av deres nyfødte som vi fulgte i 4 uker og tok bakteriologiske prøver fra navlestrengstumpen deres når de fikk omfalitt. Vi gjennomførte også en annen tverrsnittstudie (artikkel IV) der vi studerte sykehusinnlagte spedbarn opp til og med 4 ukers alder som hadde kliniske tegn på sepsis. Vi dyrket bakterier fra fødselskanals- og navlestrengstumpen (artikkel I, II og III) samt fra blod (artikkel IV). I tillegg utførte vi polymerasekjedereaksjon for å identifisere methicillinresistente *Staphylococcus aureus* (MRSA). Bakteriestammer som viste resistens mot antibiotika fra minst 3 ulike antibiotikaklasser betraktet vi som multiresistente.

Resultater: Vi rekrutterte 1472 fødende kvinner med en gjennomsnittsalder på 25 år. Nihundreogfemtifem (65%) av kvinnene hadde potensielt patogene bakterier i sin fødselskanal (artikkel I). De mest vanlige var *Escherichia coli* (35%), *Klebsiella pneumoniae* (10%) og *Staphylococcus aureus* (8%). Av de 1472 kvinnene var 57 (4%) kolonisert med utvidet-spektrum beta-laktamase (ESBL)-produserende bakterier, 45 (3%) med MRSA, og 27 (2%) med karbapenem-resistente *Enterobacteriaceae* (artikkel II). Totalt 750 (51%) av de 1472 kvinnene hadde multiresistente *potensielt patogene* bakterier i sin fødselskanal. Vi fulgte opp 769 av barna født ved klinikkene i 2-årsperioden fra juli 2016, og nesten 10% av dem utviklet omfalitt i første levemåned (artikkel III). De dominerende potensielt patogene bakteriene hos disse 65 babyene var *E. coli* (28%), *K. pneumoniae* (11%) og *Citrobacter freundii* (8%). I sykehusstudien fant vi at 46 (13%) av de 359 nyfødte med kliniske tegn på sepsis hadde positiv blodkultur (artikkel IV). De dominerende patogenene blant disse barna var *S. aureus* (63%), *E. coli* (15%) og *K. pneumoniae* (11%). Mange av disse bakteriene var resistente mot ampicillin, gentamicin, ceftriaxon og/eller methicillin.

Konklusjon: I de tre studieklinikkene var *E. coli* og *K. pneumoniae* de to vanligste bakterieartene isolert fra fødende kvinners fødselskanal. Disse var også de to vanligst isolerte artene hos studiebarn med omfalitt. I vår studie utviklet nesten 10% av babyene omfalitt i løpet av den første levemåneden. En betydelig andel av de potensielt patogene bakteriene fra kvinnenes fødselskanal og fra babyenes navlestrengstump samt av de som ble dyrket fra blodet til de nyfødte innlagt i

sykehus med klinisk sepsis var resistente mot ampicillin, gentamicin og/eller ceftriaxone, antibiotika som inngår i førstelinjebehandlingen av nyfødtsepsis. Antibiotikaresistens kan se ut til å være et betydelig problem også i sentrale deler av Uganda, og tiltak for å begrense utbredelsen vil være avgjørende for rasjonell behandling av alvorlige bakterielle infeksjoner hos disse nyfødte barna.

Original papers

The thesis is based on the following papers:

1. Tumuhamye J, Steinsland H, Tumwine JK, Namugga O, Mukunya D, Bwanga F, Sommerfelt H, Nankabirwa V. ***Vaginal colonisation of women in labor with potentially pathogenic bacteria: a cross sectional study at three primary health care facilities in Central Uganda.*** *BMC Infectious Diseases. 2020 Dec 1;20:98.*

2. Tumuhamye J, Steinsland H, Bwanga F, Tumwine JK, Ndeezi G, Mukunya D, Namugga O, Kasede AN, Sommerfelt H, Nankabirwa V. ***Vaginal colonization with antimicrobial-resistant bacteria among women in labor in central Uganda: prevalence and associated factors.*** Antimicrobial resistance and infection control. 2021;10(1):37.

3. Tumuhamye J, Sommerfelt H, Mukunya D, Tumwine JK, Namugga O, Bwanga F, Steinsland H, Nankabirwa V. ***Umbilical cord stump infections in central Uganda: incidence, bacteriological profile and risk factors.*** Int. J. Environ. Res. Public Health 2022, 19(23), 16055; https://doi.org/10.3390/ijerph192316055

4. Tumuhamye J, Sommerfelt H, Bwanga F, Ndeezi G, Mukunya D, Napyo A, Nankabirwa V, Tumwine JK. ***Neonatal sepsis at Mulago national referral hospital in Uganda: Etiology, antimicrobial resistance, associated factors and case fatality risk.*** *PloS one. 2020;15(8):e0237085.*

Acknowledgements

I would like to thank Prof. James K. Tumwine for giving me an opportunity to pursue this PhD. He chose me from the many mentees he had to join the Survival Pluss team to start this journey. Thank you for seeing the potential in me, nurturing it and believing in me. You introduced me to the scientific community that I know today. Thank you for the fatherly role you have played to ease this journey.

I would also like to thank my supervisor Dr Freddie Bwanga for mentoring me in the field of microbiology. Thank you for encouraging and guiding me. You always availed yourself to respond to my questions or any logistical concerns about the microbiological studies to ensure their smooth running.

I thank my main supervisor Dr Victoria Nankabirwa for inspiring me with your great achievements and hard work. You have taught me to work hard and aim highest against all odds. You introduced me to the world of epidemiology and made me fall in love with it. Thank you for the opportunity to help in your classes and in doing so, I unlearned and learned many things. Thank you for the opportunity to coordinate the Chlorhexidine trial in which my studies were nested. In doing so, my eyes opened up to a different side of science I never knew. I got a better understanding of methodological aspects and many concepts that I would have never appreciated in the theoretical Epi classes. Thank you for teaching me to go the extra mile in my work. Thank you for your unwavering support when I was pregnant and my children are lucky to call you Jjaja.

Special thanks to Prof. Halvor Sommerfelt for your invaluable input in my work. The words here cannot fully express my utmost sincere gratitude. You are one of the most brilliant and meticulous individuals that I know and I am privileged to have been supervised by you. You have taught me to write, to pay attention to detail, to be excellent in everything that I do (and I am still learning). I know I will get there. Thank you for taking time off your very busy schedule to always review and discuss my work. I looked forward to your feedback and your long (tough) emails kept me on the edge, pushed me out of my comfort zone and

critically think about the concepts I was writing about. During our discussions and deliberations, I unlearned and learned many things.

I am very grateful to Hans Steinsland who helped me when I was stuck with my datasets as well as review my work. Hans played an important role in organizing my laboratory dataset, and because of him, I was able to have it in a format that I could easily merge with the trial dataset that had been cut out for me. Thank you for the helping me with data analysis, how to present the data and how to write it up. You have taught me how to write in a precise minor through our interactions during the preparations of my manuscripts. You have greatly improved my writing skills.

I would like to thank Prof. Thorkild Tylleskar for the support and guidance through the periodic Survival Pluss progress meetings. You were not my academic supervisor but you were very interested in my work and gave me constructive feedback during my presentations. You were very instrumental at the start of the PhD when I travelled to Bergen. You picked us (my colleagues and I) from the airport, you tried to prepared us for the culture shock and thank you for the practical information you always gave us. I also would like to appreciate you and your dear wife Karen for the delicious meals you made us in your home and for the great hospitality.

Special thanks to Prof. Grace Ndezi for also supporting me through the Survival PLUSS project.

I thank my Survival Pluss colleagues David Mukunya, Justin Bruno Tongun, Agnes Napyo, Agnes Arach, Sr.Vincentina Achora, Beatrice Odongokara, Vivian Zalwango and Alice Mugisha for being there during the journey. Peer reviewing each other's work, sharing ideas and office space was awesome. A special thank you to David for teaching me so much especially during our discussions regarding methodological concepts in my studies and the data analysis sessions we had for my manuscripts. You read through every version of my manuscripts. Thank you for the support system you have been: as my prayer partner, a best friend and the most awesome husband. Thank you for the encouragement you provided in moments when I felt so low and discouraged.

I also thank my CISMAC-CHX study colleagues Rhoda, Olive, Joan and Kagongwe for the support and making the data collection journey easier. Without my colleagues, it would have been very difficult to balance the data collection, writing and other obligations that I had. I am grateful to the data collection team: the CISMAC study nurses at all the study sites, the health facility midwives and in-charges, the Mulago hospital team and the MBN Clinical Laboratory team. I am grateful to the microbiology laboratory team; Emma Aboce, Winnie Binoga, Denis Mango and Mary Lunyolo for your commitment, and Alfred for all the help in the molecular laboratory.

To the Bergen team, I would like to appreciate the administrative staff for Centre for International Health; Gunhild Koldal, Catherine Schwinger, Ane Straume and Daniel Gundersen for all the administrative support. Thank you for responding to my emails and making sure I got the help I needed. I will always remember your kindness.

I thank my parents for the sacrifices they made all these years. Special thanks to my mother who didn't live to see the completion of this work. Special thanks to my very large family; Judith Tumuramye, Moses Asiimwe, Agaba Isaac Kakuru, Arinda Ivan, Nancy Kamusiime, Joan Atukunda, Denis Niwagaba, Viva Niwagaba, Gerald Muzoora, Tom Mugabe, Gerald Lubowa and aunties; Peace, Charity, Dorcus, Jennifer, uncles, grandparents, nephews, nieces and the rest of the family for your prayers, encouragement and support. You have been such a great support system.

Introduction

Global neonatal mortality continues to be unacceptably high at approximately 18 deaths per 1000 live births and contributes to 2.4 million annual child deaths (1). Approximately 2 million of these deaths occur in low- and lower-middle income countries, about 1.1 million of them in sub-Saharan Africa (2). In the last two decades, progress in reducing neonatal mortality has been slow; Uganda has only seen an 18% reduction, from 33 to 27 deaths per 1000 live births (3). Neonatal infections, preterm births and intrapartum related complications are the leading causes of neonatal deaths (4). Some of the most common infections that affect newborns include sepsis, meningitis, and pneumonia. These account for about 33% of all neonatal deaths in sub-Saharan Africa (5). Therefore, reducing these infections is a prerequisite for reducing neonatal mortality and thereby achieving the third sustainable development goal.

Newborns are highly susceptible to infections because of their immature immune system and incomplete skin barrier (6). The immature immune system and incomplete skin barrier predispose the neonate to infections from pathogens colonizing the mother's birth canal and the neonate's cord stump. Moreover, treating neonatal infections is increasingly problematic, due to the emergence of multidrug-resistant strains globally. The absence of local data concerning the etiology and antimicrobial resistance of maternal and neonatal infections has exacerbated challenges in the management of neonatal infections. Data on the etiology and antimicrobial resistance is key to informing targeted treatment of infections and the timely revision of management guidelines. Therefore, this study sought to describe the potential pathogens colonizing women in labor as well as those colonizing the umbilical cord stumps of newborns with omphalitis. We also describe the pathogens causing neonatal sepsis and explore factors associated with neonatal infections.

Vaginal colonization

Some of the studies in this thesis are centered on describing vaginal colonization among women in labor. The mother's birth canal is an important source of bacteria from which the umbilical cord is contaminated during birth (7). The vagina in healthy women of reproductive age has a well-balanced ecosystem of normal flora consisting of diverse communities of bacterial populations predominated by *Lactobacilli* species (8). The

Lactobacilli produce lactic acid which maintains the vaginal pH at 4.5, and has antimicrobial properties (9). The composition of vaginal flora among women differs based on several factors such as hormonal differences, vaginal hygiene practices and genetic factors (10). Disruption of the normal vaginal flora may cause physiological changes predisposing women to colonization by opportunistic pathogens (11). Potentially pathogenic bacteria that frequently colonize the vaginal tract include "*Staphylococcus aureus,* group B *Streptococcus* (GBS)*,* group A *Streptococcus* (GAS), *Enterococcus* species, *Citrobacter* species, *Escherichia coli, Enterobacter* species, *Pseudomonas* species and *Klebsiella pneumoniae"* (12).

The risk of neonatal colonization with potentially pathogenic bacteria is highest during labor and the subsequent post-partum period (13). During labor, the neonate comes into contact with pathogens colonizing the birth canal of the mother, and their nares, conjunctiva, ears, gut and umbilical cord can be colonized; a process known as "seeding" (14). The effect of vertical transmission of potential pathogens colonizing the birth canal is most pronounced in the first days of life, especially among babies born vaginally (15). Infants born vaginally usually acquire bacteria similar to that colonizing the mother's vagina (15, 16, 17) and such infants are predisposed to neonatal infections from potentially pathogenic bacteria colonizing their mothers (18). These infections may be localized, such as omphalitis, but could also become systemic. In addition, these infections could be resistant to commonly used antibiotics (19, 20, 21).

Neonatal infections

The studies in this thesis are based on two types of neonatal infections including omphalitis and clinical sepsis.

Omphalitis

Omphalitis is an infection of the umbilicus and umbilical cord stump or surrounding skin characterized by pus discharge, redness (a sign of inflammation) and swelling of the umbilical cord stump and surrounding skin at its base. The severity of omphalitis is based on a combination of the clinical signs (22, 23, 24). However, in this thesis, omphalitis was defined as presence of pus at the umbilical cord stump. The umbilical cord is a structure made of three blood vessels (two arteries and one vein) and surrounding tissue that connect

the mother's placenta to the fetus in the uterus, through which the fetus gets its nutrients, exchanges gases and a channel for getting rid of waste (7). After birth, it is cut and the remaining stump dries up, falling off and leaving a wound that subsequently heals. This remaining stump or wound is often a route of infection into the blood stream of the neonate through the open vessels.

The skin of a neonate is colonized by both pathogenic and non-pathogenic bacteria soon after birth, but the bacterial profile varies depending on the hygienic circumstances surrounding birth and cord care practices in the postpartum period (25). The bacteria colonizing the skin and cord stump of the neonate may cause local and systemic neonatal infections (26, 27). Studies from south-east Asia (Bangladesh and Pakistan) show *S. aureus*, *E. coli, K. pneumoniae* and *Pseudomonas* spp. as the most frequent colonizers of the umbilical cord stump (25, 28). Pathogenic bacteria such as *E. coli, K. pneumoniae, Pseudomonas* spp. and *Streptococci* species track up along the umbilical vessels causing infection of the cord stump.

Risk factors for omphalitis include maternal, neonatal and behavioral factors. Maternal factors include lower education level, age, intrapartum infections, prolonged labor and home birth while neonatal risk factors include very low birth weight (29). Behavioral factors are unhygienic umbilical cord care practices around the time of childbirth, which often facilitate bacterial contamination and increase the risk of infection. The unhygienic umbilical cord care practices include cutting/tying the cord with unclean equipment and/or applying unhygienic substances on the umbilical cord stump or surrounding area (30, 31). Evidence suggests that use of topical low-cost interventions such as chlorhexidine lowers the incidence of omphalitis among neonates, especially those born at home, by reducing bacterial colonization of the skin or umbilical cord stump (32).

Neonatal sepsis

In this thesis, neonatal clinical sepsis is defined as a clinical syndrome characterized by systemic signs and symptoms of infection in the first 28 days of life (33) while culture-positive sepsis is defined as isolation of an organism in blood in addition to the clinical features. The source of the causative organism could be attributed to an intrapartum

infection, acquisition of vaginal flora or organisms from the hospital or community. The timing of exposure, inoculum size, immune status of the infant, and virulence of the causative agent influence the clinical manifestation of neonatal sepsis (34). Neonatal sepsis can be characterized as early-onset and late-onset. Early-onset sepsis refers to infections in the first 72 hours of life and is acquired before and during birth (35). Late-onset sepsis refers to infections that occur 3 days after birth, commonly caused by pathogens acquired from hospital or community settings (36).

Neonatal sepsis is one of the three major causes of deaths among newborns, and accounts for 3 million cases globally, every year (37). The global incidence rate of neonatal sepsis is estimated at 48 cases per 100,000 person years annually (38) while the risk of neonatal sepsis in sub-Saharan Africa is approximately 7.5% annually (39). Sepsis in newborns accounts for approximately 37% of all neonatal deaths in sub-Saharan Africa (4).

Etiology

Early-onset sepsis is commonly caused by GBS or *E. coli* (35) while late-onset sepsis is commonly caused by pathogens such as *Klebsiella* species and *S. aureus* (36). Pathogens that cause early-onset sepsis are often acquired before and during childbirth whereas pathogens that cause late-onset sepsis are often acquired from the environment (40). However, the bacterial profile between early- and late-onset sepsis does not differ significantly (41). Studies indicate that *E. coli*, *Klebsiella* spp., *S. aureus* and coagulase-negative *Staphylococcus* are the most common causes of early- and late-onset sepsis and mortality in low-income countries (42, 43).

Risk factors

Neonatal risk factors

Premature birth or low birth weight are the most important factors predisposing the neonates to sepsis. Premature babies with low birth weight are up to ten times more at risk of sepsis compared to term normal weight babies (34). Preterm babies require longer hospital stays, insertion of devices (catheters, probes, feeding tubes) which impair the skin barrier and provide a port of entry increasing the risk of infection (44). Other neonatal factors include, fetal distress, resuscitation, low Apgar score, omphalitis and first birth order (45).

Maternal risk factors

They include maternal previous exposure to infections, bacterial colonization and obstetric factors such as prolonged rupture of membranes (>18 hours), chorioamnionitis, intrapartum fever (>38°C) and GBS colonization in late pregnancy (46).

Antimicrobial resistance

Antimicrobial resistance is a natural phenomenon of bacteria and a typical example of bacterial adaptation. Bacterial pathogens have a remarkable genetic plasticity, which allows them to respond to environmental threats. This plasticity can result into specific responses including mutations or acquisition of foreign genetic material containing resistance determinants, hence the capability to produce resistance to virtually all antibiotics of clinical importance.

Antimicrobial resistance is a global public health crisis and 214,000 neonatal sepsis deaths are attributed to resistant pathogens each year globally (47). Antimicrobial resistance is also a cause of substantial morbidity (48, 49). Nearly half of the pathogens that cause possible serious bacterial infections are resistant to first-line (ampicillin or penicillin and gentamicin) and second-line (third generation cephalosporins) antibiotics recommended by the WHO (50). In sub-Saharan Africa, the proportion of neonates that die from infections caused by selected resistant pathogens is unknown, due to lack of surveillance antimicrobial resistance data and inadequate routine microbiological testing services such as blood culture.

Antimicrobial resistance affects all branches of medicine and imposes a huge economic burden. In 2017, WHO published a list of priority pathogens to guide research and development of new antibiotics (51). These multi-drug resistant pathogens include carbapenem-resistant *Enterobacteriaceace, Pseudomonas aeruginosa*, *and Acinetobacter baumanii*, extended-spectrum beta-lactamase (ESBL) producing bacteria, methicillin resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), and vancomycin-resistant *Enterococci* (VRE). Hospital surveillance data on children from low-income countries suggests that resistance to WHO recommended first-line antibiotics (gentamicin and ampicillin) ranges between 71% to 100% in *Klebsiella spp* isolates and 60%-70% in *E. coli* isolates (52, 53). A systematic review of the etiology of invasive bacterial infections among neonates in 26 countries from sub-Saharan Africa estimated resistance to beta-lactams at 68%, and aminoglycosides at 27% (5).

There are two important considerations in regard to antimicrobial resistance: the intrinsic resistance and the acquired resistance. Intrinsic resistance refers to innate resistance in which the bacteria are generally insensitive to a specific or class of antimicrobial agents. This is due to inaccessibility of the antimicrobial agents to their targets. For instance, vancomycin is the treatment of choice for MRSA infections but it is completely ineffective against gram-negative bacteria (54). This is because vancomycin is relatively large and cannot penetrate the outer membrane of gram-negative bacteria which acts as a permeability barrier (55). Another example is *Enterococci* species which are intrinsically resistant to folate inhibitors such as trimethoprim and sulfonamides because they use exogenous folates (56). While intrinsic resistance is genus- or species- specific, acquired resistance is strain-specific.

Acquired antimicrobial resistance

Acquired resistance refers to the ability of bacteria to develop resistance to antibiotics to which they were previously susceptible. Acquired resistance usually results from chromosomal mutations or acquisition of genetic materials containing resistance determinants most likely from intrinsically resistant bacteria in the environment (57). An example is the spread of plasmids harboring beta-lactamase genes, which allow an organism to acquire resistance to beta-lactam antibiotics (58, 59). The rapid spread of antimicrobial resistance worldwide is attributed to selection pressure from irrational use of antibiotics in humans, husbandry and aquaculture, in crop farming and environmental pollution of waste products from pharmaceutical industries (60). As a result of selection pressures, bacteria develop mechanisms to evade antibiotics, including inhibition of cell wall synthesis, interference with nucleic acid synthesis, inhibition of metabolic pathways, and causing leakage of bacterial cell membranes (61).

Resistance to beta-lactam antibiotics

In this thesis, we mainly report on resistance to beta-lactam antibiotics including ESBL, MRSA, and carbapenem resistant bacteria. Beta-lactam antibiotics are the most frequently prescribed antibiotics and the major categories include penicillins, monobactams, cephalosporins, carbapenems, and cephamycins (62). Resistance to beta-lactams is due to four mechanisms: i) production of beta-lactamases (destruction of antibiotic molecule), ii)

modification of antibiotic target, iii) change in cell wall permeability and iv) modification of the antibiotic molecule. In many instances, several of these mechanisms combine to give a high level of resistance to a specific or class of antibiotics. We briefly review each of these mechanisms below.

Production of beta-lactamase enzymes

The production of plasmid-mediated broad-spectrum beta-lactamase enzymes is the major mechanism of resistance against cephalosporins among *Enterobacteriaceace* (63). Types of broad-spectrum beta-lactamase enzymes include ESBL, metallo-beta-lactamases or carbapenemases. Beta-lactam antibiotics consist of a four membered beta-lactam ring on their structure that the broad-spectrum beta-lactamases hydrolize, thus interfering with the cell wall synthesis of bacteria resulting in resistance (64). ESBL enzymes are inactivated by beta-lactamase inhibitors such as clavulanic acid, tarzobactam, sulbactam and are widely spread in *Enterobacteriaceace* especially *K. pneumoniae* and *E. coli*.

Enzymatic modification of antibiotic binding site

Methicillin-resistance of *S. aureus* is a mechanism of resistance involving enzymatic modificaton of antibiotic binding target. This is the most important acquired resistance among clinically important *S. aureus* isolates. Antibiotics containing the beta-lactam ring bind to the penicillin-binding protein (PBP) which is essential for synthesis of bacterial cell wall and inhibits peptidoglycan formation, leading to bacterial cell lysis. Resistance to beta-lactam antibiotics in MRSA is due to acquisition of mobile genetic element staphylococcal cassette chromosome (SCCmec) carrying the *mecA* gene, which encodes for an altered penicillin-binding protein (PBP2a) (65). PBP2a has very low affinity for beta-lactam antibiotics and as a result, catalyzes the cell wall biosynthesis enabling survival and growth of the bacteria (66).

Change in cell wall permeability

This mechanism of resistance mainly occurs in gram-negative bacteria, because the antibiotic must penetrate first the outer layer and cytoplasmic layer, before exerting the antimicrobial effect. Hydrophilic molecules such as beta-lactams, floroquinolones and tetracyclines are affected by changes in permeability, because they use water filled channels

such as porins to cross the cell wall barrier (67). Gene mutations encoding the membrane proteins such as porins cause alterations, rendering the pathogen resistant.

Modification of antibiotics

Some bacteria acquire mobile genetic elements that encode aminoglycoside-modifying enzymes which modify the hydroxyl or amino groups of the aminoglycoside antibiotic resulting in resistance. Examples of aminoglycoside-modifying enzymes include: acetyltransferases, which are mainly found in *Enterobacteriaceace* conferring resistance to amikacin and gentamicin and phosphtransferases, which confer resistance to kanamycin and streptomycin. Adenyltransferase confers resistance to tobramycin and gentamicin (68, 69).

Laboratory diagnosis of neonatal infections

Early and effective diagnosis of infections is essential for improving neonatal health, survival and improving antimicrobial stewardship. When a bacterial infection is present, these tests elucidate the etiology and antibiotic susceptibility profile which is an important step in optimizing and narrowing the antibiotic treatment as quickly as possible (70). There are different methods used for diagnosing neonatal infections.

Conventional culture methods

Conventional culture methods are the most commonly used tests in low-income countries. However, they are very slow, typically providing results after 48 hours or more, are laborious and costly. In addition, blood culture has low sensitivity compared to molecular methods (71, 72). Absence of bacterial growth in blood culture may result from a low number of bacteria in the blood stream, which may be caused by maternal or neonatal use of antibiotics, and small sample volumes. Combining culture with other techniques increases sensitivity and specificity for detecting organisms in clinical specimens (72, 73).

Molecular methods

Molecular tests include multiplex real time PCR, DNA hybridization, whole genome sequencing and others (71, 72, 74, 75). They can significantly reduce the turnaround time of results. These methods are considered to be superior compared to the conventional phenotypic methods because they allow detection of more organisms in small specimen volumes. However, these methods have limitations: they are very expensive, they need

specialized personnel and equipment, and they have high rates of false positives. There is need for point of care diagnostic tests that directly identify pathogens from a clinical sample in a much shorter time and possibly before administration of treatment.

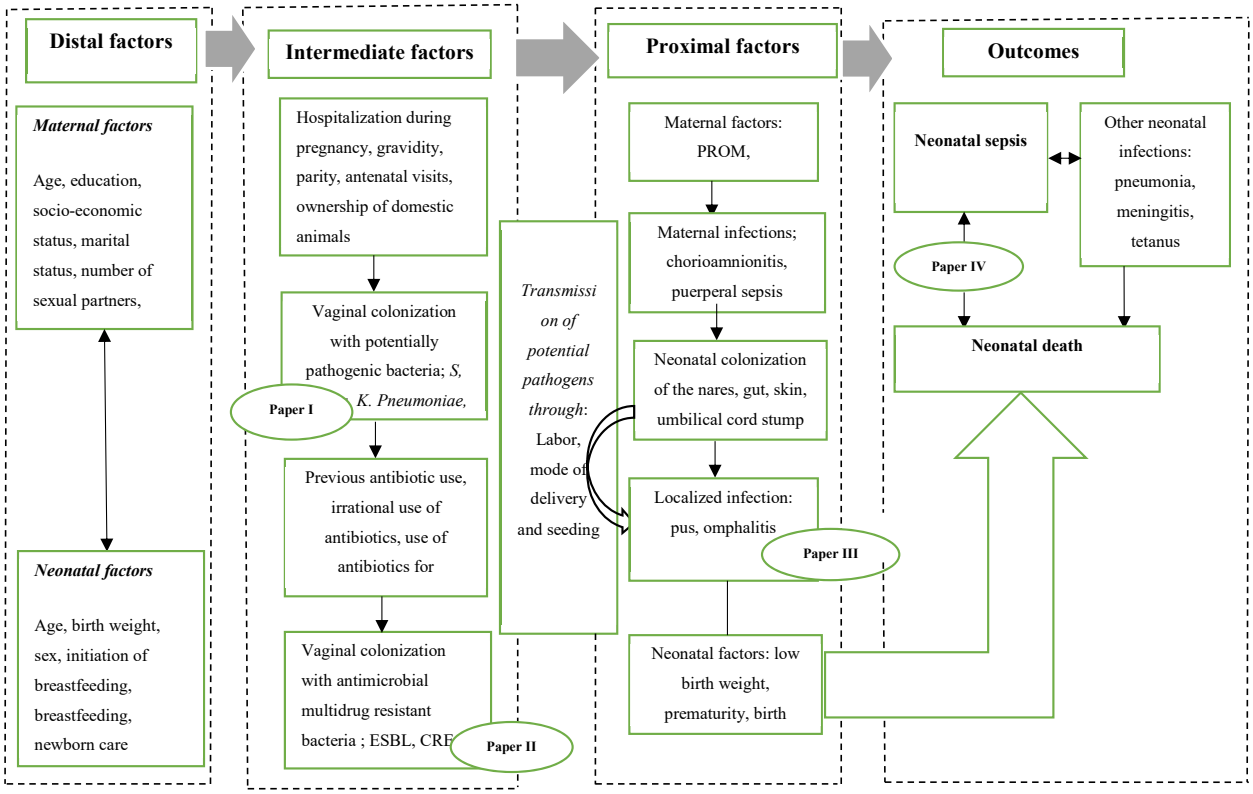

Figure 1: conceptual framework

Conceptual framework

Narrative of the conceptual framework

Figure 1 is a conceptual framework in which I sought to argue why the topic of this thesis matters. It generated research questions which guided me to choose methods used in the studies herein. The conceptual framework built towards these purposes was based on literature and theory according to Ravitch and Riggan (76). My studies aimed to explore potentially pathogenic bacteria colonizing the birth canal of women at the time of labor and associated factors. In addition, we studied umbilical cord stump colonization among neonates with omphalitis, the bacterial etiology of sepsis, associated factors and immediate clinical outcomes among neonates with sepsis. Vaginal colonization with potentially pathogenic bacteria and neonatal infections is important for several reasons. Firstly, the timing in which we sample the women (during labor) is crucial to understand what microorganisms could be passed on to the neonate. Secondly, to understand what pathogens cause infections among newborns and their antimicrobial susceptibility profiles. Lastly, to explore factors associated with both vaginal colonization and neonatal infections. Therefore, we studied these aspects among women in labor and their newborns. The factors we studied include socio-demographic factors such as age, education, and socio-economic status, which we considered distal because they indirectly influence vaginal colonization by acting through intermediate factors. For instance, literature shows an association between maternal age and vaginal colonization (77, 78). Socio-economic status also indirectly affects neonatal outcomes through mechanisms such as poor maternal nutrition status, poor sanitation conditions and other intermediate determinants. Intermediate factors such as hospitalization during pregnancy, especially in resource-limited settings with poor infection control measures increase the exposure of pregnant women to more virulent multidrug resistant bacterial strains prevalent in health facility settings, hence predisposing both the baby and mother to serious bacterial infections. Another example of intermediate exposures is previous or irrational use of antibiotics, which is a driver for antimicrobial resistance. Multidrug resistant pathogens increase the risk of infections and death. Proximal factors are known predictors of maternal infections and possible subsequent neonatal infections and mortality. Figure 1 shows the conceptualization of variables in this thesis. It explains the

inter-relationships between variables and multiple potential pathways between exposure and outcomes.

Rationale of the studies

Understanding the profile of potential pathogens colonizing the birth canal and those causing neonatal infections is important. There is a hypothesized, but poorly understood, link between pathogens colonizing the birth canal of pregnant women and those causing neonatal infections. In addition, risk factors for maternal colonization especially with multidrug resistant pathogens are not well described. Infections caused by antimicrobial resistant pathogens are of great concern and have consequences including long and expensive hospital stays, expensive treatment and increased mortality (79, 80). Antimicrobial resistance context-specific data is important and needed in our setting. The studies in this thesis sought to gain insight into the epidemiology of vaginal colonization, omphalitis and neonatal sepsis. We generate context specific data on colonization, etiology and resistance profiles, which contributes to the growing body of evidence in Uganda. Such local data may guide in antimicrobial resistance surveillance, as well as guide clinicians in making informed decisions when treating these infections.

Study objectives

General objective (Aim)

To describe the bacteriological profile of potential pathogens colonizing the vagina of women in labor and the umbilical cord stump of their newborns as well as the bacteriological profile of pathogens isolated from blood cultures of neonates with sepsis in Kampala and Mukono districts in central Uganda.

Specific objectives

At three primary health care facilities in Kampala and Mukono districts in central Uganda, we sought:

1. To describe vaginal colonization with potentially pathogenic bacteria among women in labor.
2. To describe vaginal colonization with potentially pathogenic resistant bacteria among women in labor.
3. To identify factors associated with vaginal colonization with potentially pathogenic and antimicrobial resistant bacteria.
4. To describe the bacteriological profile of bacteria isolated from umbilical cord stumps of newborns with omphalitis.
5. To estimate the incidence of omphalitis and its association with vaginal colonization of women in labor.

And at Mulago national referral hospital, we sought:

6. To estimate the proportion of newborns with sepsis who had a positive blood culture.
7. To describe the bacteriological profile of bacteria isolated from newborns with sepsis
8. To identify factors associated with culture-positive sepsis among newborns admitted with symptoms and signs of sepsis.

Study subjects and methods

Study area

The studies were conducted in two settings; studies for papers I-III were based at three primary health care settings: Kawaala health centre (HC) III, Kitebi HC III and Mukono HC IV while the study for paper IV was based at the pediatric emergency care unit of Mulago national referral hospital in Kampala. The study sites are located within Kampala and Mukono districts, both in central Uganda. Kampala is the capital city of Uganda with a resident population of 2 million, while Mukono district has a largely rural population of almost 200,000 people. Mukono general hospital conducted more complicated births including cesarean sections, while Kitebi and Kawaala HCs only conducted vaginal births. These HCs did not have facilities to perform cesarean section but could do vacuum or forceps extractions when required. They are located in urban slum areas of Kampala district. These three health facilities when combined have a monthly average of 1200 births. Mulago hospital is a national referral hospital and a teaching hospital for Makerere University and is also located in Kampala. Its pediatric emergency care unit is an emergency unit that admits critically ill children around the clock, before they are transferred to general pediatric wards.

The microbiological investigations performed in the studies were conducted at MBN Clinical Laboratories (81), a clinical and research accredited laboratory with specialization in molecular diagnostics of infectious diseases.

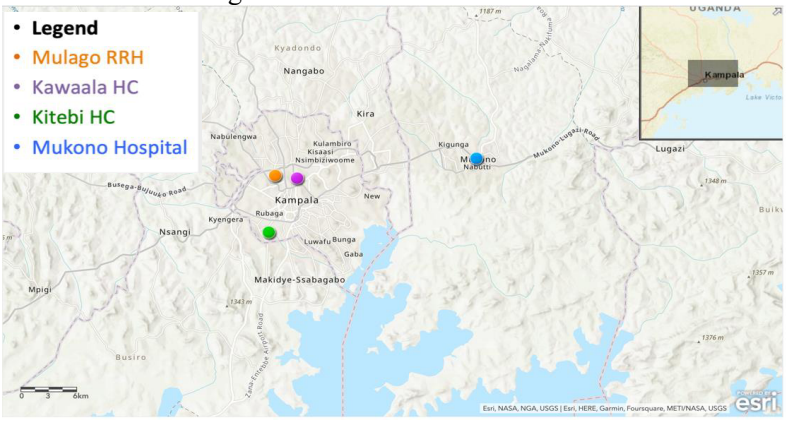

Figure 2: Map showing the four study sites

Methods

We conducted four observational studies: three (Paper I, II and III) were nested within the chlorhexidine study (22). The forth study (Paper IV) was conducted at Mulago national referral hospital. A summary of the methods used in these studies is provided in table 1.

Table 1: Summary of study methods

Paper	Study design	Sample size	Study population	Study sites	Exposure variables (s)	Outcome(s)
I	Cross-sectional study	1,472	Women in labor	Kawaala HC III, Kitebi HC III and Mukono general hospital	Maternal education, maternal age, marital status, socio-economic status, hospitalization during pregnancy, number of previous pregnancies, antenatal tetanus toxoid vaccination, antenatal care visits and possession of domestic animals at home	▪ Vaginal colonization with potentially pathogenic bacteria.
II	Cross-sectional study	1,472	Women in labor		Parity, maternal education, maternal age, hospitalization during pregnancy, antenatal care attendance, possession of domestic animals at home, socio-economic status	▪ Prevalence estimates of vaginal colonization with ESBL, carbapenamase-resistant bacteria, MRSA, VRSA, VRE and MDR.
III	Prospective cohort study	800	Neonates		Bacterial pathogens isolated from the umbilical swabs birth weight, sex, application of any substance on the cord, initiation of breastfeeding, maternal age, maternal education, hand washing practices and socio-economic status	▪ Incidence of omphalitis ▪ Bacteriological profile of bacteria isolated from neonates with omphalitis ▪ Association between vaginal colonization and omphalitis
IV	Cross-sectional study	359	Neonates	Mulago national referral hospital	Birthweight, omphalitis, lethargy, hypothermia, fever, inability to breastfeed, initiation of breastfeeding, previous antibiotic use, re-admission, length of hospitalization, presence of antimicrobial resistant pathogens in blood, MDR, sex, age	▪ Prevalence of culture-positive sepsis ▪ Bacteriological profile of pathogens causing neonatal sepsis ▪ Case fatality risk

Study participants

The participants in the study of bacterial colonization of the vagina (papers I and II) included 1,472 HIV negative women in labor, whose neonates were recruited into the chlorhexidine (CHX) trial at Kawaala HC III, Kitebi HC III and Mukono general hospital. They were enrolled if they gave informed consent within 12 hours after giving birth to babies enrolled into the CHX trial. For the omphalitis study (paper III), we included babies enrolled in the dry cord care arm (standard of care for umbilical cord care) of the CHX trial. The inclusion criteria into the trial included a birth weight of at least 1.5 kgs, no obvious cord stump infection at birth, no severe congenital anomaly and no severe illness requiring hospitalization immediately after birth. The standard of care in Uganda for umbilical cord stump is dry cord care but some mothers in addition apply other home remedies to the cord stumps. The home remedies include smoked banana leave, clarified butter, cow dung, saliva, and miscellaneous herbs. The neonatal sepsis study (paper IV) included neonates admitted to the emergency care unit of Mulago hospital who presented during weekday daytime with any of the following symptoms and signs of serious illness according to the integrated management of childhood illnesses guidelines: inability to breastfeed, convulsions, fast breathing (more than 60 breaths per minute), severe lower chest in-drawing, lethargy, fever (axillary temperature ≥37.5 ºC), and low body temperature (axillary temperature <35.5 ºC) (82).

Study procedures

In the studies nested in the CHX trial (Paper I, II and III), trained study nurses collected vaginal specimens from women during labor and umbilical swabs from their newborns. Participant characteristics were collected using structured electronic questionnaires on mobile phones using the Open Data Kit (ODK) software. For participants who missed a scheduled visit, there were window periods within which they would still provide information and be examined. The window period included day 2 to day 4 for day 3 visit, day 5 to day 9 for day 7 visit and day 10 to day 18 for day 14 visit. The window period for day 28 was from day 22 to day 40 visit. In the neonatal sepsis study (Paper IV), babies who presented at the emergency care unit at Mulago national referral hospital with symptoms and signs of sepsis upon admission were enrolled.

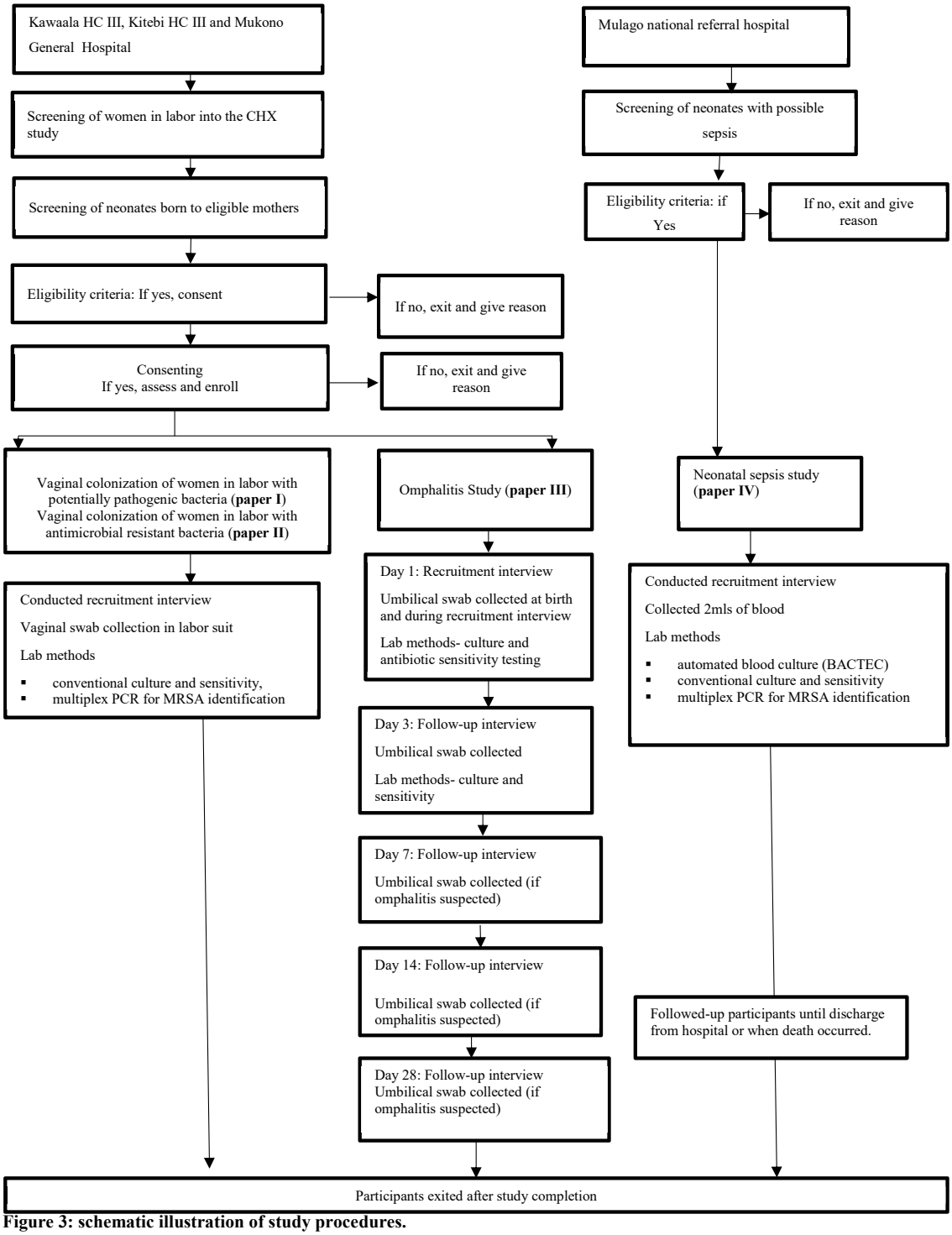

Figure 3: schematic illustration of study procedures.

A trained nurse obtained written informed consent and using a questionnaire, collected data on demographic and clinical characteristics from primary caretakers of the study participants. We followed up the participants until they were discharged and upon discharge, we collected outcome information as illustrated in figure 3.

Specimen collection

Trained midwives collected vaginal swabs (paper I and II) from women during labor and umbilical swabs (paper II) from their neonates. We used Regular Rayon sterile swabs prepacked with Amies agar gel, without charcoal transport medium (Copan Diagnostics Inc., Murrieta, CA). For vaginal specimen collection, a swab was carefully inserted into the vagina and gently pressed towards the vaginal walls and rotated to ensure that it was thoroughly coated. The midwives were careful to remove the swab, to avoid contact with the skin and the anal area. We also collected umbilical swabs from babies with omphalitis (presence of pus at the umbilical cord stump), during the visits scheduled on days 3, 7, 14 and 28 of life and at any other time point when a mother brought the baby to the study clinic with signs of omphalitis. To collect the specimen, the swab was moved around the cord stump and immediately put into a tube containing the transport medium, whereafter the tubes with the swab were kept in a cool box in which they were transported to the laboratory within 24 hours. In the sepsis study (paper IV), two milliliters of venous blood were drawn aseptically from the neonates and inoculated into pediatric blood culture bottles (BD Bactec ™ Peds Plus™/F) for culture. Within 8 hours of blood collection, the blood culture bottles were transported to the laboratories.

Bacterial identification

Paper I, II and III

We conducted bacterial species identification using conventional microbiological techniques. Primary inoculation of the vaginal swabs was done on chocolate agar, 5% sheep blood agar and on MacConkey agar (BioLab Zrt., Budapest, Hungary), followed by aerobic incubation at 35°C to 37°C for up to 72 hours to allow for slow growing bacterial colonies. From these plates, we sub-cultured morphologically distinct colonies onto new agar plates and used colonies from this sub-culture for further species identification and characterization.

Identification of gram-positive bacteria: *S. aureus* were identified based on positive catalase, coagulase and DNase tests. Beta-hemolytic *Streptococci* were identified based on their distinct characteristics such as beta hemolytic zones around colonies grown on blood agar plates, Gram stain positivity and a negative catalase test. The beta-haemolytic colonies were further Lancefield grouped into different species/groups (*Streptococcus* A-D), using the Streptococcal grouping kit (Oxoid Ltd., Basingstoke, Hants, UK). *Enterococcus* species were identified by a positive bile esculin test.

Identification of gram-negative bacteria: these were identified based on the following biochemical tests: lactose fermentation, triple sugar iron agar, sulfur-indole-motility, citrate, oxidase and urease tests.

Paper IV

Blood culture

Pediatric blood culture bottles with blood from the sick babies were incubated in an automated incubator (BD BACTEC™ FX40, Becton, Dickinson and Company Microbiology Systems, Merryland, USA) at 37°C for up to five days before considering them negative. For bottles flagged positive, blood culture aliquots were sub-cultured on blood, chocolate and MacConkey agar (BioLab Budapest, Hungary) and incubated at 35°C to 37°C for up to 96 hours. Bacterial identification was performed as described above for papers I, II and III.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) of the bacterial isolates was performed using Kirby-Bauer disk diffusion method according to the Clinical Laboratory Standard Institute guidelines (CLSI) (83). To identify ESBL-resistant bacteria, we used the combination disk method (84). To identify macrolide-lincosamide-streptogramin B (MLSB) resistance phenotype among *S. aureus* isolates, we performed the D test (83). *S. aureus* isolates were considered to have a constitutive MLSB resistance phenotype (cMLSB) if they were resistant to both erythromycin and clindamycin. Alternatively, if the *S. aureus* isolates were resistant to erythromycin and susceptible to clindamycin but there was a D-shaped inhibition zone around the clindamycin disk, we considered the isolates to have an inducible

MLSB resistance phenotype (iMLSB). To identify methicillin-resistant *S. aureus* (MRSA), we performed multiplex PCR, as described by McClure *et al.* (85). In this assay, the presence of the *mecA* methicillin resistance gene was used to identify MRSA, while the presence of the gene for the Panton-Valentine Leukocidin virulence factor indicated that the MRSA is capable of destroying white blood cells, inducing necrosis and apoptosis (86). The PCR products (the amplicons) were separated on a 2% agarose gel stained with ethidium bromide and visualized by using a UV trans-illuminator.

Study variables

Paper I: The main outcome was vaginal colonization with potentially pathogenic bacteria. Colonization with potentially pathogenic bacteria was defined as isolation of at least one of the following types of bacteria from the vaginal swab: *S. aureus, E. coli*, *K. pneumoniae,* GAS*,* GBS, *Enterococcus* spp*., Pseudomonas* spp*., Enterobacter* spp*., Citrobacter* spp*., Proteus* spp*.,* and/or *Acinetobacter* spp. Other isolated bacteria considered to represent commensal strains include: *Micrococcus* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Bacillus* spp., *Bukolderia* spp., *Serratia* spp., and coagulase-negative *Staphylococcus*. Such isolates were not included in the analyses.

The exposure and descriptive variables included level of maternal education, maternal age, marital status, number of previous pregnancies, antenatal tetanus toxoid vaccination, number of antenatal care visits, hospitalization during pregnancy, possession of domestic animals at home, and socio-economic status. Socio-economic status was represented by a wealth index variable which was generated by performing principal component analysis on data about household ownership of cupboards, radios, televisions, a mobile phone, refrigerator, motorcycle, car, ownership of a house and/or land, and presence of cemented walls, type of toilet, and three or more rooms in the house. Five quintiles of the wealth index variables were generated with the poorest belonging to quintile 1, and the least poor to quintile 5.

Paper II: The main outcome of this study was colonization with clinically important AMR bacteria with resistance patterns of MDR, ESBL and MRSA. The descriptive variables were the same as those described under paper 1 above.

Paper III: The main outcome of this study was omphalitis defined as presence of pus at the umbilical cord stump. A swab was considered culture-positive if one or more of the following potential pathogens were isolated; *S. aureus, E. coli*, *K. pneumoniae, S. pyogenes* (GAS)*, Group* B *Streptococcus* (GBS), *Enterococcus* spp.*, Pseudomonas* spp.*, Enterobacter* spp.*,* and *Citrobacter* spp. The main exposure was vaginal colonization with potentially pathogenic bacteria as previously defined in paper I (87). We collected data on other exposure and descriptive variables such as maternal age, maternal education, socio-economic status, mode of delivery, gravidity, sex of the baby, birth weight, ingestion of breastmilk, initiation of breastfeeding.

Paper IV: In the neonatal sepsis study, the main outcome of the study was culture-positive sepsis. A positive blood culture was defined by the isolation of at least one of the following pathogens in blood; *S. aureus, E. coli, K. pneumoniae, S. pneumoniae, N. meningitides,* GBS*, S. pyogenes, C. freundii, Enterococcus* spp.*, Salmonella* spp.*, Acinetobacter* spp.*, Pseudomonas* spp.*,* and *Enterobacter* spp. Secondary outcomes included: antibiotic resistance of the isolated pathogens and death of the neonates participating in the study. The independent variables included delivery mode, birth weight, sex, antibiotic use before admission, prior hospitalization and its duration, maternal history of fever, foul smelling vaginal discharge during their last trimester of pregnancy.

Number of study participants and statistical precision

For the three studies (papers I, II, III) nested into the CHX trial, we included mothers and their children who were recruited in the CHX trial up to July 2018.

Paper I and II: We enrolled 1,472 women. With this sample size, we obtained a very high (0.7% to 2.6%) absolute precision for the study outcomes (i.e. the difference between the upper limit and the lower limit of the 95% confidence interval (CI) for prevalence values ranging from 2% to 50%).

Paper III: The sample size was limited by the size of the parent CHX trial. We aimed to include 800 children from the dry cord care arm of the trial when its sample size was planned at 1600 children. We enrolled 769 into our study. With this sample size, we

obtained a high (1.0% to 2.5%) observed absolute precision for omphalitis risk values ranging from 2% to 15%.

Paper IV: Sample size was calculated based on the main objective, which was to estimate the proportion of neonates with culture-positive sepsis among those with admitted with clinical signs of sepsis. We estimated a sample size of 359 neonates with clinical sepsis given the prevalence of culture proven septicemia was 37% (88) and a precision of 5%.

Data analysis

The data were analyzed using Stata version 14.0, 15.0 and 17.0 (StataCorp LLC, College Station, TX, USA). We summarized categorical variables as proportions and Chi-squared tests were used to compare them. We described continuous variables using means and their standard deviations (SD) or, when asymmetrically distributed, medians with their interquartile ranges. We performed unadjusted and multivariable (adjusted) regression analyses to determine the association between the independent and dependent variables. When fitting the individual models, we kept independent variables that are known to be predictors of the dependent variable and those that gave *p-values* of <0.25 when tested individually in unadjusted regression analyses as well as those that changed the odds ratio of any other exposure variable in the model by >10%. Crude odds ratios (cOR) or crude risk ratios (cRR) and 95% confidence intervals (95% CI) were estimated for each independent variable. We used the *estat vif* command in Stata to test the models for potential multicollinearity between the independent variables, as indicated by one or more variance inflation factor estimates being >10. None of our models had multicollinearity issues.

Paper I: We obtained an estimate of the overall vaginal colonization prevalence by dividing the number of women with a positive vaginal culture by the total number enrolled women. To explore the associations between the vaginal colonization and exposures, we performed unadjusted and multivariable logistic regression analyses to estimate odds ratios (OR) and 95% confidence intervals for each independent variable.

Paper II: We obtained the overall prevalence of colonization with MDR bacteria by dividing the number of women colonized with MDR bacteria by the total number of women enrolled into the study. To obtain the overall prevalence of resistance with clinically

important bacteria such as MRSA, MLSB-resistant *S. aureus*, VRSA, VRE, ESBL and carbapenem-resistant bacteria, we divided the number of women colonized with such bacteria by the total number of enrolled women in the study. All proportions were reported with their respective 95% confidence intervals. We conducted multivariable logistic regression analyses to explore the association between the different independent variable, and the main outcomes of vaginal colonization with MDR bacteria, ESBL-producing bacteria and MRSA.

Paper III: We defined the neonatal incidence risk of omphalitis as the proportion of neonates who had at least one episode of omphalitis during their first 28 days of life. When calculating the incidence risk for each specific follow-up period (from birth to day 3, day 4 to 7, day 8 to 14 and day 15 to 28), we based the calculations on the mid-period population size. We calculated person-time under observation by summing up the time each neonate contributed up to 28 days of life or until they were censored (got omphalitis, died or were lost to follow-up). Time to the outcome was calculated as the age of the neonate, in days, at the time they were first diagnosed with omphalitis. Neonates who did not develop omphalitis contributed person-time up until their last registered visit to the clinic. The neonates did not contribute person-time during missed visits because for these neonates, we based the diagnosis of omphalitis on information obtained from the mothers during the first subsequent visit. According to their caretaker, none of the neonates who missed a scheduled visit experienced omphalitis during that period. We defined incidence rate of omphalitis as the ratio of the number of new omphalitis cases divided by the total person-time under observation. Incidence rate was converted to risk by using the formula: incidence rate × person-time under observation.

Kaplan-Meier curves were generated using the *sts graph* code in Stata. To estimate the association between vaginal colonization during birth with potentially pathogenic bacteria and subsequent neonatal omphalitis, we fitted a Cox proportional hazard regression model to estimate the hazard ratio (HR). The final model included vaginal colonization as the main exposure, and level of education, maternal age, socio-economic status, baby sex, birthweight, and delayed initiation of breasting feeding as potential cofounders. We tested the independent variables for collinearity.

Paper IV: We conducted unadjusted and multivariable logistic regression analysis to assess factors associated with culture-positive sepsis. To assess the association between culture-positivity and risk of subsequent death, we estimated the corresponding risk ratio (RR) with a generalized linear model of the binomial family with a log link.

Ethical considerations

In all the studies, written informed consent was obtained for the interview, specimen collection and sample storage. For papers I-III, ethical approval was obtained from the Research and Ethics Committee of School of Medicine, SOMREC, Makerere University (REC 2015-118) and from the Uganda National Council of Science and Technology (HS 1927). For paper IV, we obtained ethical approval from the Mulago National Referral Hospital Research and Ethics Review Committee (MHREC-1069), and written informed consent from parents/primary caretakers of the neonates enrolled in the study. Our application for ethical approval was assessed by one of Norway's regional ethics committees, and deemed to be ineligible for consideration in Norway, because the study was done and assessed ethically in Uganda.

Main results of the studies

The first three studies were nested in the chlorhexidine trial. Figure 4 is a schematic illustration of the relationship between the first three studies and the chlorhexidine trial.

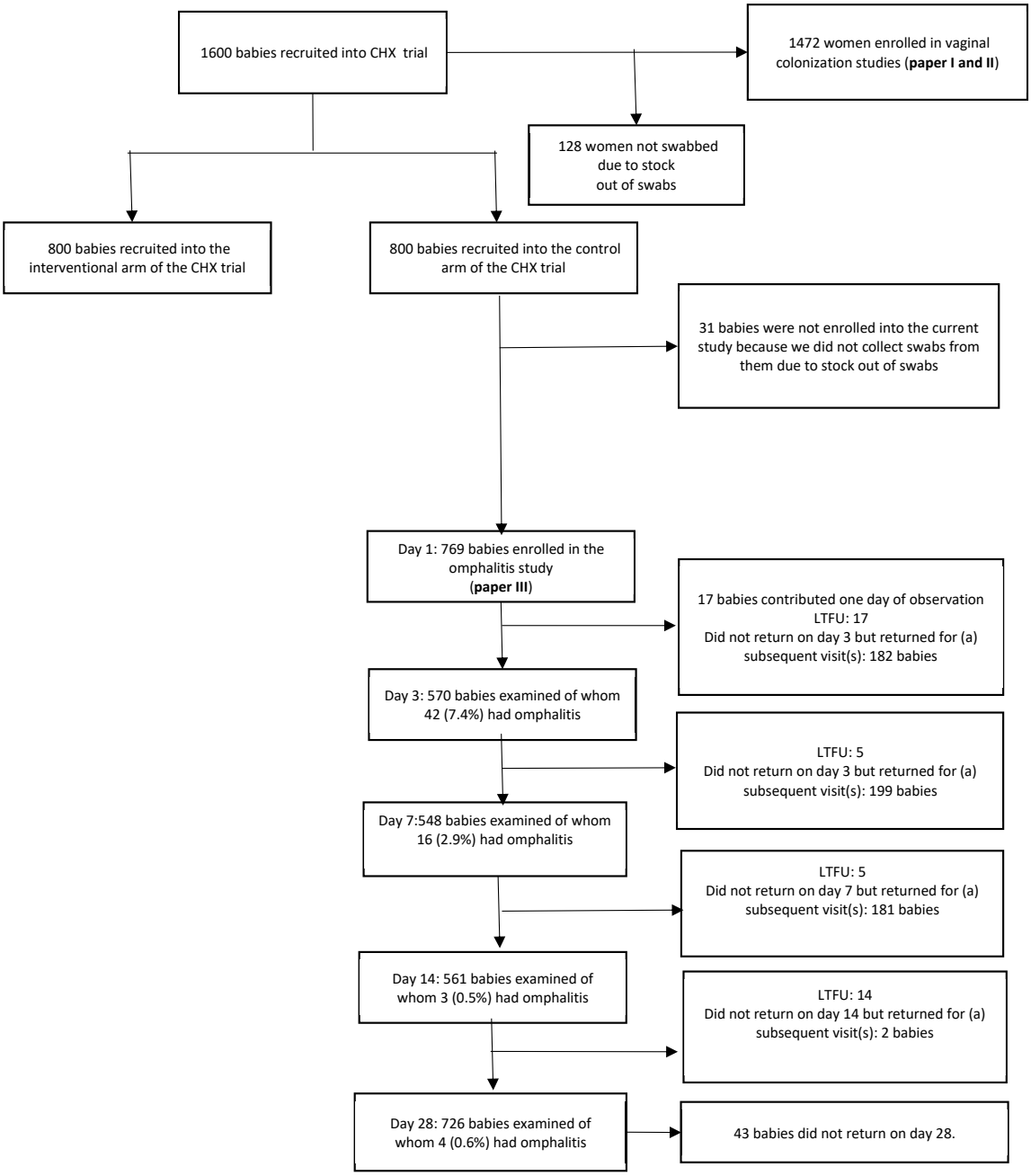

Figure 4: Schematic illustration of the relationship between the first three studies (paper I, II, III) and the chlorhexidine trial

Paper I: Vaginal colonization of women in labor

Participants: We recruited a total of 1,472 women which included 545 (37.0%) in Kawaala Health Centre III, 524 (36%) in Kitebi Health Centre III and 403 (27%) in Mukono Health Centre IV. The participants had a mean age of 25 years (5 SD) and 488 (33%) of them had a primary level education.

Vaginal colonization prevalence estimates: We obtained 1,025 potentially pathogenic bacterial isolates from vaginal specimens of 955 (65%, 95% CI 62%, 67%) of the 1,472 women. Of the women who were colonized, swabs from 878 yielded one bacterial isolate each, 69 women were colonized with two different bacterial isolates each, and three women had three bacterial isolates each. The most common isolated potential bacterial pathogens were *E. coli* (n=508; 34.5%, 95% CI 32.1%, 37.0%), *K. pneumoniae* (n=145; 9.9%, 95% CI 8.4%, 11.5%) and *S. aureus* (n=121; 8.2%, 95% CI 6.9%, 9.7%). There were no major differences in proportions of women colonized by potentially pathogenic bacteria across the three study sites.

Factors associated with vaginal colonization: We found that women who had a history of atleast five previous pregnancies, had 41% less odds of colonization by any potentially pathogenic bacteria, compared to those with only one previous pregnancy (aOR 0.59; 95% CI 0.35, 0.97). The odds of vaginal colonization with any potentially pathogenic bacteria among older women (≥30 years) were 1.52 times those among younger women (aOR 1.52; 95% CI 1.03, 2.23). There was no association between vaginal colonization with any potential pathogen and presence of domestic animals at home or wealth status of the mothers. There was no association between vaginal colonization and socioeconomic status, maternal education and attendance at antenatal care clinics.

In regard to the three most frequently isolated potentially pathogenic bacteria, we found that maternal age of ≥30 years (aOR 2.17; 95% CI 1.17, 4.00) and a history of at least 5 previous pregnancies (aOR 0.33; 95% CI 0.12, 0.88) were associated with *S. aureus* vaginal colonization. Women who owned domestic animals at home had 44% less odds of colonization by *E. coli* (aOR 0.56; 95% CI 0.3, 0.91). We found no factors associated with vaginal colonization by *K. pneumoniae*.

Paper II: Vaginal colonization with antimicrobial resistant bacteria

Participants: This study used the same cohort of participants described in study I above.

Colonization with ESBL-producing bacteria: Of the 1,472 women, 57 (3.9%; 95% CI: 3.0%, 5.1%) were colonized with ESBL-producing bacteria, all of which were *Enterobacteriaceae*. The 57 isolates recovered from these 57 women included 36 *E. coli*, 16 *K. pneumoniae,* three *Citrobacter* spp., one *K. oxytoca*, and one *Enterobacter* spp. With the exception of one *E. coli* and one *K. pneumoniae* isolate, all these ESBL-producing *Enterobacteriaceae* isolates were multi-drug resistant (MDR) strains.

Colonization with carbapenem-resistant bacteria: We found that 27 of the 1,472 women (1.8%; 95% CI: 1.2%, 2.7%) were colonized with carbapenem-resistant bacteria. Of these 27, a total of 26 were carbapenem-resistant *Enterobacteriaceae* and one was carbapenem-resistant *Acinetobacter* spp. All the carbapenem-resistant bacteria were MDR. There were no *Pseudomonas* spp. strains isolated.

MRSA, MLSB, VRSA and VRE colonization: We found that 117 of the 1,472 women were colonized with *S. aureus* of which forty-five were MRSA. All the MRSA isolates were MDR. Only 2 (3.4%) of the 45 MRSA isolates were also positive for the Panton-Valentine Leukocidin virulence factor. Three other Panton-Valentine Leukocidin-positive isolates were from methicillin sensitive *S. aureus*. Eighteen of the 1,472 women (1.2% [95% CI 0.7%, 1.9%]) were colonized with induced macrolide lincosamide-streptogramin B (iMLSB)-resistant *S. aureus* and fifteen of them were also MRSA. Twenty-four (1.6% [95% CI 1.0%, 2.4%]) women were colonized with constitutive MLSB (cMLSB)-resistant *S. aureus*. The proportions of women colonized with VRSA and VRE were the same at 0.4% (6/1,472; 95% CI 0.1%, 0.9%). All 12 isolates from these VRSA and VRE colonization were MDR.

Colonization with MDR bacteria: The prevalence of MDR colonization among women was 50.9% (750/1472; 95% CI 48.4%, 53.5%). This means that 750 of the 955 vaginally colonized women (78.5%) had multidrug resistant bacteria. The majority of colonization with MDR bacteria included *E. coli* (62.1% [426/686]), *K. pneumoniae* (13% [89/686]) and *S. aureus* (12.2% [84/686]).

Factors associated with colonization with AMR bacteria: Focusing on the three most common types of important AMR bacteria found in this study, including ESBL-producing bacteria, MRSA, and MDR bacteria, we found few factors that were associated with an increased or decreased odds of colonization in these women. Among the most significant associations, we found that being ≥30 years old was associated with increased odds of being colonized with MRSA (aOR: 3.03 [95% CI: 1.51, 6.07]) or MDR (aOR: 1.56 [95% CI: 1.09, 2.24]), compared to being aged between 20 and 24 years.

Paper III: Omphalitis in neonates

Participants: Out of 800 targeted for recruitment into the study, 769 neonates were included in the study. The 31 neonates were not swabbed because of stockouts but these neonates were similar to the swabbed neonates. Their mean birth weight was 3.2 kg (SD 0.4). Nearly all participants (99.8%) had a normal vaginal birth. Most participants 95.7% (736/769) initiated breastfeeding within the first hour and 13.8% reported to have applied a potentially unclean substance to the umbilical cord stump during the study period.

Incidence of omphalitis: Sixty-five (8.5%) of the 769 babies developed omphalitis during follow-up and majority (58 of 65; 89.2%) of the 65 cases occurred in the first week of life. The risk of omphalitis was 6.3% by day 3 (42/670; 95% CI 4.0%, 7.3%), 2.9% from day 4 to day 7 (16/559; 95% CI 1.3%, 3.5%) and 1.1% between day 8 and day 28 (7/644; 95% CI 0.40%, 2.0%). Overall, the neonatal incidence rate of omphalitis was 0.095 cases per 28 child-days (95% CI 0.073, 0.121), translating into a neonatal cumulative incidence, i.e., risk, of 9.5% (95% CI 7.3%, 12.1%).

Etiology: Of the 65 infants with omphalitis who had a bacterial culture done, we isolated atleast one species of potentially pathogenic bacteria from 41 (63.1%, 41/65) neonates. Of the 41 neonates, we isolated 43 potentially pathogenic bacteria, 39 neonates were colonized with one bacterial species and 2 neonates were colonized with two bacterial species each. The most commonly isolated potentially pathogenic bacterial species from neonates with omphalitis was *E. coli* 27.7% (18/65), *K. pneumoniae* 15.4% (10/65), *C. freundii*., 7.7% (5/65) and *Enterobacter* spp. 6.1% (4/65). Other bacterial species isolated included *Acinetobacter* spp., 4.6% (3/65), *S. aureus* 3.1% (2/65) and *K. oxytoca* 1.5% (1/65)

Antimicrobial resistance profiles: Among the *Enterobacteriaceace* isolates, 86.8% (33/38) were resistant to ampicillin, 73.7% (28/38) were resistant to amoxicillin-clavulanic acid and (60.5%, 23/38) were resistant to trimethoprim-sulfamethoxazole. There was relatively low proportion of strains that were resistant to third generation cephalosporins such as ceftriaxone (6/38), ceftazidime (4/38) and to gentamicin (4/38). All bacteria but one were susceptible to imipenem. One of the two *S. aureus* isolate in this study was methicillin resistant (MRSA) and phenotypically expressed erythromycin inducible clindamycin resistance (D-test positive). We did not find extended-spectrum beta-lactamase and carbapenem resistant gram-negative bacteria.

Factors associated with omphalitis: We found that neonates of women who were vaginally colonized with potentially pathogenic bacteria were almost equally likely to get omphalitis compared to those of women who were not colonized (adjusted hazard ratio (aHR) 1.1; 95% CI 0.63, 1.9). The hazard of omphalitis among neonates initiated late on breast milk were approximately 3 times that among neonates who were initiated early (aHR 3.1; 95% CI 1.3, 7.3). Other maternal and neonatal characteristics were not strongly associated with omphalitis.

Paper IV: Neonatal sepsis study

Participants: We recruited 359 neonates with clinical sepsis whose mean age was 8 days (sd 7.1). Their mean birth weight was 3.1 kg (sd 0.6) and the median duration of hospitalization was 5.4 (IQR 4, 6) days.

Bacterial etiology: Thirteen percent of neonates, 12.8% (46 /359; 95 % CI 9.5%, 16.7%) had a positive blood culture and one pathogen was isolated per participant. Of the 46 infants with sepsis, 15 (32.6%) had early-onset, whereas 31 (67.4%) had late-onset sepsis. *S. aureus* 63% (29/46) was the most common pathogen. Other isolated pathogens included *E. coli* isolates 15.2% (7/46), *K. pneumoniae* isolates 10.7% (5/46), *Enterococcus* spp. 4.3% (2/46), *S. pneumoniae* 2.2% (1/46), *Neisseria* spp 2.2% (1/46). and *C. freundii* isolate 2.2% (1/46).

Antimicrobial resistance profiles: Forty-one percent (19/46; 95% CI 27.0%, 56.8%) of the babies with culture-positive sepsis had MRSA. Overall, the proportion of participants with MRSA was 5.3% (19/359; 95% CI 3.2%, 8.1%). Three gram-negative bacterial pathogens phenotypically exhibited ESBL resistance translating to a proportion of 6.5% (3/46; 95% CI

1.4%, 17.9%) among neonates with positive-culture sepsis. All gram-negative bacteria isolated in this study were susceptible to carbapenem antibiotics. All *K. pneumoniae* and six *E. coli* isolates were resistant to ampicillin while resistance to gentamicin was observed in two (28.6%) of seven *E. coli* isolates and in two (40%) of five *K. pneumoniae* isolates. Three of the *K. pneumoniae* isolates, one *E. coli* and one *Neisseria* spp. isolates were resistant to third-generation cephalosporins (ceftriaxone and ceftazidime). Overall, the proportions of babies with pathogens resistant to first-line was 13.9% (50/359, 95% CI; 10.5, 17.9) and to second-line antibiotics was 1.1% (4/359, 95% CI; 0.3, 2.8).

Factors associated with culture-positive sepsis: The odds of sepsis among neonates born by cesarean section were three times among those born by spontaneous vaginal delivery (aOR 3.45, 95% CI; 1.19, 10.05).

Case fatality risk: The case fatality risk for culture-positive sepsis was 15.2% (7/46; 95% CI 6.3%, 28.9%) and 8.6% (27/313; 95% CI 5.8%, 12.3%) in the culture negative babies. Four out of the seven neonates that died and were culture-positive had either MRSA or ESBL-producing pathogens in their blood culture.

Discussion

In this thesis, we undertook studies on the maternal colonization and neonatal infections in central Uganda. The studies focused on the bacteriological profile of vaginal colonization of women in labor, factors associated with vaginal colonization, incidence of omphalitis and associated risk factors, and bacteriological profile of neonatal culture-positive sepsis, factors associated with neonatal culture-positive sepsis as well their clinical outcomes. It is the findings from these studies that we discuss below.

Discussion of main findings

Prevalence of vaginal colonization

About two-thirds (65%) of the women we studied were colonized by at least one potentially pathogenic bacterial species. This estimate is similar to that observed among Indian women in labor (89), among women with obstructed labor in western Uganda (90) and among

Bangladeshi women in labor (91). The most common potentially pathogenic bacteria isolated from the women in labor were *E. coli, K. pneumoniae* and *S. aureus*. These findings are in agreement with studies in western Uganda, Nigeria and the Bangladesh (91, 92, 93). Evidence suggests that bacteria that colonize the vagina of women in labor play an important role in neonatal health (94). The distribution of pathogens from the women we studied was similar to that from the neonates in our study (95, 96). This observation could be suggestive of a link between maternal colonization and neonatal infections but this link was not studied in this thesis.

In this study, we found a very low proportion of the participants colonized by GBS, contrary to studies from southwestern Uganda reporting higher recto-vaginal colonization in pregnant women (90, 97). Unlike those studies, we did not use Todd Hewitt medium for GBS isolation which could explain the difference. GBS colonization among pregnant women is reported to vary geographically, ranging from 4% to 35% worldwide (98). Although GBS is an important cause of neonatal infections, a systematic review reported *S. aureus*, *K. pneumoniae* and *E. coli* to be the most common cause of neonatal infections in 26 countries in sub-Saharan Africa (5). GBS screening in late pregnancy and intrapartum antibiotic prophylaxis during labor (usually intravenous penicillin) is practiced as a preventive measure to reduce neonatal GBS-related morbidity and mortality (99). However, implementation of such strategies varies across countries. For instance, in the United States, there is universal antenatal screening of pregnant women for GBS colonization (100), while in the United Kingdom, there is risk based screening (101). Asymptomatic carriage of GBS also causes significant maternal and perinatal morbidity (102). These raise a question as to whether resource limited settings such as Uganda should conduct routine screening and treatment of pregnant women for both GBS and non-GBS pathogens. Screening women could prevent early-onset neonatal sepsis but would be expensive and may overload the weak health care systems in resource-limited settings.

Vaginal colonization with antimicrobial resistant bacteria

Of great concern, about half of the women in our study were colonized by MDR bacteria including MRSA, vancomycin-resistant *Enterococci* or *Staphylococci* species and carbapenem-resistant E*nterobacteriaceae*. It is worth noting that the women we studied

were healthy women in labor, the majority of whom had no symptoms from the infections we identified. Although direct comparison of results between studies is complicated by the lack of consensus on how MDR should be defined, the internationally acknowledged MDR definition we used makes our findings possible to compare to a similar study (103). Antimicrobial susceptibility testing is not standardized internationally as there is no universal antimicrobial testing policy. There are several guidelines including European committee on antimicrobial susceptibility testing (EUCAST), CLSI and Food and drug administration (FDA). These guidelines provide different breaking points and interpretation of microbial susceptibility or resistance. For instance, comparing the definition of multidrug resistance globally is problematic, as not all antibiotics are available and tested by laboratories in different countries.

The colonization prevalence of ESBL-producing bacteria was low, similar to estimates by Rettedal S *et al*. in Norway (104) and Villar HE *et al.* in Argentina (19). However studies from Mwanza (105), Dhaka (106) and central India (107) report prevalence estimates three times higher than that reported in this study. This discrepancy may be explained by differences in methodological methods such as some studies detecting ESBL using molecular methods, others by conventional procedures, as well as genuine epidemiologic differences. Vaginal colonization prevalences of carbapenem-resistant bacteria, VRSA, erythromycin-inducible clindamycin resistant bacteria in this study were similar to those reported from other comparable studies (19, 104, 108, 109, 110, 111, 112). However, the prevalence of MRSA vaginal colonization was twice that reported by Andrews WW *et al*. among pregnant women (113) and Chen KT et al (114) among postpartum women. However, these studies were conducted in high-income countries and the women they studied were older compared to ours.

Colonization of the birth canal by multidrug resistant bacteria during labor is worrisome as it increases the risk of adverse delivery outcomes. One of the common complications during delivery is PROM, which is associated with an increased risk of ascending infections, such as chorioamnionitis (115). In the event that the mother is colonized with MDR bacteria, ascending infections resulting from PROM could be difficult to treat using current guidelines. The current recommendation suggests that, women who experience PROM are

managed with prophylactic erythromycin, ampicillin and benzylpenicillin (116). However, our study shows resistance to these antibiotics. Our study findings build on the existing body of knowledge and can be used to influence change in health policies regarding antibiotic use.

Incidence, bacteriological profile and risk factors of omphalitis

With regard to the babies born to the women in our study, we found that the risk of omphalitis in the study participants was almost 10% in the first 28 days of life. This finding is worrying because omphalitis is associated with neonatal sepsis in 2 out of every 100 cases (28). A cohort study in Pakistan found results similar to our study (24). However, previous randomized control studies in Africa and Asia estimated the omphalitis incidence proportions to range from 1% to 8% (24, 117, 118, 119). The differences in the incidence proportions could result from different case definitions of omphalitis across the studies. Our study had a strict case definition in which we defined omphalitis as presence of pus while other studies (Tanzania and Bangladesh) defined omphalitis based on redness and/or swelling with or without pus. In addition, we determined the potentially pathogenic bacteria that could have caused omphalitis and their antimicrobial susceptible patterns.

Gram-negative bacteria were the most common bacteria isolated from the omphalitis cases, similar to what was observed in other Indian studies (120, 121). However, other community-based studies in Southeast Asia identified gram-positive bacteria as the most common cause of omphalitis (28, 122, 123). It is not clear why we predominately isolated gram-negative bacteria rather than the expected gram-positive bacteria. Most (87%) of the isolated gram-negative bacteria in our study exhibited resistance to the first-line antibiotics used for treating neonatal infections. We also observed resistance to third generation cephalosporin antibiotics. These findings are consistent to those in a cohort study in Pakistan (28). There is limited published microbiological data on omphalitis. The WHO recommends ampicillin and gentamicin as first-line treatment for serious neonatal infections and cloxacillin is the first-line treatment for umbilical infections when *S. aureus* is the presumed cause (28, 123). However, cloxacillin will not work when the umbilical cord stumps of neonates with omphalitis are primarily colonized with gram-negative bacteria.

Our findings advocate for periodic and context specific culture and sensitivity results to guide generic treatment options.

We found that neonates who were initiated late on breastmilk had increased risk of omphalitis compared to neonates who were initiated early. These findings are similar to those of a study conducted in Pemba (124), which found that neonates who breastfed in the first hour of life had lower risk of omphalitis. Initiating breastfeeding in the first hour of life reduces neonatal morbidity and mortality, possibly through the transfer of protective antibodies to the babies through colostrum (125, 126). Alternatively, there could be reverse causality in which the babies with an unidentified omphalitis were unable to suckle, therefore initiating breastfeeding late but I find this an unlikely explanation because we observed the umbilical cord stumps of our study babies very carefully and babies born with signs of omphalitis were excluded from the study. Contrary to what has been found elsewhere (7), we did not observe an association between vaginal colonization of women in labor and omphalitis in this study.

Bacteriology profile of culture-positive sepsis and associated factors

The study of neonatal sepsis (paper IV) aimed to determine the proportion of newborns who had a positive blood culture among newborns with signs and symptoms of clinical sepsis at the Ugandan national referral hospital. It also aimed to describe the bacteria isolated in septic neonates, and the antimicrobial resistance profiles of isolated bacteria as well as factors associated with culture-positive sepsis. The proportion of neonates with culture-positive sepsis among those with symptoms and signs of sepsis was 13%. The most common pathogen isolated from babies with culture-positive sepsis was *S. aureus*. These findings were similar to those from previous studies in East Africa (79, 127). In addition, *S. aureus* is the main cause of neonatal sepsis in other African studies compared to studies from South Asia where gram-negative bacteria, especially *Acinetobacter* spp. and *K. pneumoniae* were the main cause (53, 128). The predominance of *S. aureus* among neonates with culture-positive sepsis in our study may indicate vertical transmission, because this pathogen was commonly observed among babies with early-onset sepsis in our study.

We observed extensive bacterial resistance to antibiotics commonly used to treat neonatal sepsis. Ampicillin and gentamicin were the first-line antibiotics used to treat almost all the

neonates in our study. Among the pathogens isolated from the blood of participants, almost three quarters were resistant to ampicillin and almost one quarter to gentamicin. This is similar to what has been reported in similar settings in Tanzania (127), Ethiopia (129), Zambia (130). On the contrary, a Ugandan study previously conducted at the same hospital reported resistance to gentamicin to be lower than that in our study (88). The differences observed could be explained by the continuous spread of antimicrobial resistance through remarkable and worrisome acquisition of antimicrobial resistance genes. Four of 14 (29%) gram-negative pathogens in our study were resistant to third-generation cephalosporins. Although imprecisely estimated, the proportions of resistance to third-generation cephalosporin in our study were similar to those observed in other African studies (129, 131).

ESBL producers accounted for 6.5% of the isolated pathogens. Other studies in low-income settings reported proportions of ESBL pathogens ranging from 11% to 25% among pathogens from neonates with sepsis (105, 132, 133, 134). The presence of multidrug resistant bacteria such as ESBL, MRSA in the blood of neonates with sepsis is an important risk factor as it associated with long hospitalization stays and high fatality risk (135, 136). In-fact, two of the 3 neonates with ESBL in this study died.

We found that the odds of developing culture-positive sepsis among neonates born through cesarean section were three times those born through vaginal delivery. Our findings are similar to those from other studies (129, 137) that found an association between cesarean section and neonatal sepsis. This finding is not surprising, especially in settings with inadequate infection prevention and control practices. This association may be explained by underlying indications for cesarean section such as obstructed labor, PROM and prolonged labor since such indications predispose the neonate to infection. In contrast to other studies (45, 138), we did not find strong associations between low birth weight, PROM and culture-positive sepsis. We did not assess preterm birth in our study although is an important risk factor for sepsis.

Methodological considerations

Internal validity

Here, I discuss methodological aspects that may be a threat to the validity of our findings. These aspects are related to the study design, data collection, analysis process and they include; selection bias, information bias, confounding and random error.

Selection bias and confounding

Participants in papers I and II were women whose babies were enrolled into the CHX trial while participants in paper III were newborns enrolled into the dry cord care arm of the trial. Thirty-one babies were not enrolled into the study in paper III because of random stockout of swabs. We think selection bias was minimal because these babies similar to those who were swabbed. For paper IV, we enrolled 359 of the 596 neonates with a diagnosis of clinical sepsis admitted from 8am to 5pm hours during weekdays during a one year period. There was a possibility of selection bias in paper IV if the neonates not enrolled into our study were different from those enrolled but we did not collect any data from the neonates not enrolled. We do not think that neonates admitted during day and weekdays are different from those admitted at night and weekends. Therefore, we do not think that selection bias significantly affected our findings. We used multivariable regression analysis as the main method for controlling for confounding. We based on literature to select the confounders we included in the analysis. We acknowledge that there may well have been residual confounding that was not adjusted for.

Follow-up

For paper III in which we conducted a prospective cohort study, 726 out of 769 participants completed the 28 days of follow up therefore loss to follow up was minimal.

Information bias

We put measures in place to reduce on the occurrence of measurement errors. At the start of the study, questionnaires were translated into Luganda; a local language that is spoken by majority of people in central Uganda. Questionnaires were pre-tested to increase clarity of the questions. During the data collection process, the study nurses had regular refresher trainings on interviewing skills to ensure that they asked the questions exactly as they were

written in the questionnaire in a neutral manner without leading the participants towards particular answers. Data were entered directly into electronic questionnaires on the ODK on mobile phones, such direct data entry circumvented the need to transfer information written on paper forms into the data base, thereby reducing the amount of data entry errors. All study procedures or measurements were performed based on standard operating procedures. For the laboratory-based studies in this thesis, bacterial identification was based on culture methods, which is the gold standard. Culture method has poor sensitivity but relatively high specificity compared to PCR based assays. Culture methods have produced reliable findings from many important microbiological studies. We could have underestimated the proportion of neonates with neonatal sepsis, because blood culture in itself has a poor sensitivity (139) and because we did not collect cerebrospinal fluid to diagnose meningitis. Furthermore, we did not use Tod Hewitt selective medium for GBS isolation, which might have underestimated the proportion of GBS isolates in our study.

Statistical precision

We enrolled a large sample, which yielded estimates with high statistical precision. In addition to a large sample size, the estimates had a very good precision because we successfully obtained blood samples from a large proportion of sick newborns with very minimal contamination (2%), transported and processed the samples using good microbiological techniques. In papers I and II, we analyzed many outcomes including overall-colonization, *E. coli* colonization, *S. aureus* colonization and *K. pneumoniae* colonization for paper I; and MDR, ESBL, MRSA outcomes for paper II. We assessed associations between several exposures and those outcomes. We, however, think it was unnecessary to adjust for multiple comparisons in these studies because we performed an exploratory analysis and were not trying to prove such associations. Further studies are needed to test the nature and strength of the associations we identified.

External validity

The studies in this thesis were conducted in Kampala and Mukono districts and our findings can be generalized to populations with similar socio-demographic characteristics. The socio-demographic characteristics of the mothers and babies we studied were described in papers I, II, III and IV.

In studies I, II and III, we included low risk HIV negative women delivering from three primary health care facilities in and close to Kampala in Uganda and infants born to such women therefore our findings may to a great extent be generalizable to HIV negative pregnant women in labor in similar settings.

Conclusion

Almost two thirds of women in labor in central Uganda may be vaginally colonized by potentially pathogenic bacteria. Nearly 10% of their neonates experienced omphalitis during the first month of life. Factors associated with vaginal colonization included age, parity and possession of domestic animals in a household. Delayed initiation of breastfeeding was associated with a higher likelihood of omphalitis while cesarean section was associated with culture-positive sepsis in the neonates. The predominant bacteria isolated in the vagina of women in labor were *E. coli*, *K. pneumoniae* and *S. aureus*. A substantial proportion of bacteria isolated from vagina, the umbilical cord stumps and blood from the septic neonates were resistant to first line antibiotics and about one half of the vaginal isolates were multi-drug resistant. This speaks to the expanding body of evidence in support of an urgent need to curb a growing antimicrobial drug resistance pandemic.

Recommendations

1. Further studies to examine the relationship between colonization of the birth canal and subsequent neonatal infections to guide policies on prophylactic or presumptive treatment after rapid diagnosis of relevant infections.

2. Revision of policies regarding antibiotic regimes used to manage infections based on findings from contextually relevant data on antimicrobial resistance, including the studies presented in this thesis.

3. Antimicrobial stewardship programs at hospital and other health-facility levels, to help in generating real-time antimicrobial resistance data and monitoring resistance patterns and trends.

References

1. GBD 2019 Under-5 Mortality Collaborators. Global, regional, and national progress towards Sustainable Development Goal 3.2 for neonatal and child health: all-cause and cause-specific mortality findings from the Global Burden of Disease Study 2019. Lancet (London, England). 2021;398(10303):870-905.
2. Every newborn progress report 2019. Geneva: World Health Organization and the United Nations Children's Fund (UNICEF), 2020. Licence: CC BY-NC-SA 3.0 IGO
3. Uganda Bureau of Statistcs - UBOS. *Uganda Demographic and Health Survey 2016*. Kampala, Uganda and Rockville, Maryland, USA: UBOS and ICF; 2018.
4. Population-based rates, timing, and causes of maternal deaths, stillbirths, and neonatal deaths in south Asia and sub-Saharan Africa: a multi-country prospective cohort study. The Lancet Global health. 2018;6(12):e1297-e308.
5. Okomo U, Akpalu ENK, Le Doare K, Roca A, Cousens S, Jarde A, et al. Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines. The Lancet Infectious diseases. 2019.
6. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. Nature reviews Immunology. 2007;7(5):379-90.
7. UN General Assembly TowtAfSD, 21 October 2015, A/RES/70/1, available at: https://www.refworld.org/docid/57b6e3e44.html [accessed 6 May 2019].
8. Larsen B, Monif GR. Understanding the bacterial flora of the female genital tract. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2001;32(4):e69-77.
9. Galask RP, Larsen B, Ohm MJ. Vaginal flora and its role in disease entities. Clinical obstetrics and gynecology. 1976;19(1):61-81.
10. Barrientos-Durán A, Fuentes-López A, de Salazar A, Plaza-Díaz J, García F. Reviewing the Composition of Vaginal Microbiota: Inclusion of Nutrition and Probiotic Factors in the Maintenance of Eubiosis. Nutrients. 2020;12(2).
11. Redelinghuys MJ, Ehlers MM, Dreyer AW, Kock MM. Normal flora and bacterial vaginosis in pregnancy: an overview. Critical reviews in microbiology. 2016;42(3):352-63.
12. Larsen B, Galask RP. Vaginal microbial flora: composition and influences of host physiology. Annals of internal medicine. 1982;96(6 Pt 2):926-30.
13. Naderi S, Gettner S, Zeighami E, Khairandish MH. Microbiology of the female genital tract during pregnancy and parturition. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 1976;14(1):81-5.
14. Backhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. Cell host & microbe. 2015;17(6):852.
15. Dogra S, Sakwinska O, Soh SE, Ngom-Bru C, Bruck WM, Berger B, et al. Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. mBio. 2015;6(1).
16. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across

multiple body habitats in newborns. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(26):11971-5.
17. Biasucci G, Benenati B, Morelli L, Bessi E, Boehm G. Cesarean delivery may affect the early biodiversity of intestinal bacteria. The Journal of nutrition. 2008;138(9):1796s-800s.
18. Russell NJ, Seale AC, O'Sullivan C, Le Doare K, Heath PT, Lawn JE, et al. Risk of Early-Onset Neonatal Group B Streptococcal Disease With Maternal Colonization Worldwide: Systematic Review and Meta-analyses. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;65(suppl_2):S152-s9.
19. Villar HE, Aubert V, Baserni MN, Jugo MB. Maternal carriage of extended-spectrum beta-lactamase-producing Escherichia coli isolates in Argentina. Journal of chemotherapy (Florence, Italy). 2013;25(6):324-7.
20. Chereau F, Herindrainy P, Garin B, Huynh BT, Randrianirina F, Padget M, et al. Colonization of extended-spectrum-beta-lactamase- and NDM-1-producing Enterobacteriaceae among pregnant women in the community in a low-income country: a potential reservoir for transmission of multiresistant Enterobacteriaceae to neonates. Antimicrobial agents and chemotherapy. 2015;59(6):3652-5.
21. Top KA, Buet A, Whittier S, Ratner AJ, Saiman L. Predictors of Staphylococcus aureus Rectovaginal Colonization in Pregnant Women and Risk for Maternal and Neonatal Infections. Journal of the Pediatric Infectious Diseases Society. 2012;1(1):7-15.
22. Nankabirwa V, Tylleskar T, Tumuhamye J, Tumwine JK, Ndeezi G, Martines JC, et al. Efficacy of umbilical cord cleansing with a single application of 4% chlorhexidine for the prevention of newborn infections in Uganda: study protocol for a randomized controlled trial. Trials. 2017;18(1):322.
23. Mullany LC, Darmstadt GL, Khatry SK, LeClerq SC, Katz J, Tielsch JM. Impact of umbilical cord cleansing with 4.0% chlorhexidine on time to cord separation among newborns in southern Nepal: a cluster-randomized, community-based trial. Pediatrics. 2006;118(5):1864-71.
24. Soofi S, Cousens S, Imdad A, Bhutto N, Ali N, Bhutta ZA. Topical application of chlorhexidine to neonatal umbilical cords for prevention of omphalitis and neonatal mortality in a rural district of Pakistan: a community-based, cluster-randomised trial. Lancet (London, England). 2012;379(9820):1029-36.
25. Mullany LC, Saha SK, Shah R, Islam MS, Rahman M, Islam M, et al. Impact of 4.0% chlorhexidine cord cleansing on the bacteriologic profile of the newborn umbilical stump in rural Sylhet District, Bangladesh: a community-based, cluster-randomized trial. The Pediatric infectious disease journal. 2012;31(5):444-50.
26. Mullany LC, Darmstadt GL, Katz J, Khatry SK, LeClerq SC, Adhikari RK, et al. Risk factors for umbilical cord infection among newborns of southern Nepal. American journal of epidemiology. 2007;165(2):203-11.
27. Mullany LC, Darmstadt GL, Katz J, Khatry SK, Leclerq SC, Adhikari RK, et al. Risk of mortality subsequent to umbilical cord infection among newborns of southern Nepal: cord infection and mortality. The Pediatric infectious disease journal. 2009;28(1):17-20.
28. Mir F, Tikmani SS, Shakoor S, Warraich HJ, Sultana S, Ali SA, et al. Incidence and etiology of omphalitis in Pakistan: a community-based cohort study. Journal of infection in developing countries. 2011;5(12):828-33.

29. Celebi Celik F, Tuzun F, Duman N, Keskinoglu P, Kumral A, Ozkan H. Current factors affecting the risk of omphalitis in newborns: A prospective case-control study. International journal of clinical practice. 2021;75(5):e14071.
30. Mullany LC, El Arifeen S, Winch PJ, Shah R, Mannan I, Rahman SM, et al. Impact of 4.0% chlorhexidine cleansing of the umbilical cord on mortality and omphalitis among newborns of Sylhet, Bangladesh: design of a community-based cluster randomized trial. BMC pediatrics. 2009;9:67.
31. Mullany LC, Darmstadt GL, Tielsch JM. Role of antimicrobial applications to the umbilical cord in neonates to prevent bacterial colonization and infection: a review of the evidence. The Pediatric infectious disease journal. 2003;22(11):996-1002.
32. Sankar MJ, Chandrasekaran A, Ravindranath A, Agarwal R, Paul VK. Umbilical cord cleansing with chlorhexidine in neonates: a systematic review. Journal of perinatology : official journal of the California Perinatal Association. 2016;36 Suppl 1(Suppl 1):S12-20.
33. Odabasi IO, Bulbul A. Neonatal Sepsis. Sisli Etfal Hastan Tip Bul. 2020;54(2):142-58.
34. Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. Lancet (London, England). 2017;390(10104):1770-80.
35. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. Clinical microbiology reviews. 2014;27(1):21-47.
36. Dong Y, Speer CP. Late-onset neonatal sepsis: recent developments. Archives of disease in childhood Fetal and neonatal edition. 2015;100(3):F257-63.
37. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N. The global burden of paediatric and neonatal sepsis: a systematic review. The Lancet Respiratory medicine. 2018;6(3):223-30.
38. Popescu CR, Cavanagh MMM, Tembo B, Chiume M, Lufesi N, Goldfarb DM, et al. Neonatal sepsis in low-income countries: epidemiology, diagnosis and prevention. Expert review of anti-infective therapy. 2020;18(5):443-52.
39. Seale AC, Blencowe H, Manu AA, Nair H, Bahl R, Qazi SA, et al. Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and Latin America for 2012: a systematic review and meta-analysis. The Lancet Infectious diseases. 2014;14(8):731-41.
40. Satar M, Ozlu F. Neonatal sepsis: a continuing disease burden. The Turkish journal of pediatrics. 2012;54(5):449-57.
41. Bandyopadhyay T, Kumar A, Saili A, Randhawa VS. Distribution, antimicrobial resistance and predictors of mortality in neonatal sepsis. Journal of neonatal-perinatal medicine. 2018;11(2):145-53.
42. collaboration IotDNISD. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. The Lancet Global health. 2016;4(10):e752-60.
43. Zaidi AK, Thaver D, Ali SA, Khan TA. Pathogens associated with sepsis in newborns and young infants in developing countries. The Pediatric infectious disease journal. 2009;28(1 Suppl):S10-8.
44. Chan GJ, Lee AC, Baqui AH, Tan J, Black RE. Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. PLoS Med. 2013;10(8):e1001502.
45. Schrag SJ, Cutland CL, Zell ER, Kuwanda L, Buchmann EJ, Velaphi SC, et al. Risk factors for neonatal sepsis and perinatal death among infants enrolled in the prevention of

perinatal sepsis trial, Soweto, South Africa. The Pediatric infectious disease journal. 2012;31(8):821-6.
46. Puopolo KM, Draper D, Wi S, Newman TB, Zupancic J, Lieberman E, et al. Estimating the probability of neonatal early-onset infection on the basis of maternal risk factors. Pediatrics. 2011;128(5):e1155-63.
47. Laxminarayan R, Matsoso P, Pant S, Brower C, Rottingen JA, Klugman K, et al. Access to effective antimicrobials: a worldwide challenge. Lancet (London, England). 2016;387(10014):168-75.
48. Blomberg B, Manji KP, Urassa WK, Tamim BS, Mwakagile DS, Jureen R, et al. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. BMC infectious diseases. 2007;7:43.
49. Blomberg B, Jureen R, Manji KP, Tamim BS, Mwakagile DS, Urassa WK, et al. High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. Journal of clinical microbiology. 2005;43(2):745-9.
50. Downie L, Armiento R, Subhi R, Kelly J, Clifford V, Duke T. Community-acquired neonatal and infant sepsis in developing countries: efficacy of WHO's currently recommended antibiotics--systematic review and meta-analysis. Archives of disease in childhood. 2013;98(2):146-54.
51. Organization WH. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. Geneva: World Health Organization 2017(WHO/EMP/IAU/2017.12). (Licence: CC BY-NC-SA 3.0 IGO.. 2017.
52. Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. Lancet (London, England). 2005;365(9465):1175-88.
53. Waters D, Jawad I, Ahmad A, Luksic I, Nair H, Zgaga L, et al. Aetiology of community-acquired neonatal sepsis in low and middle income countries. Journal of global health. 2011;1(2):154-70.
54. Rice LB. Mechanisms of resistance and clinical relevance of resistance to β-lactams, glycopeptides, and fluoroquinolones. Mayo Clinic proceedings. 2012;87(2):198-208.
55. Zgurskaya HI, Löpez CA, Gnanakaran S. Permeability Barrier of Gram-Negative Cell Envelopes and Approaches To Bypass It. ACS infectious diseases. 2015;1(11):512-22.
56. Schwarz S, Loeffler A, Kadlec K. Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine. Veterinary dermatology. 2017;28(1):82-e19.
57. Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. Microbiology spectrum. 2016;4(2).
58. Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ. Molecular mechanisms of antibiotic resistance. Nature reviews Microbiology. 2015;13(1):42-51.
59. Fernandes R, Amador P, Prudêncio CJRiMM. β-Lactams: chemical structure, mode of action and mechanisms of resistance. 2013;24(1):7-17.
60. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. BioMed research international. 2014;2014:249856.
61. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi journal of biological sciences. 2015;22(1):90-101.

62. Walsh C, Wencewicz T. Antibiotics: challenges, mechanisms, opportunities: John Wiley & Sons; 2020.
63. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrobial agents and chemotherapy. 2010;54(3):969-76.
64. Yu WL, Chuang YC, Walther-Rasmussen J. Extended-spectrum beta-lactamases in Taiwan: epidemiology, detection, treatment and infection control. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi. 2006;39(4):264-77.
65. Paterson GK, Harrison EM, Holmes MA. The emergence of mecC methicillin-resistant Staphylococcus aureus. Trends in microbiology. 2014;22(1):42-7.
66. Foster TJ. Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. FEMS microbiology reviews. 2017;41(3):430-49.
67. Arzanlou M, Chai WC, Venter H. Intrinsic, adaptive and acquired antimicrobial resistance in Gram-negative bacteria. Essays in biochemistry. 2017;61(1):49-59.
68. Power BH, Smith N, Downer B, Alisaraie L. Insight into the mechanism of chemical modification of antibacterial agents by antibiotic resistance enzyme O-phosphotransferase-IIIA. Chemical biology & drug design. 2017;89(1):84-97.
69. Boehr DD, Jenkins SI, Wright GD. The molecular basis of the expansive substrate specificity of the antibiotic resistance enzyme aminoglycoside acetyltransferase-6'-aminoglycoside phosphotransferase-2". The role of ASP-99 as an active site base important for acetyl transfer. The Journal of biological chemistry. 2003;278(15):12873-80.
70. Reviews N. Microbiology. 2017. Report No.: 11.
71. Reier-Nilsen T, Farstad T, Nakstad B, Lauvrak V, Steinbakk M. Comparison of broad range 16S rDNA PCR and conventional blood culture for diagnosis of sepsis in the newborn: a case control study. BMC pediatrics. 2009;9:5.
72. Schaub N, Boldanova T, Noveanu M, Arenja N, Hermann H, Twerenbold R, et al. Incremental value of multiplex real-time PCR for the early diagnosis of sepsis in the emergency department. Swiss medical weekly. 2014;144:w13911.
73. Tann CJ, Nkurunziza P, Nakakeeto M, Oweka J, Kurinczuk JJ, Were J, et al. Prevalence of bloodstream pathogens is higher in neonatal encephalopathy cases vs. controls using a novel panel of real-time PCR assays. PloS one. 2014;9(5):e97259.
74. Greninger AL, Chatterjee SS, Chan LC, Hamilton SM, Chambers HF, Chiu CY. Whole-Genome Sequencing of Methicillin-Resistant Staphylococcus aureus Resistant to Fifth-Generation Cephalosporins Reveals Potential Non-mecA Mechanisms of Resistance. PloS one. 2016;11(2):e0149541.
75. Kong Z, Zhao P, Liu H, Yu X, Qin Y, Su Z, et al. Whole-Genome Sequencing for the Investigation of a Hospital Outbreak of MRSA in China. PloS one. 2016;11(3):e0149844.
76. Ravitch SM, Riggan M. Reason & rigor: How conceptual frameworks guide research: Sage Publications; 2016.
77. Orrett FA. Colonization with Group B streptococci in pregnancy and outcome of infected neonates in Trinidad. Pediatrics international : official journal of the Japan Pediatric Society. 2003;45(3):319-23.
78. Khan MA, Faiz A, Ashshi AM. Maternal colonization of group B streptococcus: prevalence, associated factors and antimicrobial resistance. Annals of Saudi medicine. 2015;35(6):423-7.
79. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC pediatrics. 2010;10:39.

80. Mshana SE, Hain T, Domann E, Lyamuya EF, Chakraborty T, Imirzalioglu C. Predominance of Klebsiella pneumoniae ST14 carrying CTX-M-15 causing neonatal sepsis in Tanzania. BMC infectious diseases. 2013;13:466.
81. MBN Clinical laboratories [Available from: https://mbnlab.com.
82. World Health O. Handbook : IMCI integrated management of childhood illness. Geneva: World Health Organization; 2005.
83. Institute. CaLS. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition. . Clinical and Laboratory Standards Institute, Wayne, PA.; (2009).

84. Coutinho LM, Scazufca M, Menezes PR. Methods for estimating prevalence ratios in cross-sectional studies. Revista de saude publica. 2008;42(6):992-8.
85. McClure-Warnier JA, Conly JM, Zhang K. Multiplex PCR assay for typing of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. Journal of visualized experiments : JoVE. 2013(79).
86. Seni J, Bwanga F, Najjuka CF, Makobore P, Okee M, Mshana SE, et al. Molecular characterization of Staphylococcus aureus from patients with surgical site infections at Mulago Hospital in Kampala, Uganda. PloS one. 2013;8(6):e66153.
87. Tumuhamye J, Steinsland H, Tumwine JK, Namugga O, Mukunya D, Bwanga F, et al. Vaginal colonisation of women in labour with potentially pathogenic bacteria: a cross sectional study at three primary health care facilities in Central Uganda. BMC infectious diseases. 2020;20(1):98.
88. Mugalu J, Nakakeeto MK, Kiguli S, Kaddu-Mulindwa DH. Aetiology, risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. African health sciences. 2006;6(2):120-6.
89. Elliyas S, Gaind R, Kanwal SK, Singh S, Arya S. Bacterial Colonization of Vagina in Indian Women During Labor and Its Association With Puerperal and Neonatal Sepsis: A Tertiary Hospital Study. Cureus. 2021;13(3):e13943.
90. Ngonzi J, Bebell LM, Bazira J, Fajardo Y, Nyehangane D, Boum Y, et al. Risk Factors for Vaginal Colonization and Relationship between Bacterial Vaginal Colonization and In-Hospital Outcomes in Women with Obstructed Labor in a Ugandan Regional Referral Hospital. International journal of microbiology. 2018;2018:6579139.
91. Chan GJ, Modak JK, Mahmud AA, Baqui AH, Black RE, Saha SK. Maternal and neonatal colonization in Bangladesh: prevalences, etiologies and risk factors. Journal of perinatology : official journal of the California Perinatal Association. 2013;33(12):971-6.
92. Javanian M, Rad ZA, Mojaveri MH, Shiadeh AG, Ebrahimpour S. Maternal recto vaginal colonization in term and preterm deliveries. Electronic physician. 2017;9(10):5434-8.
93. Ekwempu CC, Lawande RV, Egler LJ. Microbial flora of the lower genital tract of women in labour in Zaria, Nigeria. Journal of clinical pathology. 1981;34(1):82-3.
94. Gabriel I, Olejek A, Stencel-Gabriel K, Wielgos M. The influence of maternal vaginal flora on the intestinal colonization in newborns and 3-month-old infants. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2018;31(11):1448-53.

95. Tumuhamye J, Sommerfelt H, Bwanga F, Ndeezi G, Mukunya D, Napyo A, et al. Neonatal sepsis at Mulago national referral hospital in Uganda: Etiology, antimicrobial resistance, associated factors and case fatality risk. PloS one. 2020;15(8):e0237085.
96. Tumuhamye J, Sommerfelt H, Tumwine JK, Mukunya D, Ndeezi G, Namugga O, et al. Umbilical Cord Stump Infections in Central Uganda: Incidence, Bacteriological Profile, and Risk Factors. International journal of environmental research and public health. 2022;19(23).
97. Namugongo A, Bazira J, Fajardot Y, Joseph N. Group B Streptococcus Colonization among Pregnant Women Attending Antenatal Care at Tertiary Hospital in Rural Southwestern Uganda. International journal of microbiology. 2016;2016:3816184.
98. Russell NJ, Seale AC, O'Driscoll M, O'Sullivan C, Bianchi-Jassir F, Gonzalez-Guarin J, et al. Maternal Colonization With Group B Streptococcus and Serotype Distribution Worldwide: Systematic Review and Meta-analyses. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;65(suppl_2):S100-s11.
99. Ohlsson A, Shah VS. Intrapartum antibiotics for known maternal Group B streptococcal colonization. The Cochrane database of systematic reviews. 2013(1):Cd007467.
100. Prevention CfDCa. Prevention of perinatal group B streptococcal disease: a public health perspective. Centers for Disease Control and Prevention. MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports. 1996;45(Rr-7):1-24.
101. Akker-van Marle ME, Rijnders ME, Dommelen P, Fekkes M, Wouwe JP, Amelink-Verburg MP, et al. Cost-effectiveness of different treatment strategies with intrapartum antibiotic prophylaxis to prevent early-onset group B streptococcal disease. BJOG : an international journal of obstetrics and gynaecology. 2005;112(6):820-6.
102. Hastings MJ, Easmon CS, Neill J, Bloxham B, Rivers RP. Group B streptococcal colonisation and the outcome of pregnancy. The Journal of infection. 1986;12(1):23-9.
103. Devi U, Barman N, Barua P, Malik V, Das J, Baruah P, et al. Vaginal carriage of antibiotic resistant Escherichia coliby pregnant women: a concern for the neonate. 2014;3(4):153.
104. Rettedal S, Lohr IH, Bernhoff E, Natas OB, Sundsfjord A, Oymar K. Extended-spectrum beta-lactamase-producing Enterobacteriaceae among pregnant women in Norway: prevalence and maternal-neonatal transmission. Journal of perinatology : official journal of the California Perinatal Association. 2015;35(11):907-12.
105. Nelson E, Kayega J, Seni J, Mushi MF, Kidenya BR, Hokororo A, et al. Evaluation of existence and transmission of extended spectrum beta lactamase producing bacteria from post-delivery women to neonates at Bugando Medical Center, Mwanza-Tanzania. BMC research notes. 2014;7:279.
106. Tarana MN, Shamsuzzaman SM. Laboratory Diagnosis of Bacterial Vaginosis and Potential Pathogens Other Than Group B Streptococcus in Vaginal Swab of Pregnant Women in Dhaka Medical College Hospital. Mymensingh medical journal : MMJ. 2018;27(4):834-42.
107. Pathak A, Chandran SP, Mahadik K, Macaden R, Lundborg CS. Frequency and factors associated with carriage of multi-drug resistant commensal Escherichia coli among women attending antenatal clinics in central India. BMC infectious diseases. 2013;13:199.

108. Danino D, Melamed R, Sterer B, Porat N, Hazan G, Gushanski A, et al. Mother-to-child transmission of extended-spectrum-beta-lactamase-producing Enterobacteriaceae. The Journal of hospital infection. 2018;100(1):40-6.
109. Cursino MA, Garcia CP, Lobo RD, Salomao MC, Gobara S, Raymundo GF, et al. Performance of surveillance cultures at different body sites to identify asymptomatic Staphylococcus aureus carriers. Diagnostic microbiology and infectious disease. 2012;74(4):343-8.
110. Hetsa BA, Kumar A, Ateba CN. Characterization of multiple antibiotic resistant clinical strains of Staphylococcus isolated from pregnant women vagina. Folia microbiologica. 2018;63(5):607-17.
111. Rawstron SA, Jackman JM, Serebro E, Johnson G, Cabbad M, Bromberg K, et al. Perirectal Screening for Carbapenem-Resistant Enterobacteriaceae Obtained From 100 Consecutive Healthy Pregnant Women in Labor at a Brooklyn Hospital: Results and Risk Factors. Infection control and hospital epidemiology. 2018;39(3):369-71.
112. Oelmeier de Murcia K, Glatz B, Willems S, Kossow A, Strobel M, Stuhmer B, et al. Prevalence of Multidrug Resistant Bacteria in Refugees: A Prospective Case Control Study in an Obstetric Cohort. Zeitschrift fur Geburtshilfe und Neonatologie. 2017;221(3):132-6.
113. Andrews WW, Schelonka R, Waites K, Stamm A, Cliver SP, Moser S. Genital tract methicillin-resistant Staphylococcus aureus: risk of vertical transmission in pregnant women. Obstetrics and gynecology. 2008;111(1):113-8.
114. Chen KT, Huard RC, Della-Latta P, Saiman L. Prevalence of methicillin-sensitive and methicillin-resistant Staphylococcus aureus in pregnant women. Obstetrics and gynecology. 2006;108(3 Pt 1):482-7.
115. Caughey AB, Robinson JN, Norwitz ER. Contemporary diagnosis and management of preterm premature rupture of membranes. Reviews in obstetrics & gynecology. 2008;1(1):11-22.
116. Health Mo. Uganda Clinical Guidelines. Government of Uganda; 2016.
117. Sazawal S, Dhingra U, Ali SM, Dutta A, Deb S, Ame SM, et al. Efficacy of chlorhexidine application to umbilical cord on neonatal mortality in Pemba, Tanzania: a community-based randomised controlled trial. The Lancet Global health. 2016;4(11):e837-e44.
118. Arifeen SE, Mullany LC, Shah R, Mannan I, Rahman SM, Talukder MR, et al. The effect of cord cleansing with chlorhexidine on neonatal mortality in rural Bangladesh: a community-based, cluster-randomised trial. Lancet (London, England). 2012;379(9820):1022-8.
119. Semrau KEA, Herlihy J, Grogan C, Musokotwane K, Yeboah-Antwi K, Mbewe R, et al. Effectiveness of 4% chlorhexidine umbilical cord care on neonatal mortality in Southern Province, Zambia (ZamCAT): a cluster-randomised controlled trial. The Lancet Global health. 2016;4(11):e827-e36.
120. Faridi MM, Rattan A, Ahmad SH. Omphalitis neonatorum. Journal of the Indian Medical Association. 1993;91(11):283-5.
121. Mason WH, Andrews R, Ross LA, Wright HT, Jr. Omphalitis in the newborn infant. The Pediatric infectious disease journal. 1989;8(8):521-5.
122. Sengupta M, Banerjee S, Banerjee P, Guchhait P. Outstanding Prevalence of Methicillin Resistant Staphylococcus aureus in Neonatal Omphalitis. Journal of clinical and diagnostic research : JCDR. 2016;10(9):Dm01-dm3.

123. Sawardekar KP. Changing spectrum of neonatal omphalitis. The Pediatric infectious disease journal. 2004;23(1):22-6.
124. Mullany LC, Faillace S, Tielsch JM, Stolzfus RJ, Nygaard KE, Kavle JA, et al. Incidence and risk factors for newborn umbilical cord infections on Pemba Island, Zanzibar, Tanzania. The Pediatric infectious disease journal. 2009;28(6):503-9.
125. Edmond KM, Zandoh C, Quigley MA, Amenga-Etego S, Owusu-Agyei S, Kirkwood BR. Delayed breastfeeding initiation increases risk of neonatal mortality. Pediatrics. 2006;117(3):e380-6.
126. Group NS. Timing of initiation, patterns of breastfeeding, and infant survival: prospective analysis of pooled data from three randomised trials. The Lancet Global health. 2016;4(4):e266-75.
127. Mhada TV, Fredrick F, Matee MI, Massawe A. Neonatal sepsis at Muhimbili National Hospital, Dar es Salaam, Tanzania; aetiology, antimicrobial sensitivity pattern and clinical outcome. BMC public health. 2012;12:904.
128. colloboration IotDNISD. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. The Lancet Global health. 2016;4(10):e752-60.
129. T GE, Moges F, Eshetie S, Yeshitela B, Abate E. Bacterial etiologic agents causing neonatal sepsis and associated risk factors in Gondar, Northwest Ethiopia. BMC pediatrics. 2017;17(1):137.
130. Kabwe M, Tembo J, Chilukutu L, Chilufya M, Ngulube F, Lukwesa C, et al. Etiology, Antibiotic Resistance and Risk Factors for Neonatal Sepsis in a Large Referral Center in Zambia. The Pediatric infectious disease journal. 2016;35(7):e191-8.
131. Musoke RN, Revathi G. Emergence of multidrug-resistant gram-negative organisms in a neonatal unit and the therapeutic implications. Journal of tropical pediatrics. 2000;46(2):86-91.
132. Eibach D, Belmar Campos C, Krumkamp R, Al-Emran HM, Dekker D, Boahen KG, et al. Extended spectrum beta-lactamase producing Enterobacteriaceae causing bloodstream infections in rural Ghana, 2007-2012. International journal of medical microbiology : IJMM. 2016;306(4):249-54.
133. Garcia C, Astocondor L, Rojo-Bezares B, Jacobs J, Saenz Y. Molecular Characterization of Extended-Spectrum beta-Lactamase-Producer Klebsiella pneumoniae Isolates Causing Neonatal Sepsis in Peru. The American journal of tropical medicine and hygiene. 2016;94(2):285-8.
134. Marando R, Seni J, Mirambo MM, Falgenhauer L, Moremi N, Mushi MF, et al. Predictors of the extended-spectrum-beta lactamases producing Enterobacteriaceae neonatal sepsis at a tertiary hospital, Tanzania. International journal of medical microbiology : IJMM. 2018;308(7):803-11.
135. Flokas ME, Karanika S, Alevizakos M, Mylonakis E. Prevalence of ESBL-Producing Enterobacteriaceae in Pediatric Bloodstream Infections: A Systematic Review and Meta-Analysis. PloS one. 2017;12(1):e0171216.
136. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. The Journal of antimicrobial chemotherapy. 2007;60(5):913-20.

137. Shehab El-Din EM, El-Sokkary MM, Bassiouny MR, Hassan R. Epidemiology of Neonatal Sepsis and Implicated Pathogens: A Study from Egypt. BioMed research international. 2015;2015:509484.
138. Murthy S, Godinho MA, Guddattu V, Lewis LES, Nair NS. Risk factors of neonatal sepsis in India: A systematic review and meta-analysis. PloS one. 2019;14(4):e0215683.
139. Cohen D, Natshe A, Ben Chetrit E, Lebel E, Breuer GS. Synovial fluid culture: agar plates vs. blood culture bottles for microbiological identification. Clinical rheumatology. 2019.

Errata

Item	Error	Correction
Paper I (Vaginal colonization)	Table 3 has a line "Sexual partners" on Page 3 of 10	It should have been "Other sexual partners" (i.e. than spouse)
Paper II (AMR vaginal colonization)	Abstract: has «WHO ((CISMAC. Centre for Intervention Science in Maternal and Child health»	It should have been WHO (World Health Organization)
Paper IV (Neonatal sepsis study)	Under "Immediate clinical outcomes" section on page 8 of 14 I write that "the odds of death and indicate adjusted RR in parenthesis"	It should have been the "the risk of death…."

Papers

Paper I
Paper II
Paper III
Paper IV

Appendices (on request from the author)

Appendix I: CHX trial questionnaires
Appendix II: Sepsis study questionnaire
Appendix III: School of medicine ethical clearance form
Appendix IV: Mulago research and ethics committee approval letter

Appendix V: UNCST ethical clearance form

Paper I

Tumuhamye et al. BMC Infectious Diseases (2020) 20:98
https://doi.org/10.1186/s12879-020-4821-6

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Vaginal colonisation of women in labour with potentially pathogenic bacteria: a cross sectional study at three primary health care facilities in Central Uganda

Josephine Tumuhamye[1*], Hans Steinsland[2], James K. Tumwine[3], Olive Namugga[4], David Mukunya[4], Freddie Bwanga[5], Halvor Sommerfelt[4] and Victoria Nankabirwa[4,6]

Abstract

Background: Potentially pathogenic bacteria that colonise the lower genital tract of women in labour can be passed to the baby during birth. While many babies become colonised with these bacteria after delivery, a few develop neonatal infections. The lower genital tract is a reservoir for potential pathogens and a source of infection for neonates. We determined the prevalence of vaginal colonisation of potentially pathogenic bacteria among women in labour in Central Uganda and identified potential risk factors associated with this colonisation.

Methods: We conducted a cross sectional study at three primary health care facilities and collected vaginal swabs from HIV-1 negative women in labour. Specimens were cultured on different selective microbiological media, and biochemical tests were used to classify bacterial isolates on the species level. Multivariable logistic regression analyses were used to estimate the association between relevant exposures and colonisation with potentially pathogenic bacteria.

Results: We recruited 1472 women in labour whose mean age was 24.6 years (standard deviation [SD] 4.9). Of these, 955 (64.9%; 95% Confidence Interval [CI] 62.4, 67%) were vaginally colonised with at least one potentially pathogenic bacterial species. The most commonly isolated species were *Escherichia coli* ($n = 508$; 34.5%), *Klebsiella pneumoniae* ($n = 144$; 9.8%) and *Staphylococcus aureus* ($n = 121$; 8.2%). Results from exploratory multivariable regression analyses indicated that having had ≥5 previous pregnancies (adjusted odds ratio [aOR] 0.59; 95% CI 0.35, 0.97) or being ≥30 years old (aOR 1.52; 95% CI 1.03, 2.23) could be associated with vaginal colonisation with any potentially pathogenic bacteria, as well as with vaginal colonisation with *S. aureus* (aOR 0.33; 95% CI 0.12, 0.88, and aOR 2.17; 95% CI 1.17, 4.00, respectively). Possession of domestic animals in a household (aOR 0.57; 95% CI 0.35, 0.92) could be associated with vaginal colonisation with *E. coli*.

Conclusions: Two-thirds of HIV-1 negative women in labour were vaginally colonised by potentially pathogenic bacteria, mainly *E. coli, K. pneumoniae*, and *S. aureus*.

Keywords: Potentially pathogenic bacteria, Vaginal colonisation, Labour, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, Uganda

* Correspondence: tphynne@gmail.com
[1]Centre for Intervention Science in Maternal and Child Health (CISMAC), Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway
Full list of author information is available at the end of the article

Background

The normal lower genital tract is inhabited by a number of different bacteria that live in well-balanced populations. In healthy women of reproductive age, the primary bacteria colonising the vagina are of the genus *Lactobacillus* [1]. They reduce the pH of the vagina to between 2 and 4, which helps to inhibit growth of pathogenic bacteria [2]. During pregnancy, physiological changes alter the homeostasis of the vaginal environment. These changes are complex and not fully understood, but they generally lead to a reduction in the *Lactobacillus* population and, thereby, facilitating the growth of potentially pathogenic bacteria such as *Staphylococcus aureus* and members of the *Enterobacteriaceae* family [3].

During vaginal delivery, a newborn comes into direct contact with the mother's flora in the lower genital tract. Eventually, the baby's umbilicus, mucous membranes and parts of the skin may be colonised with bacteria that are potentially pathogenic for the neonate [4]. This colonisation process is known as seeding, and it has implications for long term neonatal health outcomes [5]. The potentially pathogenic bacteria that are seeded to the baby often include *S. aureus*, group B *Streptococcus* (GBS), group A *Streptococcus* (GAS), *Enterococcus* spp., *Klebsiella pneumoniae, Escherichia coli, Enterobacter* spp., *Pseudomonas* spp. and *Citrobacter* spp. [6].

Severe infections account for 26% of neonatal deaths globally [7] and they are the leading cause of mortality among newborns in sub Saharan Africa [8]. The lower genital tract of women is an important source of pathogens causing life-threatening infections including bacteremia, meningitis, pneumonia and arthritis during the first week of life [9, 10] [11]. We conducted a cross-sectional study among women in labour at three primary health care facilities in the Central region of Uganda to estimate the prevalence and improve our understanding of the aetiology and risk factors associated with vaginal colonisation by potentially pathogenic bacteria.

Methods

Study design and setting

This cross-sectional study was conducted between July 2016 and July 2018 in three primary health care facilities in Central Uganda: Mukono Health Centre IV, Kawaala Health Centre III, and Kitebi Health Centre III. These three health care facilities have a combined monthly average of 2400 antenatal visits and 1200 deliveries. The three health facilities mostly deliver women who are considered to have low risk of obstetric complications. The women who are likely to have complicated deliveries are usually referred to tertiary hospitals. The HIV prevalence among women in reproductive age in Uganda is approximately 8% [12]. The Mukono Health Centre IV is located within Mukono district, which has a largely rural population of around 60,000 people and is located around 25 km from Uganda's capital city, Kampala, while the Kitebi Health Centre III and the Kawaala Health Centre III are located in Kampala, which has a population of approximately 1.5 million people. The study was nested within an ongoing randomised controlled trial aimed at assessing the effectiveness of a single application of 4% chlorhexidine solution on the umbilical cord stump for the prevention of omphalitis and severe illness in HIV-1 unexposed newborns [13].

Participants

We included women who became enrolled in the above-mentioned randomised controlled trial, who were HIV-1 negative, who gave birth during the daytime on a weekday, who gave consent to participate in the study before (orally) and within 12 h (written) after giving birth, and who gave birth to babies who had the following characteristics: birth weights of > 1.5 kg, no severe congenital anomalies, no obvious signs of cord stump infection, and no severe illness requiring hospitalisation at birth [13]. The randomised controlled trial aims to recruit 4760 newborns, and we enrolled 1472 of these into the present study. With this sample size we would obtain a very high (0.7 to 2.6%) absolute precision, i.e. the difference between the upper limit and the lower limit of the 95% confidence interval (CI) for prevalence values ranging from 2 to 50%. Demographic characteristics of the study participants were collected through interviews, as described below, and can be found listed in Table 1.

Data collection and consent

Trained research nurses obtained verbal consent to collect specimens from women in labour, and after giving birth, obtained written informed consent to allow for the use of the collected specimens and data. Socio-demographic and clinical data were collected by using structured electronic questionnaires on mobile phones based on the Open Data Kit (ODK) software [14]. Distribution of relevant characteristics we collected can be found listed in Table 1. These include exposures associated with vaginal colonisation of mother, including premature rupture of membranes (PROM), defined as breakage of membranes of the amniotic sac before labour onset [15], prolonged labour, defined as labour beyond 24 h, parity, maternal level of education, maternal age, hospitalisation during pregnancy, marital status, antenatal care attendance, possession of domestic animals in the household, having been pregnant multiple times (multigravidity) and socioeconomic data.

Specimen collection and transportation

Trained midwives collected vaginal swab specimens from the women during labour, by using Regular Rayon sterile swabs pre-packed with Amies Agar Gel without

Table 1 Distribution of characteristics of study participants at the three study sites

Participant characteristics	*N* = 1472 (%)	Uncolonised 517 (%)	Colonised 955 (%)
Mother's age			
< =19 years	205 (13.9)	76 (14.70)	129 (13.5)
20–24 years	587 (39.9)	216 (41.8)	371 (38.9)
25–29 years	454 (30.8)	151 (29.2)	303 (31.7)
> =30 years	226 (15.4)	74 (14.3)	152 (15.9)
Education level			
Primary	488 (33.2)	185 (35.8)	303 (31.7)
Secondary	854 (58.0)	278 (53.8)	576 (60.3)
Tertiary	130 (8.8)	53 (10.4)	76 (8.0)
Marital status			
Unmarried	300 (20.4)	92 (17.8)	208 (21.8)
Married	1172 (79.6)	425 (82.2)	747 (78.2)
Wealth index			
Quintile1	489 (33.2)	184 (35.6)	305 (31.9)
Quintile2	100 (6.8)	31 (6.0)	69 (7.2)
Quintile3	298(20.2)	106 (20.5)	192 (20.1)
Quintile4	295 (20.0)	96 (18.6)	199 (20.8)
Quintile5	290 (19.7)	100 (19.3)	190 (19.9)
Gravidity			
First pregnancy	442 (30.0)	163 (31.5)	279 (29.2)
2–4 pregnancies	910 (61.8)	303 (58.6)	607 (63.6)
5 or more pregnancies	120 (8.2)	51 (9.9)	69 (7.2)
Hospitalisation during pregnancy			
No	1387 (94.2)	483 (93.4)	904 (94.7)
Yes	710 (94.5)	34 (6.6)	51 (5.3)
Antenatal visits			
Once	75 (5.1)	30 (5.8)	45 (4.7)
2–4 times	1259 (85.5)	442 (85.5)	817 (85.6)
5 or more times	138 (9.4)	45 (8.7)	93 (9.7)
Own domestic animals			
No	1355 (92.1)	472 (91.3)	883 (92.5)
Yes	117 (8.0)	45 (8.7)	72 (7.5)
Monthly income level			
< 30	205 (13.9)	76 (14.70)	129 (13.5)
30- < 60	587 (39.9)	216 (41.8)	371 (38.9)
60- < 90	454 (30.8)	151 (29.2)	303 (31.7)
≥ 90	226 (15.4)	74 (14.3)	152 (15.9)
Mode of delivery			
Spontaneous vaginal delivery	1334 (90.6)	480 (92.8)	854 (89.4)
Assisted vaginal delivery	135 (9.2)	35 (6.8)	100 (10.5)
Caesarean section	3 (0.2)	2 (0.4)	1 (0.1)
Sexual partners			
No	824 (70.3)	302 (71.1)	522 (69.9)
Yes	185 (15.8)	58 (13.7)	127 (17.0)

Table 1 Distribution of characteristics of study participants at the three study sites *(Continued)*

Participant characteristics	*N* = 1472 (%)	Uncolonised 517 (%)	Colonised 955 (%)
DNK	163 (13.9)	65 (15.3)	98 (13.1)
Premature rapture of membranes			
No	1466 (99.6)	512 (99.0)	954 (99.9)
Yes	6(0.4)	5 (1.0)	1 (0.1)
Prolonged labour			
No	1458 (99.1)	514 (99.4)	944 (98.9)
Yes	14 (0.9)	3 (0.6)	11 (1.1)
Use of mama kit			
No	1011 (68.7)	343 (66.3)	668 (69.9)
Yes	461 (31.3)	174 (33.7)	287 (30.1)
Tetanus toxoid vaccination			
No	1314 (89.3)	454 (87.8)	860 (90.1)
Yes	158 (10.7)	63 (12.2)	95 (9.9)

Charcoal transport medium (Copan Diagnostics Inc., Murrieta, CA). The swab was first carefully inserted into the vagina about halfway between the introitus and cervix. This way, contamination from the cervical mucus was avoided. The swab was then gently pressed towards the vaginal walls and rotated to ensure that it was thoroughly coated. The midwives took caution when removing the swab to avoid contact with the skin and the anal area. The vaginal swabs were immediately stored in the Amies transport medium in a specimen transport cooler. The coolers were subsequently transported within 24 h to MBN Clinical Laboratories where the specimens immediately underwent microbiological analyses [16].

Microbiological analyses

Primary inoculation of the vaginal swabs was done on 5% sheep blood agar (BioLab Zrt., Budapest, Hungary) and on MacConkey agar (BioLab Zrt.), followed by aerobic incubation between 35 °C–37 °C for 18–24 h. The blood agar plates were further incubated for a total of 72 h to allow development of slow growing bacterial colonies. From these plates, we picked and streaked one representative of each morphologically distinct colony onto new agar plates and used colonies from this sub-culture for further species identification and characterisation.

Bacterial species identification was performed by using conventional microbiological techniques.

Gram-positive bacterial identification: *Staphylococcus aureus* species was identified based on positive catalase, coagulase and DNase tests. Beta-haemolytic Streptococci were identified by having distinct colony characteristics, having transparent haemolytic zones around colonies grown on blood agar plates, being Gram stain positive and being negative in the catalase test. The beta-haemolytic colonies were further Lancefield grouped into different species/groups (*Streptococcus* A-D) using the Streptococcal Grouping Kit (Oxoid Ltd., Basingstoke, Hants, UK). *Enterococcus* species were identified by being positive in the bile esculin test [17].

Gram-negative bacteria identification: These were identified biochemically based on lactose fermentation, triple sugar iron agar, sulfur-indole-motility, citrate and urease tests [17].

Reference strains *S. aureus* ATCC 25923 for gram-positive bacteria, and *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 for gram-negative bacteria were regularly included in the identification pipeline to control the quality of the microbiological procedures

Main outcome and exposure definitions

The study's main outcome was vaginal colonisation with potentially pathogenic bacteria. Colonisation with potential pathogenic bacteria was defined as isolation of at least one of the following types of bacteria from the vaginal swab; *S. aureus, E. coli*, *K. pneumoniae*, group A *Streptococcus* (GAS), group B *Streptococcus* (GBS), *Enterococcus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Citrobacter* spp., *Proteus* spp. and/or *Acinetobacter* spp. These bacteria are known to cause infections in newborns. Other bacteria that were isolated which we considered to represent commensal strains since they rarely are found associated with newborn infections included *Candida* spp., *Micrococcus* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Bacillus* spp., *Bukolderia* spp., *Serratia* spp. and coagulase-negative *Staphylococcus*. Such isolates were not included in the analyses.

In the statistical analyses, described below, we tested for associations between different exposures and 4 different outcomes, including colonization with any potential pathogen, with *E. coli*, with *S. aureus*, and with *K.*

pneumoniae. In those analyses, we tested exposures that other studies have found to be associated with maternal colonization with potentially pathogenic bacteria [18–21], including: Maternal level of education, maternal age, hospitalisation during pregnancy, marital status, number of previous pregnancies, antenatal tetanus toxoid vaccination, number of antenatal care visits, hospitalisations during pregnancy, possession of domestic animals at home, and socioeconomic status. Socioeconomic status was represented by a wealth index variable which was generated by performing principal component analysis on data about household ownership of cupboards, radios, televisions, a mobile phone, refrigerator, motor cycle, car, ownership of a house and/or land, and presence of cemented walls, type of toilet, and three or more rooms in the house. Five quintiles of the wealth index variables were generated with the poorest belonging to quintile 1, and the least poor to quintile 5.

Statistical analysis

The data were analysed by using STATA 15.0 (StataCorp LLC, College Station, TX, USA). To obtain an estimate of the overall vaginal colonisation prevalence, we divided the number of women who had a positive vaginal culture for one or more potential pathogens by the total number of enrolled women in the study. To explore the associations between the above-mentioned outcomes and exposures, we performed bivariable (unadjusted) and multivariable (adjusted) logistic regression analyses where we estimated odds ratios (OR) and 95% confidence intervals (CIs) for each exposure. For each tested model we used the *estat vif* command in STATA to ensure there was little potential multicollinearity between the independent variables in the model, as indicated by one or more variance inflation factor estimates of > 10. None of our models appeared to have potential multicollinearity issues.

Results

We recruited a total of 1472 women including 545 (37.0%) from Kawaala Health Centre III, 524 (36%) from Kitebi Health Centre III and 403 (27%) from Mukono Health Centre IV. The characteristics of these women are listed in Table 1. All but 3 (0.2%) of the women had vaginal deliveries. The mean age of the participants was 24.6 (standard deviation 4.9) years, 1172 (80%) were married or cohabiting, 1295 (88%) earned less than 30 US dollars per month, 185 (15.8%) had other sexual partners and 488 (33%) had at least a primary education. Only 6 (0.41%) of the women experienced PROM and 14 (0.9%) prolonged labour.

Vaginal colonisation

Of the 1472 recruited women, 955 (64.9%; 95% CI 62.4, 67.3%) were colonised with at least one potential bacterial pathogen. Of the 955 colonised women, 878 were colonised with one potentially pathogenic bacteria, 69 were colonised with two potential pathogens while the remaining three women were colonised with three potential bacterial pathogens (Table 2). A total of 1025 potentially pathogenic bacterial pathogens were isolated from the colonised women. Overall, the most frequently isolated potential bacterial pathogens were *E. coli* ($n = 508$; 34.5%), *K. pneumoniae* ($n = 145$; 9.9%) and *S. aureus* ($n = 121$; 8.2%). There were no major differences in proportions of women colonised by potentially pathogenic bacteria between the three study sites (Table 3).

Exposures associated with vaginal colonisation

In the statistical analyses to identify exposures that are potentially associated with colonization with different pathogens, we found that having ≥5 previous pregnancies (aOR 0.59; 95% CI 0.35, 0.97) and maternal age of ≥30 years (aOR 1.52; 95% CI 1.03, 2.23) were associated with vaginal colonisation of women in labour with any potentially pathogenic bacteria (Table 4). Focusing these analyses on the three most commonly isolated potential pathogenic bacteria, we found that maternal age of ≥30 years (aOR 2.17; 95% CI 1.17, 4.00) and a history of at least 5 previous pregnancies (aOR 0.33; 95% CI 0.12, 0.88) were associated with *S. aureus* vaginal colonisation (Table 5). We found that possession of domestic animals in a household (aOR 0.57; 95% CI 0.35, 0.92) could be associated with vaginal colonisation by E. coli (Table 6). We found no exposures significantly associated with colonisation by *E. coli* (Table 6) and *K. pneumoniae* (Table 7).

Table 2 Number and percentage of women colonised with more than one potentially pathogenic bacterial isolates

Combination of bacteria isolated	*N* = 1472 (%)
Escherichia coli and *Klebsiella pneumoniae*	10 (0.7)
Escherichia coli and *Enterococcus* spp.	10 (0.7)
Escherichia coli and *Staphylococcus aureus*	9 (0.6)
Escherichia coli and *Enterobacter* spp.	6 (0.4)
Escherichia coli and *Citrobacter* spp.	5 (0.3)
Klebsiella pneumoniae and *Enterococcus* spp.	4 (0.3)
Escherichia coli and Escherichia coli	4 (0.3)
Staphylococcus aureus and *Staphylococcus aureus*	4 (0.3)
Klebsiella pneumoniae and *Staphylococcus aureus*	3 (0.2)
Klebsiella pneumoniae and *Citrobacter* spp.	3 (0.2)
Escherichia coli and *Klebsiella oxytoca*	2 (0.1)
Klebsiella oxytoca and *Citrobacter* spp.	2 (0.1)
Ten other combinations[a]	1 (0.1)

[a]Includes: K. pneumoniae and Pseudomonas spp.; Acinetobacter spp. and S. aureus; E. coli and Proteus mirabilis; Enterococcus spp. and Citrobacter spp.; E. coli and Acinetobacter spp.; E. coli and Pseudomonas spp.; K. pneumoniae and K. pneumoniae; E. coli, Citrobacter spp., and S. aureus; E. coli, Pseudomonas spp., and S. aureus; E. coli, Enterococcus spp., and S. aureus

Table 3 Distribution of bacterial isolates from study participants across the three study sites

Bacteria isolated	Kawaala HC III *n* = 545 (%)	Kitebi HC III *n* = 524 (%)	Mukono HC IV *n* = 403 (%)	Total n = 1472 (%)
E. coli	203 (37.2)	183 (34.9)	122 (30.3)	508 (34.5)
K. pneumoniae	50 (9.2)	48 (9.2)	47 (11.7)	145 (9.9)
S. aureus	49 9.0)	41 (7.8)	31 (7.7)	121 (8.2)
Citrobacter spp.	38 (7.0)	38 (7.0)	31 (7.7)	107 (7.3)
Enterococcus spp.	16 (2.9)	19 (3.6)	12 (3.0)	47 (3.2)
Enterobacter spp.	21 (3.9)	5 (0.9)	6 (1.5)	32 (2.2)
Acinetobacter spp.	10 (1.8)	10 (1.9)	12 (3.0)	32 (2.2)
K. oxytoca	8 (1.5)	4 (0.8)	11 (2.7)	23 (1.6)
Group B *Streptococcus*	0	3 (0.6)	0	3 (0.2)
Group A *Streptococcus*	2 (0.14)	1 (0.07)	0	3 (0.2)
Pseudomonas spp.	0	3 (0.6)	0	3 (0.2)
Proteus mirabilis	1 (0.18)	0	0	1 (0.07)

Discussion

We studied the prevalence of different potentially pathogenic bacteria colonising the vagina of women in labour at three primary health care facilities in Central Uganda and evaluated the association between potential risk factors and colonisation with these bacteria.

Sixty-five percent (65%) of the study participants were colonised by at least one potential bacterial pathogen. The prevalence of women colonised with potential pathogens in our study was higher than that reported in a similar study in Bangladesh [22]. This differences in colonisation prevalence may be due to several reasons, including differences in ethnic and geographical settings, that our study women were colonised with a wider range of pathogen species and the small sample size in the Bangladesh study. *E. coli, K. pneumoniae* and *S. aureus* were the most commonly isolated species. We found that the prevalences of individual potentially pathogenic bacteria were similar to those reported in other studies—*E. coli* was the predominant potential pathogen with a proportion similar to a study in Iran [23]. The proportion of *K. pneumoniae* isolates we found is similar to that reported in Nigeria [24] and Bangladesh [22]. Another study reported a prevalence of *S. aureus* vaginal colonisation in pregnant women similar to ours [25]. The bacteria that colonise the vagina of women in labour play an important role in newborn health such as defining their early gut microbiota [26]. A recent study has demonstrated that maternal vaginal colonisation with *E. coli* or *S. aureus* is significantly associated with pathogens isolated from the blood of neonates with early-onset sepsis [27].

In our study, the prevalence of vaginal GBS colonisation was only 0.2%, which is lower than what similar studies have reported [28, 29]. The difference could be a result of the methodological differences between our study and the other studies. We did not use the Todd Hewitt medium for GBS isolation, and did not collect anal swabs in our study, which could potentially have underestimated the GBS prevalence. The difference could also result from the fact that we use culture-based techniques to detect GBS instead of the more sensitive PCR based methods. However, vaginal colonisation varies greatly across geographical settings and a systematic review of studies from 85 countries indicates that East Africa and southern Asia have the lowest prevalence of maternal vaginal GBS colonisation compared to other regions [30]. Generally, we observed that there were no major differences in proportions of women colonised by potentially pathogenic bacteria between the study sites. This is an important finding because it indicates that this was a well-conducted large study and its findings are generalizable.

We found that women 30 years or more of age appeared more likely to be vaginally colonised with any potentially pathogenic bacteria and particularly with *S. aureus* compared to women who were 20–24 years in our study. Similar observations have been made in other studies [19, 21], where they found that older women were more often colonised than younger women. Vaginal colonisation rates during pregnancy may be attributed to several factors such as gestational age, mother's age and parity. The association we observed could possibly be due to the fact that the majority of the women aged ≤30 years in our study were multipara and multigravida. We also found that women who had had at least 5 previous pregnancies appeared less likely to be colonised with these organisms than primigravida women. In

Table 4 Exposures associated with vaginal colonisation with any potentially pathogenic bacteria of women in labour at three study sites ($N = 1472$)

Characteristics	$N = 1472$ (%)	Colonisation	
		Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (years)			
20–24	587 (39.9)	1	1
≤ 19	205 (13.9)	0.99 (0.71, 1.37)	0.97 (0.68, 1.38)
25–29	454 (30.8)	1.17 (0.90, 1.51)	1.20 (0.91, 1.58)
≥ 30	226 (15.4)	1.20 (0.86, 1.65)	1.52 (1.03, 2.23)
Education level			
Tertiary	130 (8.8)	1	1
No education	33 (2.2)	0.75 (0.35, 1.63)	0.88 (0.40, 1.94)
Primary	455 (30.9)	1.20 (0.81, 1.79)	1.38 (0.91, 2.10)
Secondary	854 (58.0)	1.47 (1.01, 2.15)	1.64 (1.11, 2.43)
Gravida			
Primigravida	442 (30.0)	1	1
2–4 pregnancies	910 (61.8)	1.17 (0.92, 1.48)	1.06 (0.80, 1.40)
≥ 5 pregnancies	120 (8.2)	0.79 (0.52, 1.19)	0.59 (0.35, 0.97)
Hospitalisation during pregnancy			
No	1387 (94.2)	1	–
Yes	710 (94.5)	0.80 (0.51, 1.25)	–
Antenatal attendance			
One time	75 (5.1)	1	–
2–4 times	1259 (85.5)	1.23 (0.77, 1.98)	–
5 or more times	138 (9.4)	1.38 (0.77, 2.47)	–
Domestic animals at home			
No	1355 (92.1)	1	1
Yes	117 (8.0)	0.86 (0.58, 1.26)	0.80 (0.51, 1.25)
Wealth index			
5th Quintile (least poor)	290 (19.7)	1	1
1st Quintile (poorest)	489 (33.2)	0.87 (0.64, 1.18)	0.81 (0.57, 1.14)
2nd Quintile	100 (6.8)	1.17 (0.72, 1.91)	1.09 (0.65, 1.83)
3rd Quintile	298 (20.0)	0.95 (0.68, 1.34)	0.87 (0.60, 1.27)
4th Quintile	295 (20.0)	1.09 (0.77, 1.54)	1.01 (0.70, 1.46)

Table 5 Exposures associated with vaginal *S. aureus* colonisation of women in labour at three study sites ($N = 1472$)

Characteristics	$N = 1472$ (%)	*S. aureus* colonisation	
		Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (years)			
20–24	587 (39.9)	1	1
≤ 19	205 (13.9)	1.10 (0.60, 2.00)	0.90 (0.48, 1.69)
25–29	454 (30.8)	1.12 (0.70, 1.78)	1.34 (0.81, 2.19)
≥ 30	226 (15.4)	1.47 (0.86, 2.51)	2.17 (1.17, 4.00)
Education level			
Tertiary	130 (8.8)	1	1
No education	33 (2.2)	0.87 (0.18, 4.22)	1.04 (0.21, 5.24)
Primary	455 (30.9)	1.33 (0.63, 2.82)	1.61 (0.73, 3.53)
Secondary	854 (58.0)	1.11 (0.54, 2.28)	1.27 (0.61, 2.67)
Gravida			
Primigravida	442 (30.0)	1	1
2–4 pregnancies	910 (61.8)	0.87 (0.58, 1.32)	0.67 (0.41 1.10)
≥ 5 pregnancies	120 (8.2)	0.64 (0.28, 1.47)	0.33 (0.12, 0.88)
Hospitalisation during pregnancy			
No	1387 (94.2)	1	–
Yes	710 (94.5)	1.04 (0.47, 2.3)	–
Antenatal attendance			
One time	75 (5.1)	1	–
2–4 times	1259 (85.5)	0.96 (0.41, 2.27)	–
5 or more times	138 (9.4)	1.30 (0.48, 3.53)	–
Domestic animals at home			
No	1355 (92.1)	1	1
Yes	117 (8.0)	0.72 (0.33, 1.58)	0.78 (0.32, 1.87)
Wealth index			
5th Quintile (Least poor)	290 (19.7)	1	1
1st Quintile (Poorest)	489 (33.2)	1.27 (0.74, 2.18)	1.14 (0.63, 2.09)
2nd Quintile	100 (6.8)	0.96 (0.40, 2.34)	0.84 (0.33, 2.13)
3rd Quintile	298 (20.0)	1.07 (0.58, 1.98)	0.99 (0.51, 1.93)
4th Quintile	295 (20.0)	1.03 (0.55, 1.92)	0.96 (0.50, 1.84)

contrast, studies in Thailand [31], Trinidad [19] and India [32] found multigravida women were more often colonised than primigravida women. These differences are difficult to explain, and given the exploratory nature of these analyses, further studies would be needed to confirm these results.

More surprising was the finding that women living with domestic animals at home were less likely to be colonised by *E. coli* than those who did not live with animals. Normally, living with animals would be considered an important risk factor for infection with *E. coli* [33, 34]. Further studies would be needed to identify the underlying reasons for why these women appeared to be protected. Few women in our study experienced premature rupture of membranes (PROM), which is an important risk factor for neonatal infections [35]. The low prevalence of PROM among the participants in our study is probably a result of the pre-delivery screening that is being done at our three

Table 6 Exposures associated with vaginal *E. coli* colonisation of women in labour at three study sites (*N* = 1472)

Characteristics	*N* = 1472 (%)	*E. coli colonisation*	
		Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (years)			
20–24	587 (39.9)	1	1
≤ 19	205 (13.9)	1.18 (0.85, 1.65)	1.10 (0.77, 1.57)
25–29	454 (30.8)	1.04 (0.80, 1.35)	1.06 (0.80, 1.40)
≥ 30	226 (15.4)	1.06 (0.77, 1.46)	1.20 (0.82, 1.75)
Education level			
Tertiary	130 (8.8)	1	1
No education	33 (2.2)	0.88 (0.37, 2.05)	0.94 (0.39, 2.26)
Primary	455 (30.9)	1.10 (0.72, 1.68)	1.15 (0.74, 1.80)
Secondary	854 (58.0)	1.33 (0.89, 1.98)	1.37 (0.91, 2.07)
Gravida			
Primigravida	442 (30.0)	1	1
2–4 pregnancies	910 (61.8)	0.96 (0.76, 1.22)	0.99 (0.74, 1.32)
≥ 5 pregnancies	120 (8.2)	0.75 (0.49, 1.17)	0.75 (0.43, 1.29)
Hospitalisation during pregnancy			
No	1387 (94.2)	1	–
Yes	710 (94.5)	0.74 (0.46, 1.21)	–
Antenatal attendance			
One time	75 (5.1)	1	–
2–4 times	1259 (85.5)	1.14 (0.69, 1.88)	–
5 or more times	138 (9.4)	0.87 (0.47, 1.59)	–
Domestic animals at home			
No	1355 (92.1)	1	1
Yes	117 (8.0)	0.64 (0.42, 0.98)	0.57 (0.35, 0.92)
Wealth index			
5th Quintile (Least poor)	290 (19.7)	1	1
1st Quintile (Poorest)	489 (33.2)	0.93 (0.69, 1.26)	0.77 (0.55, 1.09)
2nd Quintile	100 (6.8)	1.57 (0.99, 2.49)	1.30 (0.80, 2.11)
3rd Quintile	298 (20.0)	0.81 (0.57, 1.14)	0.67 (0.46, 1.0)
4th Quintile	295 (20.0)	0.96 (0.68, 1.35)	0.83 (0.57, 1.19)

Table 7 Exposures associated with vaginal *K. pneumoniae* colonisation of women in labour at three study sites (*N* = 1472)

Characteristics	*N* = 1472 (%)	*K. pneumoniae* colonisation	
		Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (years)			
20–24	587 (39.9)	1	1
≤ 19	205 (13.9)	1.12 (0.67, 1.89)	1.45 (0.83, 2.55)
25–29	454 (30.8)	1.07 (0.71, 1.61)	0.96 (0.63,1.48)
≥ 30	226 (15.4)	0.8 (0.46, 1.40)	0.81 (0.43,1.51)
Education level			
Tertiary	130 (8.8)	1	1
No education	33 (2.2)	0.77 (0.21, 2.82)	0.73 (0.19, 2.80)
Primary	455 (30.9)	0.70 (0.37, 1.31)	0.65 (0.33, 1.27)
Secondary	854 (58.0)	0.88 (0.49, 1.58)	0.83 (0.46,1.52)
Gravida			
Primigravida	442 (30.0)	1	1
2–4 pregnancies	910 (61.8)	1.28 (0.86, 1.90)	1.49 (0.93, 2.36)
≥ 5 pregnancies	120 (8.2)	0.76 (0.49, 1.67)	1.05 (0.41, 2.68)
Hospitalisation during pregnancy			
No	1387 (94.2)	1	–
Yes	710 (94.5)	1.40 (0.73, 2.7)	–
ANC attendance			
One time	75 (5.1)	1	–
2–4 times	1259 (85.5)	1.49 (0.59,3.76)	–
5 or more times	138 (9.4)	2. 10 (0.75,5.90)	–
Domestic animals at home			
No	1355 (92.1)	1	1
Yes	117 (8.0)	1.17 (0.64, 2.14)	1.52 (0.74, 3.12)
Wealth index			
5th Quintile (least poor)	290 (19.7)	1	1
1st Quintile (poorest)	489 (33.2)	1.04 (0.62, 1.76)	1.22 (0.66, 2.25)
2nd Quintile	100 (6.8)	0.83 (0.35, 2.00)	0.97 (0.38, 2.47)
3rd Quintile	298 (20.0)	1.57 (0.91, 2.70)	1.82 (0.98, 3.39)
4th Quintile	295 (20.0)	1.44 (0.83, 2.50)	1.61 (0.88, 2.95)

health facilities, where women who are considered to be at risk of experiencing PROM or other complications during delivery are early on referred to tertiary hospitals.

One of the limitations of this study is that we only enrolled HIV-1 negative women. Nevertheless, we are confident that these findings are generalisable to the majority of women in reproductive age in Uganda because 92% of women of reproductive age in Uganda are HIV-1 negative. Since we used traditional microbiological methods to identify the different potential pathogenic bacteria, our prevalence estimates are probably lower than they would have been if we instead had used molecular profiling methods, such as PCR, to detect colonization.

Conclusion

We found that among HIV-1 negative women in labour at health facilities in Central Uganda, almost two-thirds had vaginal colonisation by potentially pathogenic bacteria,

mainly *E. coli*, *K. pneumoniae*, and *S. aureus*. This is of concern since exposures to pathogenic bacteria during birth is likely to increase the risk of newborn infections. We have also identified exposures that appear to be associated with colonisation with these potentially pathogenic organisms. Further studies are needed to evaluate the virulence of the potential pathogens and the risk of neonatal infections associated with this colonisation.

Abbreviations

CI: Confidence interval; CISMAC: Centre for intervention science in maternal and child health; GAS: Group A *Streptococcus*; GBS: Group B *Streptococcus*; HIV-1: Human immunodeficiency virus type 1; NORHED: Norwegian programme for capacity development in higher education and research for development; ODK: Open data kit; OR: Odds ratio; PCR: Polymerase chain reaction; PROM: Premature rupture of membranes; SD: Standard deviation

Acknowledgements

We thank all the women who participated in our study and the midwives at Kawaala Health Centre III, Kitebi Health Centre III and Mukono Health Centre IV for their cooperation. Special thanks to the CISMAC research nurses for their hard work and commitment. We acknowledge the MBN microbiology team, including Emmanuel Aboce, Mango Wandera Dennis and Sheila Tumwesigye for their excellent work.

Authors' contributions

JT, VN, HS, JKT, and FB conceived the study. JT, VN, FB, HS and ON designed the study. JT and FB led the microbiology testing and results interpretation. HSt, VN and HS prepared the Chlorhexidine trial dataset for analysis. JT, DM, and HSt analysed the data. JT wrote the first draft of manuscript. All authors reviewed the manuscript, approved and agreed to submit the final version of the manuscript.

Funding

This work was funded by the Research Council of Norway through its Centre of Excellence scheme and the University of Bergen (UiB), Norway to the Centre for Intervention Science in Maternal and Child Health (CISMAC; project number 223269) and its funding of the parent Chlorhexidine trial. The Norwegian programme for capacity development in higher education and research for development (NORHED) through the Survival Pluss project (Project Number; UGA-13-0030) partly funded this work. The Research Council of Norway and NORHED played no role in design, analysis or interpretation of the study findings.

Availability of data and materials

Datasets used for this study can be obtained through a reasonable request from the principal investigator of the chlorhexidine trial (VN) nankabirwav@gmail.com and the corresponding author.

Ethics approval and consent to participate

Informed consent was obtained for both the interview and specimen storage. Ethical approval was obtained from the Research and Ethics Review Committee of School of Medicine, SOMREC, Makerere University (REC 2015–118) and from the Uganda National Council of Science and Technology (HS 1927).

Consent for publication

Not applicable

Competing interests

Authors declare no competing interests.

Author details

[1]Centre for Intervention Science in Maternal and Child Health (CISMAC), Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway. [2]CISMAC, Centre for International Health, Department of Global Public Health and Primary Care and Department of Biomedicine, University of Bergen, Bergen, Norway. [3]Department of Paediatric and Child Health, Makerere University, Kampala, Uganda. [4]CISMAC, Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway. [5]Department of Immunology and Molecular Biology, Makerere University, Kampala, Uganda. [6]Department of Epidemiology and Biostatics school of Public Health, Makerere University, Kampala, Uganda.

Received: 5 August 2019 Accepted: 24 January 2020
Published online: 31 January 2020

References

1. Larsen B, Monif GR. Understanding the bacterial flora of the female genital tract. Clin Infect Dis. 2001;32(4):e69–77.
2. Galask RP, Larsen B, Ohm MJ. Vaginal flora and its role in disease entities. Clin Obstet Gynecol. 1976;19(1):61–81.
3. Larsen B, Galask RP. Vaginal microbial flora: composition and influences of host physiology. Ann Intern Med. 1982;96(6 Pt 2):926–30.
4. Carroll SG, Papaioannou S, Ntumazah IL, Philpott-Howard J, Nicolaides KH. Lower genital tract swabs in the prediction of intrauterine infection in preterm prelabour rupture of the membranes. Br J Obstet Gynaecol. 1996; 103(1):54–9.
5. Backhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe. 2015;17(6):852.
6. Chan GJ, Modak JK, Mahmud AA, Baqui AH, Black RE, Saha SK. Maternal and neonatal colonization in Bangladesh: prevalences, etiologies and risk factors. J Perinatol. 2013;33(12):971–6.
7. GBD 2016 Mortality collaborators. Global, regional, and national under-5 mortality, adult mortality, age-specific mortality, and life expectancy, 1970–2016: A systematic analysis for the global burden of disease study 2016. Lancet. 2017;390(10100):1084–150.
8. The Alliance for Maternal and Newborn Health Improvement (AMANHI) mortality study group. Population-based rates, timing, and causes of maternal deaths, stillbirths, and neonatal deaths in south Asia and sub-Saharan Africa: a multi-country prospective cohort study. Lancet Glob Health. 2018;6(12):e1297–308.
9. Witkin SS, Linhares IM, Giraldo P. Bacterial flora of the female genital tract: function and immune regulation. Clin Obstet Gynaecol. 2007;21(3):347–54.
10. Ayengar V, Madhulika VSN. Neonatal sepsis due to vertical transmission from maternal genital tract. Indian J Pediatr. 1991;58(5):661–4.
11. Russell NJ, Seale AC, O'Sullivan C, Le Doare K, Heath PT, Lawn JE, Bartlett L, Cutland C, Gravett M, Ip M, et al. Risk of Early-Onset Neonatal Group B Streptococcal Disease With Maternal Colonization Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017;65(suppl_2):S152–s159.
12. Uganda population-based HIV impact assessment [https://phia.icap.columbia.edu/]. Accessed 24 August 2019.
13. Nankabirwa V, Tylleskar T, Tumuhamye J, Tumwine JK, Ndeezi G, Martines JC, Sommerfelt H. Efficacy of umbilical cord cleansing with a single application of 4% chlorhexidine for the prevention of newborn infections in Uganda: study protocol for a randomized controlled trial. Trials. 2017;18(1):322.
14. Open Data Kit: The standard for mobile data collection [https://opendatakit.org]. Accessed 3 December 2018.
15. Fishel Bartal M, Sibai BM, Ilan H, Fried M, Rahav R, Alexandroni H, Schushan Eisan I, Hendler I. Trial of labor after cesarean (TOLAC) in women with premature rupture of membranes. J Matern Fetal Neonatal Med. 2019:1–7. https://doi.org/10.1080/14767058.2019.1566312.
16. MBN Clinical laboratories [https://mbnlab.com], Accessed 16 March 2019.
17. Winn W. AS, Janda W., Koneman E., Procop G., Schreckenberger P., Woods G. Koneman's Color Atlas and Textbook of Diagnostic Microbiology., 6th Edition, edn. New York.: Lippincott Williams and Wilkins, New York.; 2006.
18. Lederer DJ, Bell SC, Branson RD, Chalmers JD, Marshall R, Maslove DM, Ost DE, Punjabi NM, Schatz M, Smyth AR, et al. Control of confounding and reporting of results in causal inference studies. Guidance for authors from editors of respiratory, sleep, and critical care journals. Ann Am Thorac Soc. 2019;16(1):22–8.
19. Orrett FA. Colonization with group B streptococci in pregnancy and outcome of infected neonates in Trinidad. Pediatr Int. 2003;45(3):319–23.
20. Stokholm J, Schjorring S, Eskildsen CE, Pedersen L, Bischoff AL, Folsgaard N, Carson CG, Chawes BL, Bonnelykke K, Molgaard A, et al. Antibiotic use during pregnancy alters the commensal vaginal microbiota. Clin Microbiol Infect. 2014;20(7):629–35.

21. Khan MA, Faiz A, Ashshi AM. Maternal colonization of group B streptococcus: prevalence, associated factors and antimicrobial resistance. Ann Saudi Med. 2015;35(6):423–7.
22. Chan GJ, Modak JK, Mahmud AA, Baqui AH, Black RE, Saha SK. Maternal and neonatal colonization in Bangladesh: prevalences, hh and risk factors. J Perinatol. 2013;33(12):971–6.
23. Javanian M, Rad ZA, Mojaveri MH, Shiadeh AG, Ebrahimpour S. Maternal recto vaginal colonization in term and preterm deliveries. Electron Physician. 2017;9(10):5434–8.
24. Ekwempu CC, Lawande RV, Egler LJ. Microbial flora of the lower genital tract of women in labour in Zaria, Nigeria. J Clin Pathol. 1981;34(1):82–3.
25. Andrews WW, Schelonka R, Waites K, Stamm A, Cliver SP, Moser S: Genital tract methicillin-resistant Staphylococcus aureus: risk of vertical transmission in pregnant women. Obstet Gynecol 2008, 111(1):113–118.
26. Gabriel I, Olejek A, Stencel-Gabriel K, Wielgos M. The influence of maternal vaginal flora on the intestinal colonization in newborns and 3-month-old infants. J Matern Fetal Neonatal Med. 2018;31(11):1448–53.
27. Kim JY, Sung JH, Chang KH, Choi SJ, Oh SY, Roh CR, Kim JH. Abnormal vaginal colonization by gram-negative bacteria is significantly higher in pregnancy conceived through infertility treatment compared to natural pregnancy. J Matern Fetal Neonatal Med. 2017;30(5):556–61.
28. Namugongo A, Bazira J, Fajardot Y, Joseph N. Group B Streptococcus colonization among pregnant women attending antenatal Care at Tertiary Hospital in rural southwestern Uganda. Int J Microbiol. 2016;2016:3816184.
29. Ngonzi J, Bebell LM, Bazira J, Fajardo Y, Nyehangane D, Boum Y, Nanjebe D, Boatin A, Kabakyenga J, Jacquemyn Y, et al. Risk factors for vaginal colonization and relationship between bacterial vaginal colonization and in-hospital outcomes in women with obstructed labor in a Ugandan regional referral hospital. Int J Microbiol. 2018;2018:6579139.
30. Russell NJ, Seale AC, O'Driscoll M, O'Sullivan C, Bianchi-Jassir F, Gonzalez-Guarin J, Lawn JE, Baker CJ, Bartlett L, Cutland C, et al. Maternal Colonization With Group B Streptococcus and Serotype Distribution Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017;65(suppl_2): S100–s111.
31. Akkaneesermsaeng W, Petpichetchian C, Yingkachorn M, Sasithorn S. Prevalence and risk factors of group B Streptococcus colonisation in intrapartum women: a cross-sectional study. J Obstet Gynaecol. 2019:1–5.
32. Sharmila V, Joseph NM, Arun Babu T, Chaturvedula L, Sistla S. Genital tract group B streptococcal colonization in pregnant women: a south Indian perspective. J Infect Dev Ctries. 2011;5(8):592–5.
33. Osman KM, Badr J, Orabi A, Elbehiry A, Saad A, Ibrahim MDS, Hanafy MH. Poultry as a vector for emerging multidrug resistant Enterococcus spp.: first report of vancomycin (van) and the chloramphenicol-florfenicol (cat-fex-cfr) resistance genes from pigeon and duck faeces. Microb Pathog. 2019;128: 195–205.
34. Lupindu AM, Dalsgaard A, Msoffe PL, Ngowi HA, Mtambo MM, Olsen JE. Transmission of antibiotic-resistant Escherichia coli between cattle, humans and the environment in peri-urban livestock keeping communities in Morogoro, Tanzania. Prev Vet Med. 2015;118(4):477–82.
35. Ocviyanti D, Wahono WT. Risk factors for neonatal Sepsis in pregnant women with premature rupture of the membrane. J Pregnancy. 2018;2018: 4823404.

Publisher's Note

Paper II

Tumuhamye *et al.*
Antimicrob Resist Infect Control (2021) 10:37
https://doi.org/10.1186/s13756-021-00897-9

Antimicrobial Resistance and Infection Control

RESEARCH

Open Access

Vaginal colonization with antimicrobial-resistant bacteria among women in labor in central Uganda: prevalence and associated factors

Josephine Tumuhamye[1,2*], Hans Steinsland[1,3], Freddie Bwanga[4], James K. Tumwine[5], Grace Ndeezi[5], David Mukunya[2], Olive Namugga[2], Agnes Napyo Kasede[2,5,6], Halvor Sommerfelt[2] and Victoria Nankabirwa[2,7]

Abstract

Background: According to WHO (CISMAC. Centre for Intervention Science in Maternal and Child health), the antimicrobial resistant bacteria considered to be clinically most important for human health and earmarked for surveillance include extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae,* carbapenem-resistant bacteria, methicillin-resistant (MRSA) and, macrolide-lincosamide-streptogramin B -resistant vancomycin-resistant (VRSA) *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* (VRE). If these bacteria are carried in the female genital tract, they may be transmitted to the neonate causing local or systemic neonatal infections that can be difficult to treat with conventionally available antimicrobials. In order to develop effective treatment strategies, there is need for updated information about the prevalence of colonization with important antimicrobial-resistant pathogens.

Objective: We sought to estimate the prevalence of vaginal colonization with potentially pathogenic and clinically important AMR bacteria among women in labour in Uganda and to identify factors associated with colonization.

Methods: We conducted a cross-sectional study among HIV-1 and HIV-2 negative women in labour at three primary health care facilities in Uganda. Drug susceptibility testing was done using the disk diffusion method on bacterial isolates cultured from vaginal swabs. We calculated the prevalence of colonization with potentially pathogenic and clinically important AMR bacteria, in addition to multidrug-resistant (MDR) bacteria, defined as bacteria resistant to antibiotics from ≥ 3 antibiotic classes.

Results: We found that 57 of the 1472 enrolled women (3.9% prevalence; 95% Confidence interval [CI] 3.0%, 5.1%) were colonized with ESBL-producing *Enterobacteriaceae,* 27 (1.8%; 95% CI 1.2%, 2.6%) were colonized with carbapenem-resistant *Enterobacteriaceae*, and 85 (5.8%; 95% CI 4.6%, 7.1%) were colonized with MRSA. The prevalence of colonization with MDR bacteria was high (750/1472; 50.9%; 95% CI 48.4%, 53.5%). Women who were ≥ 30 years of age had higher odds of being colonized with MDR bacteria compared to women aged 20–24 years (OR 1.6; 95% CI 1.1, 2.2).

*Correspondence: tphynne@gmail.com
[1] Centre for Intervention Science for Maternal and Child Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway
Full list of author information is available at the end of the article

Conclusion: Most of the women included in our study were vaginally colonized with potentially pathogenic MDR and other clinically important AMR bacteria. The high prevalence of colonization with these bacteria is likely to further increase the incidence of difficult-to-treat neonatal sepsis.

Keywords: Antimicrobial resistance, Multidrug resistance, MDR, ESBL, MRSA, MLSB, Carbapenem-resistant bacteria, Vaginal colonization

Background

The spread of infections with antimicrobial-resistant bacterial pathogens is a global public health challenge [1]. Pathogens that are responsible for most invasive neonatal infections are often resistant to commonly used antibiotics [2], and many are resistant to antibiotics from several different classes, including many last-resort drugs, which further complicates and limits the possibilities for treatment. Infections with these pathogens are associated with prolonged hospital stays, increased risk of complications and of death [3]. In the World Health Organization's recently published Global Priority Pathogens List, reducing the burden of infection with pathogenic antimicrobial resistant bacteria has been given priority, and combatting the spread of AMR is also listed as one of the main priorities in the United Nations general assembly's 2030 agenda for sustainable global health development [4].

The AMR bacteria considered to most importantly threaten neonatal health include extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae,* carbapenem-resistant bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA), macrolide-lincosamide-streptogramin B (MLSB)-resistant *S. aureus,* vancomycin-resistant *S. aureus* (VRSA), and vancomycin-resistant *Enterococcus* spp. (VRE) [5, 6]. The incidence of systemic infections with anaerobes is relatively low among neonates [2, 7, 8].

Many AMR bacteria are multidrug-resistant (MDR), which is commonly defined as being resistant to antibiotics from ≥ 3 different antibiotic classes [9]. Being infected with MDR bacteria tends to complicate or prolong treatment since the causative bacteria are resistant to commonly used antibiotics. Hospitalizations or visits to health clinics, direct contact with livestock and overuse of antibiotics are considered to be the most important risk factors for becoming infected with MDR bacteria and other clinically important AMR bacteria [4].

It is thought that pathogenic bacteria colonizes the birth canal mainly after faecal contamination [10] and are then sometimes transmitted to the baby during labour and delivery [11]. Such transmission is probably one of the main sources of neonatal bacterial infection within the first week of life, particularly if there was prolonged / obstructed labour or premature rupture of membranes (PROM) [12–14]. Having access to relevant antimicrobial resistance data for bacterial pathogens colonizing the birth canal can help clinicians make informed treatment decisions for neonatal bacterial infections, and, thus, improve chances of recovery while reducing the risk of complications and death. The availability of antimicrobial resistance surveillance data on a local level also helps to inform national health policies. There is a shortage of up-to-date data on AMR in sub-Saharan Africa. In addition, there is little knowledge about the extent of and risk factors associated with vaginal colonization with AMR bacteria. Here, we isolated potentially pathogenic bacteria from the birth canal of Ugandan woman in labour, determined the antimicrobial resistance patterns of the isolates, and estimated the prevalence of and identified risk factors associated with colonization with such bacteria.

Methods

Study design, setting and participants

We conducted a cross-sectional study between July 2016 and July 2018 at three primary health care facilities in and close to Kampala in central Uganda: Mukono General Hospital (formerly Mukono Health Centre IV), Kawaala Health Centre III, and Kitebi Health Centre III [15]. This study was nested within the Chlorhexidine Trial, which is a randomized controlled assessing whether a single application of 4% chlorhexidine solution on the umbilical cord stump immediately after birth reduces the risk of omphalitis and severe illness [16].

Of the 1658 women in labour who were screened for this study, we recruited 1472 who had already agreed to being enrolled in the above-mentioned Chlorhexidine Trial. The inclusion criteria for the trial were: mother was negative for HIV-1 and HIV-2 and gave birth on a weekday, the newborn weighed > 1.5 kgs, had no severe congenital anomalies, had no obvious signs of umbilical cord stump infection and had no severe illness on the day it was born. We enThe sample size was calculated for the trial but not for the present study. With this sample size we would obtain a very high (0.7% to 2.6%) absolute precision, i.e. the difference between the upper limit and the lower limit of the 95% confidence interval (CI) for prevalence estimates ranging from 2 to 50%.

Consent and interview

Midwives obtained oral consent to collect a specimen from women in labour. After birth, a study nurse confirmed the verbal consent by obtaining written consent. In the subsequent interview, study nurses collected socio-demographic and clinical information from study participants using Open Data Kit-based standardized questionnaires [17].

Specimen collection and processing

Trained midwives collected the vaginal swabs during labour. A Regular Rayon sterile swab (Copan Diagnostics Inc., Murrieta, CA) was carefully inserted halfway between the introitus and cervix, avoiding contamination from the cervical mucus. The swab was then gently pressed against the vaginal wall, rotated to collect the specimen, and then removed, carefully avoiding contact with other parts of the body. The swabs were immediately stored in Amies Agar Gel without Charcoal transport medium (Copan Diagnostics Inc.) and transported daily in a cold box holding a temperature of 10–25 °C to MBN Clinical Laboratories Ltd in Kampala, which is a private research and diagnostic laboratory currently undergoing accreditation, where the swabs were immediately processed. We did not culture the specimens anaerobically because of the added cost and effort and because anaerobic bacteremia is uncommon in neonates [2].

Culture methods

The specimens were streaked onto blood agar containing 5% in-house produced sheep blood and onto MacConkey agar (both Biolab Inc., Budapest, Hungary) and incubated aerobically at 35–37 °C for 18–24 h for isolation of single colonies. The blood agar plates were further incubated for a total of 72 h to enable isolation of slow-growing colonies. From each of the two plates, one representative of each morphologically distinct colony was picked and streaked onto new plates and 1–5 resulting colonies from each of them were pooled in saline solution and subjected to further species determination and classification as described below, before being stored at −80 °C in Brain Heart Infusion broth with 20% glycerol.

Species identification

Identification of bacterial species was mainly done based on colony morphology, Gram staining and on standard biochemical tests. *S. aureus* was identified with the catalase, slide coagulase, mannitol fermentation, and DNase tests. The bile esculin test was performed to identify *Enterococcus* spp. Putative streptococcal isolates were grouped into different Lancefield groups with the Streptococcal Grouping Kit (Oxoid Ltd., Basingstoke, Hants, UK). Lactose and non-lactose fermenting colonies of gram-negative bacilli were identified based on morphology on MacConkey agar, and the isolates were characterized on the species level by performing standard biochemical tests such as Sulphide Indole Motility (SIM test), gas production, citrate test, urease test and oxidase test [18]. If two isolates from the same specimen were of the same species but had different biochemical characteristics, we included both isolates in the analyses. We considered gram-negative isolates representing *E. coli*, *K. pneumoniae, Citrobacter* spp., *Enterobacter* spp., *Acinetobacter* spp., *K. oxytoca, Pseudomonas* spp., and *Proteus* spp. and gram-positive isolates representing *S. aureus, Enterococcus* spp., Group A *Streptococcus*, and Group B *Streptococcus* to be potentially pathogenic bacteria and they were thereby included in the present study.

Antimicrobial drug susceptibility determination

Antimicrobial drug susceptibility testing of the bacterial isolates was performed using the disk diffusion method as described in the 2017 Performance Standards published by the Clinical Laboratory Standard Institute (CLSI) [19]. We also tested for the antibiotics recommended by the same standard. For gram-positive isolates, we tested against the following antibiotic resistance discs purchased from Biolab Inc.: penicillin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), erythromycin (15 µg), oxacillin (1 µg), vancomycin (30 µg), ceftriaxone (30 µg), and linezolid (30 µg). For Gram-negative isolates, we tested against the following discs: trimethoprim-sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), amikacin (10 µg), ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), tetracycline (30 µg), piperacillin (100 µg), piperacillin-tazobactam (100/10 µg), colistin (10 µg), and imipenem (10 µg). The inhibition zone diameters were measured after incubation at 35–37 °C for 24 h, and we considered an isolate to be resistant (i.e. non-susceptible) if the measurements indicated resistance or intermediate resistance to the given drug.

ESBL-producing *Enterobacteriaceae* identification

To identify ESBL-producing *Enterobacteriaceae*, we used the combination disk method [20] where a combination disk containing 30 µg ceftazidime and 10 µg clavulanic acid was placed 15 mm from a 30 µg ceftazidime disk on a Mueller–Hinton agar plate. Isolates that had clear zones that were ≥ 5.0 mm larger around the combination disk than around the ceftazidime disk were considered to represent ESBL-producing bacteria.

Carbapenem-resistant bacteria identification

We considered isolates that were resistant to imipenem to be carbapenem-resistant based on CLSI guidelines.

MRSA identification

To identify methicillin-resistant *S. aureus* (MRSA) genotypically, we performed multiplex PCR-based identification of MRSA of most *S. aureus* isolates, as described by McClure et al. [21]. In this assay, the presence of the *mecA* methicillin resistance gene was used to identify MRSA, while the presence of the gene for the Panton-Valentine Leukocidin (PVL) virulence factor was a marker for community-acquired MRSA [22]. The completed reaction was separated on a 2% agarose gel stained with ethidium bromide, and the amplicons were visualized by using a UV trans-illuminator.

MLSB-resistant *S. aureus* identification

To identify *S. aureus* isolates that had the macrolide-lincosamide-streptogramin B (MLSB) resistance phenotype, we performed the D-test [23]. In this test, disks with 15 µg erythromycin and with 2 µg clindamycin were placed 15 mm apart. If the isolate was resistant to both erythromycin and clindamycin, the isolate was considered to have a constitutive MLSB resistance phenotype (cMLSB), while if it was resistant to erythromycin and susceptible to clindamycin, but there was a D-shaped inhibition zone around the clindamycin disk, we considered the isolate to have an inducible MLSB resistance phenotype (iMLSB).

VRE and VRSA identification

We considered *Enterococcus* spp. and *S. aureus* isolates that are resistant to vancomycin to represent VRE and VRSA, respectively.

MDR bacteria

We used the definition proposed by Magiorakos et al. [9], i.e. that isolates non-susceptible to ≥ 1 antibiotic in ≥ 3 of the antibiotic classes were considered MDR. The antibiotic selection was based on the 2017 Performance Standards from CLSI [19], which differs slightly from other commonly used standards, such as those published by EUCAST [24]. The antibiotic classes and antibiotics (given in parentheses) used for the MDR definition included penicillins (ampicillin, piperacillin, penicillin), penicillins and beta-lactamase inhibitors (amoxicillin-clavulanic acid), antipseudomonal penicillins and beta-lactamase inhibitors (piperacillin-tazobactam), non-extended-spectrum beta-lactams such as second generation cephalosporins (cefuroxime), extended spectrum beta lactams such as third generation cephalosporins (ceftriaxone, ceftazidime), carbapenems (imipenem), fluoroquinolones (ciprofloxacin), phenicols (chloramphenicol), folate pathway inhibitors (Trimethoprim-sulfamethoxazole), aminoglycoside (gentamicin, amikacin), anti-staphylococcal beta lactams (oxacillin), glycopeptides (vancomycin), macrolides (erythromycin), tetracyclines (tetracycline), and oxazolidinones (linezolid). As seen in Tables 1 and 2, we did not test for resistance for a given antibiotic if the species is known to be naturally resistant to the antibiotic.

Exposure factors

To identify potential risk factors for colonization with different AMR bacteria in the logistic regression models described below, we included the following exposure information, which were acquired during interviews with the mothers after they had given birth. PROM defined as rupture of membranes before the start of labour [25], parity, maternal level of education, maternal age, hospitalization during pregnancy, antenatal care attendance, ownership of domestic animals at home and socioeconomic status. As a measure of socioeconomic status, we used principal component analysis on an asset index that we generated by evaluating the woman's access to or ownership of cupboards, radios, televisions, a mobile phone, refrigerator, motorcycle, car, ownership of a house, and presence of cemented walls, flushing toilet, and having three or more rooms in the house. Socioeconomic status was divided into 5 levels, where the poorest women were categorized as belonging to the 1st quintile while the richest were categorized as belonging in the 5th quintile.

Statistical analyses

The statistical analyses were done using Stata version 15.0 (StataCorp, College Station, Texas, USA). We obtained the overall prevalence of MDR colonization by dividing the number of women colonized with MDR bacteria by the total number of women enrolled into the study. To obtain the overall prevalence of resistance with clinically important bacteria such as MRSA, MLSB-resistant *S. aureus*, VRSA, VRE, ESBL and carbapenem-resistant bacteria, we divided the number of women colonized with such bacteria by the total number of enrolled women in the study. All proportions were reported with their respective 95% confidence intervals, which were calculated using the exact method.

We performed multivariable logistic regression analyses to explore the association between selected exposures (maternal age, maternal education, socioeconomic status, gravidity, number of antenatal visits, hospitalization during pregnancy, ownership of domestic animals) and our primary outcome of vaginal colonization with MDR bacteria and secondary outcomes including ESBL and MRSA

Table 1 Vaginal colonization with antimicrobial drug-resistant potentially pathogenic gram-negative bacteria among study women in labor

Antibiotics[a]	Number of the 1472 study women who were colonized with resistant bacteria (prevalence of colonization/proportion of isolates, in %)							
	E. coli (n = 504/508)[c]	*K. pneumoniae* (n = 144/145)	*Enterobacter* spp. (n = 32/32)	*K. oxytoca* (n = 23/23)	*Citrobacter* spp. (n = 107/107)	*Acinetobacter* spp. (n = 32/32)	*Pseudomonas* spp. (n = 3/3)	*Proteus* spp. (n = 3/3)
Ampicillin	454 (30.8/89.4)	141 (9.6/97.2)	31 (2.1/96.9)	22 (1.5/95.7)	105 (7.1/98.1)	NA	0 [6]	1 (0.1/33.3)
Amoxycillin-Clavulanic acid	366 (24.9/72.0)	120 (8.2/82.8)	31 (2.1/96.9)	14 (0.9/60.9)	80 (5.4/74.8)	NA	NA	1 (0.1/33.3)
Trimethoprim-Sulfamethoxazole	354 (24.0/69.7)	90 (6.1/62.1)	17 (1.2/53.1)	9 (0.6/39.1)	56 (3.8/52.3)	21 (1.4/65.6)	NA	0 [6]
Ciprofloxacin	74 (5.0/14.6)	26 (1.8/17.9)	3 (0.2/9.4)	1 (0.1/4.3)	18 (1.2/16.8)	9 (0.6/28.1)	0 [6]	0 [6]
Chloramphenicol	103 (7.0/20.3)	36 (2.4/24.8)	5 (0.3/15.6)	4 (0.3/17.4)	18 (1.2/16.8)	4 (0.3/12.5)	0 [6]	0 [6]
Gentamicin	111 (7.5/21.9)	37 (2.5/25.5)	6 (0.4/18.8)	3 (0.2/13.0)	22 (1.5/20.6)	7 (0.5/21.9)	1 (0.1/33.3)	0 [6]
Amikacin	125 (8.5/24.6)	33 (2.2/22.8)	9 (0.6/28.1)	2 (0.1/8.7)	19 (1.3/17.8)	6 (0.4/18.8)	0 [6]	0 [6]
Ceftriaxone	92 (6.3/18.1)	42 (2.9/29.0)	4 (0.3/12.5)	4 (0.3/17.4)	31 (2.1/29.0)	NA	NA	0 [6]
Cefuroxime	366 (24.9/72.0)	92 (6.3/63.4)	28 (1.9/87.5)	8 (0.5/34.8)	83 (5.6/77.6)	NA	NA	1 (0.1/33.3)
Ceftazidime	55 (3.7/10.8)	30 (2.0/20.7)	1 (0.1/3.1)	3 (0.2/13.0)	7 (0.5/6.5)	13 (0.9/40.6)	0 [6]	0 [6]
Imipenem	41 (2.8/8.1)	19 (1.3/13.1)	9 (0.6/28.1)	1 (0.1/4.3)	27 (1.8/25.2)	2 (0.1/6.3)	0 [6]	0 [6]
Tetracycline	NA	NA	NA	NA	NA	9 (0.6/28.1)	0 [6]	NA
Piperacillin	NA	NA	NA	NA	NA	16 (1.1/50.0)	0 [6]	NA
Piperacillin-Tazobactam	NA	NA	NA	NA	NA	11 (0.7/34.4)	0 [6]	NA
Colistin	NA	NA	NA	NA	NA	NA	0 [6]	NA
ESBL-producing bacteria	36 (2.4/7.1)	16 (1.1/11.0)	1 (0.1/3.1)	1 (0.1/4.3)	3 (0.2/2.8)	NA	NA	0 [6]
Carbapenem-resistant bacteria	41 (2.8/8.1)	19 (1.3/13.1)	9 (0.6/28.1)	1 (0.1/4.3)	27 (1.8/25.2)	2 (0.1/6.3)	0 [6]	0 [6]
MDR	426 (28.9/83.9)	89 (6.0/61.4)	22 (1.5/68.8)	6 (0.4/26.1)	62 (4.2/57.9)	18 (1.2/56.3)	2 (0.1/66.7)	1 (0.1/33.3)
MDR[b]	350 (23.8/68.9)	54 (3.7/37.2)	12 (0.8/37.5)	4 (0.3/17.4)	32 (2.2/29.9)	18 (1.2/56.3)	2 (0.1/66.7)	1 (0.1/33.3)

[a] NA indicates that the antibiotic was not tested or was not relevant for the given organism

[b] MDR excluding ESBL-producing and carbapenem-resistant bacteria

[c] The two numbers in parentheses indicate number of colonized women and the number of isolates of each given species, respectively

bacteria. We chose to conduct exploratory multivariable analyses because the exposures selected potentially confounded one another.

We used the estat vif command in STATA to test the models for potential multicollinearity between the independent variables, as indicated by one or more variance inflation factor estimates being > 10. None of our models appeared to have potential multicollinearity issues.

Results

Bacterial isolates

A total of 1472 women in labour with an average age of 24.6 years (standard deviation: 4.9 years) were enrolled in the study. We obtained 1025 potentially pathogenic bacterial isolates from vaginal specimens from 955 (64.9%) of the women, including 1 isolate from 878, 2 from 69, and 3 from 3 women. The 1025 isolates represented 851 (83%) gram-negative and 174 (17%) gram-positive isolates, and included 508 (49.6%) *Escherichia coli*, 145 (14.1%) *Klebsiella pneumoniae*, 121 (11.8%) *Staphylococcus aureus*, 107 (10.4%) *Citrobacter* spp., 47 (4.6%) *Enterococcus* spp., 32 (3.1%) *Enterobacter* spp., 32 (3.1%) *Acinetobacter* spp., 23 (2.2%) *Klebsiella oxytoca*, 3 (0.3%) *Pseudomonas* spp., 3 (0.3%) group A *Streptococcus*, 3 (0.3%) group B *Streptococcus*, and 1 (0.1%) *Proteus* sp. Nine of the 955 specimens yielded more than one isolate for a given species. These included four specimens with two *E. coli* isolates, one

Table 2 Vaginal colonization with antimicrobial drug-resistant potentially pathogenic gram-positive bacteria among study women in labor

Antibiotics[a]	Number of the 1472 study women who were colonized with bacteria resistant to one or more antibiotics (prevalence of colonization /proportion of isolates, in %)			
	Enterococcus spp. (n = 47/47)[c]	*S. aureus* (n = 117/121)	Group A *Streptococcus* (n = 3/3)	Group B *Streptococcus* (n = 3/3)
Trimethoprim-Sulfamethoxazole	NA	77 (5.2/63.6)	2 (0.1/66.7)	1 (0.1/33.3)
Ciprofloxacin	40 (2.7/85.1)	48 (3.3/39.7)	NA	NA
Chloramphenicol	19 (1.3/40.4)	26 (1.8/21.5)	0 [6]	1 (0.1/33.3)
Gentamicin	NA	38 (2.6/31.4)	NA	NA
Penicillin	18 (1.2/38.3)	93 (6.3/76.9)	0 [6]	1 (0.1/33.3)
Ampicillin	NA	NA	NA	1 (0.1/33.3)
Tetracycline	30 (2.0/63.8)	70 (4.8/57.9)	3 (0.2/100)	3 (0.2/100)
Erythromycin	30 (2.0/63.8)	89 (6.0/73.6)	2 (0.1/66.7)	3 (0.2/100)
Vancomycin	6 (0.4/12.8)	6 (0.4/5.0)	1 (0.1/33.3)	1 (0.1/33.3)
Ceftriaxone	NA	NA	0 [6]	0 [6]
Linezolid	8 (0.5/17.0)	NA	NA	NA
Oxacillin	37 (2.5/78.7)	85 (5.8/70.2)	1 (0.1/33.3)	1 (0.1/33.3)
MRSA	NA	85 (5.8/70.2)	NA	NA
VRSA/VRE	6 (0.4/12.8)	6 (0.4/5.0)	NA	NA
iMLSB-resistant *S. aureus*	NA	18 (1.2/14.9)	NA	NA
cMLSB-resistant *S. aureus*	NA	24 (1.6/19.8)	NA	NA
MDR	37 (2.5/78.7)	85 (5.8/70.2)	1 (0.1/33.3)	1 (0.1/33.3)
MDR[b]	31 (2.1/66.0)	0 [6]	1 (0.1/33.3)	1 (0.1/33.3)

[a] NA indicates that the information is not relevant for the given resistance pattern

[b] MDR excluding MRSA, VRSA, VRE, iMLSB- and cMLSB-resistant *S. aureus*

[c] The two numbers in parentheses indicate number of colonized women and the number of isolates of each given species, respectively

specimen with two *K. pneumonia* isolates, and four specimens with two *S. aureus* isolates.

Antimicrobial resistance profiles of the potentially pathogenic bacteria

Antimicrobial resistance results are listed in Tables 1 and 2. Of the 1472 women, 976 (66.3%; 95% CI 63.8%, 68.7%) were colonized with bacteria resistant to at least one of the first-line antibiotics used for treating severe neonatal infections in Uganda, including ampicillin and gentamicin.

Colonization with ESBL-producing bacteria

Of the 1472 women, 57 (3.9%; 95% CI: 3.0%, 5.1%) were colonized with ESBL-producing bacteria, all of which were *Enterobacteriaceae* (Table 1). The 57 isolates recovered from these 57 women included 36 *E. coli*, 16 K. *pneumoniae,* 3 *Citrobacter* spp., 1 K. *oxytoca*, and 1 *Enterobacter* spp. Except for 1 *E. coli* and 1 K. *pneumoniae*, all these ESBL-producing *Enterobacteriaceae* isolates were MDR.

Colonization with carbapenem-resistant bacteria

We found that 27 of the 1472 women (1.8%; 95% CI: 1.2%, 2.7%) were colonized with carbapenem-resistant bacteria. All the carbapenem-resistant bacteria were *Enterobacteriaceae* including: 10 *E. coli,* 7 *Citrobacter* spp., 5 K. *pneumoniae*, 3 *Enterobacter* spp., and 1 K. *oxytoca* isolates; except one carbapenem-resistant *Acinetobacter* spp. None of the *Pseudomonas* spp. strains were carbapenem-resistant. All the carbapenem-resistant bacteria were MDR.

MRSA, MLSB, VRSA and VRE colonization

We found that 117 of the 1472 women were colonized with *S. aureus.* Among these, 85 (5.8% [95% CI 4.6, 7.1]) were colonized with MRSA. Forty-five of 55 *S. aureus* isolates (81.8%) tested positive in our PCR-based MRSA assay, but of these only 2 (3.4%) were positive for the Panton-Valentine Leukocidin (PVL) virulence factor. The three other PVL positive isolates were from methicillin sensitive *S. aureus*. All MRSA isolates were MDR. Eighteen of the 1472 women (1.2% [95% CI 0.7%, 1.9%]) were colonized with induced macrolide

lincosamide-streptogramin B (iMLSB)-resistant *S. aureus* while 24 (1.6% [95% CI 1.0%, 2.4%]) were colonized with constitutive MLSB (cMLSB)-resistant *S. aureus*. Fifteen of the 18 iMLSB-resistant *S. aureus* (83.3%) isolates were also MRSA. The proportion of women colonized with VRSA and VRE was both 0.4% (6/1472; 95% CI 0.1%, 0.9%). All 12 isolates from these VRE and VRSA colonization were MDR (Fig. 1).

Colonization with MDR bacteria

We found that 750 (50.9%; 95% CI 48.4%, 53.5%) of the women were colonized with MDR bacteria (Tables 1 and 2). The majority of colonizations with MDR bacteria included *E. coli* (56.8% [426/750]), *K. pneumoniae* (11.9% [89/750]) and *S. aureus* (11.2% [84/750]). The distribution of characteristics of women colonized with MDR bacteria is shown in Table 3.

Even when omitting the clinically important AMR bacteria (ESBL-producers, carbapenem- and MLSB-resistant bacteria, MRSA, VRSA and VRE), as many as 506 (34.5%) of the women were colonized with MDR bacteria.

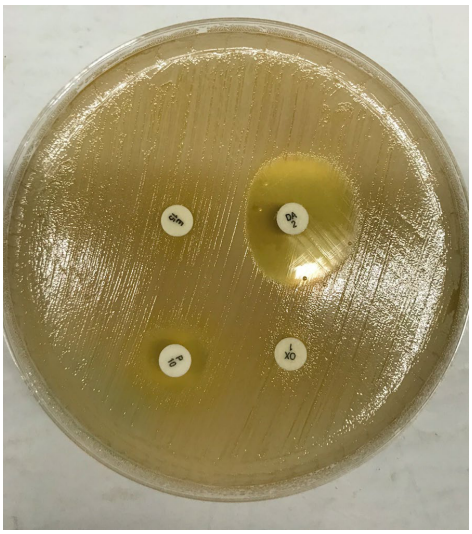

Figure 1 Example of a disk diffusion test for inducible clindamycin resistance in a *S. aureus.* isolate. Inducible clindamycin resistance is indicated by the flattened border between the clindamycin disk (top right) and the erythromycin disk (top left), resulting in a characteristic 'D'-shaped area cleared of *S. aureus.* around the clindamycin disk. The two lower disks were included to test the isolate for penicillin (left) and oxacillin (right) resistance.

Exposures associated with colonization with AMR bacteria

We found that being ≥30 years old was associated with an increased odds of being colonized with MRSA (adjusted OR: 3.03 [95% CI: 1.51, 6.07]) or MDR (adjusted OR: 1.56 [95% CI: 1.09, 2.24]) compared to 20–24 year olds. Wealth level was not associated with AMR colonization (Table 3).

Discussion

In this study, we investigated the prevalence of vaginal colonization with clinically important AMR bacteria among women in labour at three primary health care facilities in and close to Kampala in central Uganda; and established exposures associated with AMR. The prevalence of colonization with MDR bacteria in our study was 46.6%, similar to those reported from Malawi and Ethiopia [26–28]. Although direct comparison of results between studies are complicated by the lack of consensus on how MDR should be defined, we used an internationally acknowledged MDR definition, making our findings comparable to those from similar studies [29, 30]. With the main exceptions of gentamicin, ampicillin and ceftriaxone, most of the antibiotics included in this study are not commonly used to treat serious bacterial infection in children in Uganda. Nevertheless, the antibiotic classes of all relevant first- and second-line antibiotics used in Uganda should be represented in this study.

The prevalence of women colonized with ESBL-producing bacteria in our study was low (4%). Our findings are similar to those reported from other comparable studies [31–34]. However, studies in similar settings such as Tanzania [35] and India [36] found a prevalence of ESBL vaginal colonization to be higher (15%) and a study in Bangladesh found a prevalence of women colonized with ESBL-producing bacteria to be 21% [37]. It is not clear why there is a large difference in prevalence of vaginal colonization with these ESBL-producing bacteria between these studies, but since ESBL encoding genes are easily spread between bacteria [38], it is possible that the differences in prevalence could be effects of differences in antibiotic use and, thereby, selective pressures between the study populations. The discrepancies may also be explained by the differences in microbiological methods, sampling error and study populations as the women we studied were low risk and averagely young women aged about 25 years.

The prevalence of colonization with carbapenem-resistant bacteria in our study was 2%, similar to that reported among Algerian pregnant women [39] and among women in labour at Brooklyn, New York Hospital [40]. These studies including ours suggest the importance to assess maternal colonization with carbapenem-resistant

Table 3 Association between exposures and vaginal colonization with important antibiotic-resistant bacteria among study women in labor

Participant characteristic	No. of women (%), N = 1472	Adjusted odds ratio (95% CI) of being colonized with bacteria		
		ESBL-producing *Enterobacteriaceace*	MRSA	MDR
Mother's age				
20–24 years	587 (39.9)	1	1	1
< = 19 years	205 (13.9)	0.69 (0.26, 1.79)	1.26 (0.59, 2.68)	1.16 (0.82, 1.63)
25–29 years	454 (30.8)	1.32 (0.69, 2.54)	1.68 (0.93, 3.02)	1.27 (0.98, 1.65)
> = 30 years	226 (15.4)	0.91 (0.34, 2.43)	3.03 (1.51, 6.07)	1.56 (1.09, 2.24)
Education level				
Tertiary	130 (8.8)	1	1	1
Primary	488 (33.2)	1.15 (0.39, 3.33)	1.91 (0.74, 4.93)	1.31 (0.86, 1.98)
Secondary	854 (58.0)	1.08 (0.40, 2.89)	1.44 (0.58, 3.53)	1.42 (0.96, 2.09)
Wealth index				
Quintile5	290 (19.7)	1	1	1
Quintile1	489 (33.2)	0.82 (0.36, 1.85)	1.09. (0.54, 2.20)	0.82 (0.59, 1.13)
Quintile2	100 (6.8)	0.42 (0.09, 1.97)	0.85 (0.29, 2.51)	0.85 (0.53, 1.38)
Quintile3	298 (20.2)	0.86 (0.36, 2.09)	1.07 (0.50, 2.31)	0.86 (0.60, 1.23)
Quintile4	295 (20.0)	1.06 (0.45, 2.49)	0.97 (0.45, 2.06)	0.86 (0.61, 1.22)
Gravidity				
Primigravida	442 (30.0)	1	1	1
2–4 pregnancies	910 (61.8)	0.73 (0.37, 1.44)	0.84 (0.46, 1.55)	0.95 (0.73, 1.25)
5 or more pregnancies	120 (8.2)	0.68 (0.18, 2.59)	0.39 (0.13, 1.19)	0.62 (0.37, 1.02)
Hospitalization during pregnancy				
No	1387 (94.2)	1	1	1
Yes	710 (94.5)	1.35 (0.47, 3.33)	1.32 (0.55, 3.17)	0.99 (0.64, 1.55)
Antenatal visits				
Once	75 (5.1)	1	1	1
2–4 times	1259 (85.5)	0.53 (0.20, 1.41)-	0.78 (0.30, 2.02)	1.15 (0.71, 1.85)
5 or more times	138 (9.4)	0.28 (0.06, 1.26)-	1.29 (0.42, 3.95)	1.12 (0.63, 1.99)
Own domestic animals				
No	1355 (92.1)	1	1	1
Yes	117 (8.0)	0.16 (0.02, 1.27)	0. 89 (0.34, 2.35)	0. 77 (0.50, 1.20)

bacteria during labour as there is potential for vertical transmission of these bacteria to newborns.

Overall, the prevalence of colonization with MRSA was 5.8%, which is somewhat higher than that observed in other similar studies that reported a prevalence 0.5%-3.5% [41–47]. This may be explained by differences in geographical setting as well as differences in microbiological testing methods used in our study compared to other similar studies. MRSA is usually resistant to majority of beta-lactam drugs including penicillins, beta-lactamase inhibitors, cephalosporin and carbapenems. However, MRSA are sensitive to ceftaroline suggesting general overuse and/or misuse of antibiotics. Resistance to these drugs occurs because of acquisition of genes that encode drug-inactivating enzymes. However, a majority of the *S. aureus* isolates in our study were highly susceptible to vancomycin. Vancomycin is still the first line drug for treating MRSA infections in many countries, but it remains an expensive drug to be used in resource limited settings like Uganda.

The very low prevalence (0.4%) of VRSA in our study is similar to that observed (0.9%) in a South African cohort of pregnant women [47]. Relatively new anti-MRSA drugs (linezolid, tigecycline, daptomycin and quinupristin-dalfopristin) were introduced after the emergence of VRSA but they are expensive, especially in resource limited settings. Increased spread of MRSA and VRSA are of public health concern since they render treatment efforts with affordable beta lactam antibiotics futile. The prevalence of PVL positive MRSA was 0.3% in our study which

is comparable to the 0.9% reported among Bangladeshi pregnant women [37].

In our study, we assessed for MLSB resistance also known as erythromycin-inducible clindamycin resistance. We observed a prevalence of vaginal colonization with erythromycin-inducible clindamycin resistance among study participants to be 1.2% similar to the 0.7% observed among pregnant women in an Egyptian study [48]. The majority of studies on vaginal colonization of pregnant women with MLSB resistant bacteria report such resistance among GBS compared to *S. aureus* in our study; hence making it difficult to directly compare our findings. We reported a low prevalence of 0.4% among study participants and our findings are similar to those of others [49]. However, our findings differ from those in Ethiopia and the discrepancy may be due to differences in antibiotic prescription and or antibiotic use, geographical settings, study populations and differences in VRE detection methods. Vaginal colonization of women in labour with VRE is of concern to the newborn in case of vertical transmission because it would greatly limit the options of effective treatment of serious infections, leading to poor clinical outcomes among the neonates.

Generally, unregulated access of antibiotics over the counter and their increased use in domestic and commercial animal farming contributes significantly to the antimicrobial resistance problem, also in resource limited settings [50]. Irrational use of antibiotics is a major public health problem globally and is associated with increase in antibiotic resistance [51]. A multi-site study conducted in Uganda found that 41% of antibiotics were issued over the counter without prescription [52]. This suggests that irrational use of antibiotics is very common in Uganda.

In the present study, we observed that women aged 30 or more years were more likely to be colonized by MDR or MRSA than 20–24 years old women. Our findings are similar to another study that found that Moroccan women who were older were more likely to be colonized with multi-drug resistant bacteria [53]. We tested exposures that other studies found to be associated with vaginal colonization [54–56]. Unlike those studies, we did not find substantial associations between colonization with antimicrobial resistant bacteria and exposures such as living with domestic animals, prior hospitalization or prior health care facility visits. One potential explanation could be the differences in geographical settings between the studies. Another potential explanation is the likely absence of power to study these exposures given our relatively wide confidence intervals.

We conducted a large study with 1472 women recruited from three health facilities in and close to Kampala. The study had some limitations. We did not perform molecular antimicrobial resistance assays except for MRSA (*mecA* PCR) to confirm the phenotypic resistance patterns we observed using the disk diffusion method. Given that the resistance phenotype of some bacteria may be conditional on the culture condition, we cannot rule out the possibility that we may have underestimated the prevalences of colonization with AMR bacteria in this study given we have only tested the isolates for resistance by using the disk diffusion method. By not performing anaerobe cultures, we probably missed colonization with some pathogenic bacteria that could contribute to severe infections in neonates, and it may have reduced our ability to identify a few facultative anaerobic bacteria that may be easier to identify when grown under anaerobic conditions [7]. Finally, our use of traditional biochemical tests to determine the species of each isolate may not always give accurate results. Consequently, we may have somewhat underestimated the true prevalence of some of the infections.

Conclusion

The prevalence of vaginal colonization with potentially pathogenic MDR and other clinically important AMR bacteria among HIV-negative women in labour at three primary health care facilities was high. The finding of extensive colonization with multidrug-resistant bacteria including ESBL-producing *Enterobacteriaceace*, carbapenem-resistant bacteria, methicillin-resistant *S. aureus*, erythromycin-inducible clindamycin resistant-*S. aureus*, vancomycin-resistant *S. aureus* and vancomycin-resistant *Enterococci* in our study raises a question whether we should conduct routine screening of pregnant women or exposed newborns for carriage/colonization. However, screening women during antenatal would be expensive and identifying the exposed infants would increase on the existing huge workload for health workers. Our findings have implications for possible prophylactic treatment to pregnant women colonized with such multidrug resistant bacteria, the prevention and management of early on-set neonatal sepsis including providing local data to guide choice of antibiotics for treating early-onset neonatal sepsis and vaccine development in similar settings. There is need to investigate whether there is vertical transmission of these multidrug-resistant bacteria to the babies.

Acknowledgements

The authors thank all the women who participated in our study and the midwives at Kawaala HC III, Kitebi HC III and Mukono HC IV for their cooperation. Special thanks to the CISMAC research nurses for their hard work and commitment. We also acknowledge MBN microbiology and molecular laboratory staff for the support rendered to our project.

Authors' contributions

JT, VN, HS, JKT, and FB conceived the study. JT, VN, FB, HS and ON designed the study. JT and FB led the microbiology testing and results interpretation. HSt, VN and HS prepared the dataset for analysis. JT, DM, and HSt analysed the data. JT wrote the first draft of manuscript. All authors reviewed, approved

and agreed to submit the final version of the manuscript. All authors read and approved the final manuscript.

Funding
JT was supported by a grant from the State Educational Loan Fund in Norway through the Quota Program. This study was conducted with financial support from the Centre for Interventional Science in Maternal and Child Health through the Chlorhexidine trial. This funding played a role in design of the study, data and specimen collection and analysis process. The contribution by HS, HSt and VN was supported by CISMAC (project number 223269), which is funded by the Research Council of Norway through its Centres of Excellence scheme and the University of Bergen, Norway. Part of the laboratory work was funded by the Norwegian programme for capacity development in higher education and research for development (NORHED) through the Survival Pluss project (Project Number; UGA-13-0030).

Availability of data and materials
Datasets used during the current study can be obtained through a reasonable request from the principle investigator of the Chlorhexidine Trial (VN) nankabirwav@gmail.com and the corresponding author.

Ethical approval and consent to participate
Informed consent was obtained for both the interview and specimen collection and storage. Ethical approval was obtained from the Research and Ethics review Committee of School of Medicine, SOMREC, Makerere University (REC REF 2015-118) and from the Uganda National Council of Science and Technology (HS 1927).

Consent for publication
Not applicable.

Competing interests
Authors declare no competing interests.

Author details
[1] Centre for Intervention Science for Maternal and Child Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway. [2] Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway. [3] Department of Biomedicine, University of Bergen, Bergen, Norway. [4] Department of Medical Microbiology, Makerere University College of Health Sciences, Kampala, Uganda. [5] Department of Paediatric and Child Health, Makerere University College of Health Sciences, Kampala, Uganda. [6] Department of Public Health, Busitema University, Busitema, Uganda. [7] Department of Epidemiology and Biostatistics School of Public Health, Makerere University, Kampala, Uganda.

Received: 13 September 2020 Accepted: 21 January 2021
Published online: 17 February 2021

References

1. Laxminarayan R, et al. Antibiotic resistance—the need for global solutions. Lancet Infect Dis. 2013;13(12):1057–98.
2. Okomo, U., et al., *Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines.* Lancet Infect Dis, 2019.
3. Coque, T.M., F. Baquero, and R. Canton, *Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe.* Euro Surveill, 2008. **13**(47).
4. UN General Assembly, T.o.w.t.A.f.S.D., 21 October 2015, A/RES/70/1, available at: https://www.refworld.org/docid/57b6e3e44.html. Accessed 6 May 2019.
5. Breijyeh, Z., B. Jubeh, and R. Karaman, *Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it.* Molecules, 2020. **25**(6).
6. Rice, L.B., *Antimicrobial resistance in gram-positive bacteria.* Am J Infect Control, 2006. **34**(5 Suppl 1): p. S11–9; discussion S64–73.
7. Messbarger N, Neemann K. Role of anaerobic blood cultures in neonatal bacteremia. J Pediatric Infect Dis Soc. 2018;7(3):e65–9.
8. Iwata K, Takahashi M. Is anaerobic blood culture necessary? If so, who needs it? Am J Med Sci. 2008;336(1):58–63.
9. Magiorakos AP, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.
10. Meyn LA, Krohn MA, Hillier SL. Rectal colonization by group B Streptococcus as a predictor of vaginal colonization. Am J Obstet Gynecol. 2009;201(1):76.e1-7.
11. Chereau F, et al. Colonization of extended-spectrum-beta-lactamase- and NDM-1-producing Enterobacteriaceae among pregnant women in the community in a low-income country: a potential reservoir for transmission of multiresistant Enterobacteriaceae to neonates. Antimicrob Agents Chemother. 2015;59(6):3652–5.
12. Jimenez-Ramila C, et al. Vagino-rectal colonization and maternal-neonatal transmission of Enterobacteriaceae producing extended-spectrum beta-lactamases or carbapenemases: a cross-sectional study. J Hosp Infect. 2019;101(2):167–74.
13. Marando R, et al. Predictors of the extended-spectrum-beta lactamases producing *Enterobacteriaceae neonatal* sepsis at a tertiary hospital Tanzania. Int J Med Microbiol. 2018;308(7):803–11.
14. Herindrainy P, et al. Acquisition of extended spectrum beta-lactamase-producing enterobacteriaceae in neonates: a community based cohort in Madagascar. PLoS ONE. 2018;13(3):e0193325.
15. Tumuhamye J, et al. Vaginal colonisation of women in labour with potentially pathogenic bacteria: a cross sectional study at three primary health care facilities in Central Uganda. BMC Infect Dis. 2020;20(1):98.
16. Nankabirwa V, et al. Efficacy of umbilical cord cleansing with a single application of 4% chlorhexidine for the prevention of newborn infections in Uganda: study protocol for a randomized controlled trial. Trials. 2017;18(1):322.
17. *Open Data Kit: The standard for mobile data collection*. [cited 2018 3rd December]; Available from: https://opendatakit.org.
18. Winn W., A.S., Janda W., Koneman E., Procop G., Schreckenberger P., and Woods G. , *Koneman's Color Atlas and Textbook of Diagnostic Microbiology.* 6th Edition, ed. 2006, New York.: Lippincott Williams and Wilkins, New York.
19. Clinical and Laboratory Standards Institute (CLSI) (2017): Performance standards for antimicrobial susceptibility testing; Twenty-seventh informational supplement. CLSI document M100-S27. Wayne, P.C.a.L.S.
20. Coutinho LM, Scazufca M, Menezes PR. Methods for estimating prevalence ratios in cross-sectional studies. Rev Saude Publica. 2008;42(6):992–8.
21. McClure JA, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. J Clin Microbiol. 2006;44(3):1141–4.
22. Seni J, et al. Molecular characterization of Staphylococcus aureus from patients with surgical site infections at Mulago Hospital in Kampala, Uganda. PLoS ONE. 2013;8(6):e66153.
23. Institute., C.a.L.S., Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition. , in CLSI Document M2-A10. . (2009). Clinical and Laboratory Standards Institute, Wayne, PA.
24. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, v., 2020. [cited 2020 8 May]; Available from: http://www.eucast.org/clinical_breakpoints/.
25. Fishel Bartal, M., et al., *Trial of labor after cesarean (TOLAC) in women with premature rupture of membranes().* J Matern Fetal Neonatal Med, 2019: p. 1–7.
26. Admas A, et al. Proportion of bacterial isolates, their antimicrobial susceptibility profile and factors associated with puerperal sepsis among postpartum/aborted women at a referral Hospital in Bahir Dar Northwest Ethiopia. Antimicrob Resist Infect Control. 2020;9:14.
27. Gallaher JR, et al. Colonization with Multidrug-Resistant Enterobacteriaceae is Associated with Increased Mortality Following Burn Injury in Sub-Saharan Africa. World J Surg. 2018;42(10):3089–96.
28. Feleke T, et al. Multidrug-resistant bacterial isolates from patients suspected of nosocomial infections at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. BMC Res Notes. 2018;11(1):602.

29. Assefa S, Desta K, Lema T. Group B streptococci vaginal colonization and drug susceptibility pattern among pregnant women attending in selected public antenatal care centers in Addis Ababa, Ethiopia. BMC Pregnancy Childbirth. 2018;18(1):135.
30. Teerawattanapong, N., et al., *Prevention and control of multidrug-resistant gram-negative bacteria in adult intensive care units: a systematic review and network meta-analysis.* Clin Infect Dis, 2017. **64**(suppl_2): p. S51-s60.
31. Rettedal S, et al. Extended-spectrum beta-lactamase-producing Enterobacteriaceae among pregnant women in Norway: prevalence and maternal-neonatal transmission. J Perinatol. 2015;35(11):907–12.
32. Danino D, et al. Mother-to-child transmission of extended-spectrum-beta-lactamase-producing Enterobacteriaceae. J Hosp Infect. 2018;100(1):40–6.
33. Villar HE, et al. Maternal carriage of extended-spectrum beta-lactamase-producing Escherichia coli isolates in Argentina. J Chemother. 2013;25(6):324–7.
34. Cursino MA, et al. Performance of surveillance cultures at different body sites to identify asymptomatic Staphylococcus aureus carriers. Diagn Microbiol Infect Dis. 2012;74(4):343–8.
35. Nelson E, et al. Evaluation of existence and transmission of extended spectrum beta lactamase producing bacteria from post-delivery women to neonates at Bugando Medical Center Mwanza-Tanzania. BMC Res Notes. 2014;7:279.
36. Pathak A, et al. Frequency and factors associated with carriage of multidrug resistant commensal Escherichia coli among women attending antenatal clinics in central India. BMC Infect Dis. 2013;13:199.
37. Tarana MN, Shamsuzzaman SM. Laboratory diagnosis of bacterial vaginosis and potential pathogens other than group B streptococcus in vaginal swab of pregnant women in Dhaka Medical College Hospital. Mymensingh Med J. 2018;27(4):834–42.
38. Blake DP, et al. Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ileal conditions. J Appl Microbiol. 2003;95(3):428–36.
39. Mairi A, et al. Carbapenemase-producing Enterobacteriaceae among pregnant women and newborns in Algeria: prevalence, molecular characterization, maternal-neonatal transmission, and risk factors for carriage. Am J Infect Control. 2019;47(1):105–8.
40. Rawstron SA, et al. Perirectal screening for carbapenem-resistant enterobacteriaceae obtained from 100 consecutive healthy pregnant women in labor at a brooklyn hospital: results and risk factors. Infect Control Hosp Epidemiol. 2018;39(3):369–71.
41. Gray JW, Suviste J. Three years' experience of screening for meticillin-resistant Staphylococcus aureus in obstetrics. J Hosp Infect. 2013;83(1):61–3.
42. Top KA, et al. Predictors of Staphylococcus aureus Rectovaginal colonization in pregnant women and risk for maternal and neonatal infections. J Pediatric Infect Dis Soc. 2012;1(1):7–15.
43. Ghanim N, et al. Maternal-neonatal outcome with Staphylococcus aureus rectovaginal colonization. J Reprod Med. 2011;56(9–10):421–4.
44. Andrews WW, et al. Genital tract methicillin-resistant Staphylococcus aureus: risk of vertical transmission in pregnant women. Obstet Gynecol. 2008;111(1):113–8.
45. Chen KT, et al. Prevalence of methicillin-sensitive and methicillin-resistant Staphylococcus aureus in pregnant women. Obstet Gynecol. 2006;108(3 Pt 1):482–7.
46. Dammeyer AH, et al. Clinical relevance of colonization with antimicrobial-resistant bacteria (AMRB) and methicillin susceptible Staphylococcus aureus (MSSA) for mothers during pregnancy. Arch Gynecol Obstet. 2019;300(5):1303–16.
47. Hetsa BA, Kumar A, Ateba CN. Characterization of multiple antibiotic resistant clinical strains of Staphylococcus isolated from pregnant women vagina. Folia Microbiol (Praha). 2018;63(5):607–17.
48. Shabayek SA, Abdalla SM, Abouzeid AM. Vaginal carriage and antibiotic susceptibility profile of group B Streptococcus during late pregnancy in Ismailia, Egypt. J Infect Public Health. 2009;2(2):86–90.
49. Oelmeier de Murcia, K., et al., *Prevalence of Multidrug Resistant Bacteria in Refugees: A Prospective Case Control Study in an Obstetric Cohort.* Z Geburtshilfe Neonatol, 2017. **221**(3): p. 132–136.
50. Eagar H, Swan G, van Vuuren M. A survey of antimicrobial usage in animals in South Africa with specific reference to food animals. J S Afr Vet Assoc. 2012;83(1):16.
51. Wise, R., et al., *Antimicrobial resistance. Is a major threat to public health.* Bmj, 1998. **317**(7159): p. 609–10.
52. Mukonzo JK, et al. Over-the-counter suboptimal dispensing of antibiotics in Uganda. J Multidiscip Healthc. 2013;6:303–10.
53. El Mekes A, et al. The clinical and epidemiological risk factors of infections due to multi-drug resistant bacteria in an adult intensive care unit of University Hospital Center in Marrakesh-Morocco. J Infect Public Health. 2020;13(4):637–43.
54. Khan MA, Faiz A, Ashshi AM. Maternal colonization of group B streptococcus: prevalence, associated factors and antimicrobial resistance. Ann Saudi Med. 2015;35(6):423–7.
55. Chan GJ, et al. Maternal and neonatal colonization in Bangladesh: prevalences, hh and risk factors. J Perinatol. 2013;33(12):971–6.
56. Darabi R, et al. The prevalence and risk factors of group B streptococcus colonization in Iranian pregnant women. Electron Physician. 2017;9(5):4399–404.

Publisher's Note

Paper III

International Journal of
Environmental Research and Public Health

MDPI

Article

Umbilical Cord Stump Infections in Central Uganda: Incidence, Bacteriological Profile, and Risk Factors

Josephine Tumuhamye [1,*], Halvor Sommerfelt [1,2], James K. Tumwine [3,4], David Mukunya [5], Grace Ndeezi [3], Olive Namugga [1], Freddie Bwanga [6], Hans Steinsland [1,7] and Victoria Nankabirwa [1,8]

1 Centre for Intervention Science for Maternal and Child Health, Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, 5020 Bergen, Norway
2 Cluster for Global Health, Division for Health Services, Norwegian Institute of Public Health, 0430 Oslo, Norway
3 Department of Paediatric and Child Health, Makerere University, Kampala P.O. Box 7062, Uganda
4 School of Medicine, Kabale University, Kabale P.O. Box 317, Uganda
5 Department of Community and Public Health, Busitema University, Mbale P.O. Box 236, Uganda
6 Department of Immunology and Molecular Biology, Makerere University, Kampala P.O. Box 7062, Uganda
7 Department of Biomedicine, University of Bergen, 5020 Bergen, Norway
8 Department of Epidemiology, Biostatics School of Public Health, Makerere University, Kampala P.O. Box 7062, Uganda
* Correspondence: tphynne@gmail.com

Abstract: Umbilical cord stump infection (omphalitis) is a risk factor for neonatal sepsis and death. We assessed the incidence of omphalitis, described the bacteriological and antibiotic-resistance profile of potentially pathogenic bacteria isolated from the umbilical cord stump of omphalitis cases, and evaluated whether bacteria present in the birth canal during birth predicted omphalitis. We enrolled 769 neonates at birth at three primary healthcare facilities and followed them for 28 days with scheduled visits on days 3, 7, 14, and 28. Cox regression models were used to estimate the rates of omphalitis associated with potential risk factors. Sixty-five (8.5%) neonates developed omphalitis, with an estimated incidence of 0.095 cases per 28 child-days (95% CI 0.073, 0.12). Potentially pathogenic bacteria were isolated from the cord stump area of 41 (63.1%) of the 65 neonates with omphalitis, and the most commonly isolated species were *Escherichia coli* (n = 18), *Klebsiella pneumoniae* (n = 10), *Citrobacter freundii* (n = 5), and *Enterobacter* spp. (n = 4). The *Enterobacteriaceace* isolates were resistant to gentamicin (10.5%, 4/38), ampicillin (86.8%, 33/38), and ceftriaxone (13.2%, 5/38). Delayed initiation of breastfeeding was associated with an increased risk of omphalitis (aHR 3.1; 95% CI 1.3, 7.3); however, vaginal colonization with potentially pathogenic bacteria did not predict omphalitis.

Keywords: omphalitis; umbilical cord stump infections; neonates; newborns; incidence rate; incidence proportion; antimicrobial-resistance

Citation: Tumuhamye, J.; Sommerfelt, H.; Tumwine, J.K.; Mukunya, D.; Ndeezi, G.; Namugga, O.; Bwanga, F.; Steinsland, H.; Nankabirwa, V. Umbilical Cord Stump Infections in Central Uganda: Incidence, Bacteriological Profile, and Risk Factors. *Int. J. Environ. Res. Public Health* **2022**, *19*, 16055. https://doi.org/10.3390/ijerph192316055

Academic Editor: Paul B. Tchounwou

Received: 24 October 2022
Accepted: 28 November 2022
Published: 30 November 2022

1. Introduction

Globally, 5.1 million children die before five years of age, and of these deaths, 2.4 million occur during the first month of life [1]. In Uganda, it has been estimated that out of every 1000 live births, almost 307 die within the first 28 days of life [2], i.e., as neonates. Infections are the leading cause and account for approximately one-third of all neonatal deaths in sub-Saharan Africa [3]. Omphalitis is clinically characterized by the presence of redness, swelling, and/or pus at the umbilical cord stump [4]. The stump can act as a portal of entry for invasive pathogenic bacteria into the bloodstream [5]. Furthermore, it contains necrotic tissue, which provides a suitable medium for bacterial growth. The mother's birth canal is one of the most important sources of these bacteria, where the umbilical cord is contaminated during birth [6]. Other known maternal risk factors associated

with omphalitis include intrapartum infections such as chorioamnionitis, a prolonged rapture of membranes, and home delivery; neonatal risk factors include very low birth weight and improper cord care, such as the application of home remedies to speed up cord separation, which often facilitates bacterial contamination and increases the risk of infection [7]. Potentially pathogenic bacteria that are commonly found colonizing the umbilical cord include *Staphylococcus aureus*, group A *streptococci*, group B *streptococci*, and gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, and *Pseudomonas* species.

Omphalitis remains an important risk factor for severe illness and death among neonates in developing countries [4,8,9]. Four large community-based trials in developing countries indicate that the incidence risk of moderate to severe omphalitis during the neonatal period may range widely, from 1% to 7.6% [10–13]. Although neonatal infections are a major problem in developing countries, data on the incidence, bacterial etiology, antimicrobial resistance patterns, and risk factors associated with the development of neonatal omphalitis are scarce. Previous studies focus mainly on the incidence of omphalitis [10–12,14–16] but few report on the risk factors, bacterial etiology, and antimicrobial resistance patterns [7,15,16]. We sought to estimate its incidence among HIV-unexposed babies born at three primary healthcare facilities in Uganda. We also describe the bacteriological profile of the cord stump of the omphalitis cases. Finally, we estimate the association between vaginal colonization and potentially pathogenic bacteria of women in labor and omphalitis amongst their neonates and sought to describe other factors that may be associated with neonatal omphalitis.

2. Materials and Methods

2.1. Study Design and Setting

We conducted a prospective cohort study between July 2016 and July 2018 at three primary healthcare facilities in Central Uganda: Mukono general hospital (formerly Mukono Health Centre IV), Kawaala Health Centre III, and Kitebi Health Centre III. The two health centers are located in urban slum areas in Kampala district, the capital city of Uganda. Both these health facilities mostly conducted normal deliveries during the study period, while Mukono general hospital, which is located within Mukono district about 20 km away from Kampala, also conducted more complicated deliveries, including caesarian sections. This study was nested within a randomized controlled trial assessing the effectiveness of a single application of 4% chlorhexidine solution on the umbilical cord stump for the prevention of omphalitis and severe illness in neonates whose mothers did not have HIV infection [17]. We enrolled participants from the standard care (control) arm of this clinical trial, where the cord stump was left dry. The present study includes data from two previous studies that characterized potentially pathogenic bacteria isolated from vaginal swabs from the mothers during labor [18,19].

2.2. Recruitment, Enrollment, and Follow-Up

During recruitment, a study midwife obtained permission verbally from women in labor to collect vaginal swabs. After giving birth, a study nurse informed the women about the chlorhexidine trial [17] and obtained informed consent for enrolment into the trial and the current cohort study. The exclusion criteria were birth weight less than 1.5 kg, omphalitis at birth, severe congenital anomaly, and severe illness requiring immediate hospitalization. In the current study, we aimed to include the 800 newborn babies enrolled in the dry cord care (control) arm of the trial when its sample size was planned to be 1600 [17].

After enrollment, the neonate was scheduled for follow-up, on day 3, day 7, day 14, and day 28. During these interviews, a study nurse examined the baby for various health indicators, including signs of omphalitis, and asked the mother about past symptoms and illnesses. The scheduled visits had specified window periods around them, i.e., day 2 to day 4 for the day 3 visit, day 5 to day 9 for the day 7 visit, and day 10 to day 18 for the day

14 visit. The window period for the day 28 visit was from day 22 to day 40. If a mother did not turn up for an interview, reminder phone calls were made, and if this failed then study staff made home visits to speak directly with the mother and bring her and her baby to the study clinic. If no interviews could be conducted within the specified time window, the participant was considered to be lost to follow-up for the scheduled visit.

We set out to enroll 800 neonates in the control arm of the trial from mid-2016 to mid-2018, i.e., half of its initial sample size of 1600. This would yield a high (1.0% to 2.5%) absolute precision (half-width of the 95% confidence interval) for omphalitis risk in the range of 2% to 15%.

2.3. Data Collection, Management, and Quality Control

As described earlier [19], we used electronic questionnaires generated using the Open Data Kit (ODK) software [20] on mobile phones to capture our data. The pre-coded digital questionnaires had inbuilt checks to prevent progression if erroneous data were entered. The completed questionnaires were saved on mobile phones and uploaded onto a server at the end of each day. During enrollment, we collected baseline data, including birth weight, baby sex, breastfeeding initiation (early: within one hour after birth, after which we characterized it as delayed), and the mother's age, education, and socioeconomic status as well as mode of delivery (vaginal or caesarean section).

Omphalitis was in the current study defined by the presence of pus on the umbilical cord stump [17]. Before study began, we trained the study nurses in the study procedures, including the use of structured electronic questionnaires and yearly refresher sessions during the study to ensure data quality and completeness.

2.4. Specimen Collection

We collected umbilical cord stump swabs for bacterial culture from babies with omphalitis during the follow-up visits scheduled for days 3, 7, 14, and 28 after birth. Using Regular Rayon sterile swabs pre-packed with Amies agar gel transport medium without charcoal (Copan Diagnostics Inc., Murrieta, CA, USA), a trained midwife moved a swab gently around the cord stump after which she placed it into a specimen tube containing the transport medium and kept it refrigerated for up to 8 h before the bacteriological analyses were undertaken.

2.5. Bacterial Isolation and Identification

The bacterial isolation and identification were performed as described previously [18,19]. Briefly, primary inoculation of the umbilical swab was performed on 5% sheep blood agar and MacConkey agar (both from BioLab Zrt., Budapest, Hungary), followed by aerobic incubation between 35 °C to 37 °C for 18 to 24 h for MacConkey plates and blood agar plates up to 72 h to allow growth of slow-growing bacteria. In this cohort study, we considered the following species to be potentially pathogenic: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, group A *Streptococcus* (GAS), group B *Streptococcus* (GBS), *Enterococcus* spp., *Pseudomonas* spp., *Enterobacter* spp., *Citrobacter* spp., *Proteus* spp., and *Acinetobacter* spp., since they are known to cause infections in neonates. We considered all other species of bacteria that were isolated in this study as being commensals since they rarely are associated with neonatal infections, and these isolates were therefore excluded from further analyses. These included isolates of *Micrococcus* spp., *Corynebacterium* spp., *Lactobacillus* spp., *Bacillus* spp., *Bukolderia* spp., *Serratia* spp., and coagulase-negative Staphylococci.

2.6. Antimicrobial Susceptibility Testing

Antimicrobial drug susceptibility testing of the bacterial isolates was performed on Mueller-Hinton agar plates by using the disk diffusion method as described earlier [18] based on the Clinical Laboratory Standard Institute (CLSI) guidelines [21]. All antibiotic disks were produced by and purchased from BioLab Zrt.

2.7. Statistical Analysis

Data were analyzed by using Stata version 17.0 (StataCorp, College Station, TX, USA). We summarized categorical variables as proportions and continuous variables as means and compared them by using Student's t-tests. We defined the neonatal incidence risk of omphalitis as the proportion of newborns who had at least one episode of omphalitis during their first 28 days of life. When calculating the incidence risk for each specific follow-up period (from birth to day 3, day 4 to 7, day 8 to 14, and day 15 to 28), we based the calculations on the mid-period population size. For example, the mid-period population for the birth to day 3 period was calculated as the study population count at enrollment plus the number of neonates who completed the day 3 examination, divided by two.

We calculated person-time under observation by summing up the time each neonate contributed up to 28 days of life or until they were censored (developed omphalitis, died, or were lost to follow-up). Time to the outcome was calculated as the age of the neonate, in days, at the time they were first diagnosed with omphalitis. Neonates who did not develop omphalitis contributed person-time up until their last registered visit to the clinic. The neonates did not contribute person-time during missed visits because of these neonates, we based the diagnosis of omphalitis on information obtained from the mothers during the first subsequent visit. According to their caretaker, none of the neonates who missed a scheduled visit experienced omphalitis during that period. We defined incidence rate of omphalitis as the ratio of the number of new omphalitis cases divided by the total person-time under observation. Incidence rate was converted to risk by using the formula: incidence rate $\times$ person-time under observation [22].

Kaplan-Meier curves were generated using the sts graph code in Stata. To estimate the association between vaginal colonization during birth and potentially pathogenic bacteria and subsequent neonatal omphalitis, we fitted a Cox proportional hazard regression model to estimate the hazard ratio (HR). The final model included vaginal colonization as the main exposure, and level of education, maternal age, socio-economic status, baby sex, birthweight, and delayed initiation of breastfeeding as potential cofounders. We evaluated the covariates for collinearity, defined as presence of a variance inflation factor greater than 10 [23].

3. Results

3.1. Participant Characteristics

Of the 800 neonates in the control arm of the chlorhexidine trial, we enrolled 769 (96.1%), the remaining 31 could not be examined because swabs were occasionally out of stock (Figure 1). The participants' mean birth weight was 3.2 kg (standard deviation 0.4). Half of them (52.7%) were males (405/769) and only three were delivered by caesarian section, the rest vaginally. Most (95.7%) study babies (736/769) initiated breastfeeding within the first hour and 13.8% (106/769) of neonates had home remedy substances applied to the umbilical cord stump. Follow-up proportions were 570/769 (74.2%) on the visits scheduled for day 3, 548/769 (71.3%) for day 7, 561/769 (73.0%) for day 14, and 726/769 (94.4%) for day 28. The mothers of the neonates had a mean age of 25 years (SD 4.8) at birth. Two-thirds were vaginally colonized with at least one potentially pathogenic bacterium during labor (Table 1).

3.2. Incidence of Omphalitis

Sixty-five (8.5%) of the 769 babies developed omphalitis during follow-up and the majority (58 or 89.2%) of the 65 cases occurred in the first week of life. The risk of omphalitis was 6.3% by day 3 (42/670; 95% CI 4.0%, 7.3%), 2.9% from day 4 to day 7 (16/559; 95% CI 1.3%, 3.5%) and 1.1% between day 8 and day 28 (7/644; 95% CI 0.40%, 2.0%). The majority (89.2%) of the 65 cases occurred in the first week of life. Overall, the neonatal incidence rate of omphalitis was 0.095 cases per 28 child-days (95% CI 0.073, 0.12), translating to a neonatal cumulative incidence, i.e., risk, of 9.5% (95% CI 7.3%, 12.1%). All cases of omphalitis included in these calculations were new, i.e., incident cases.

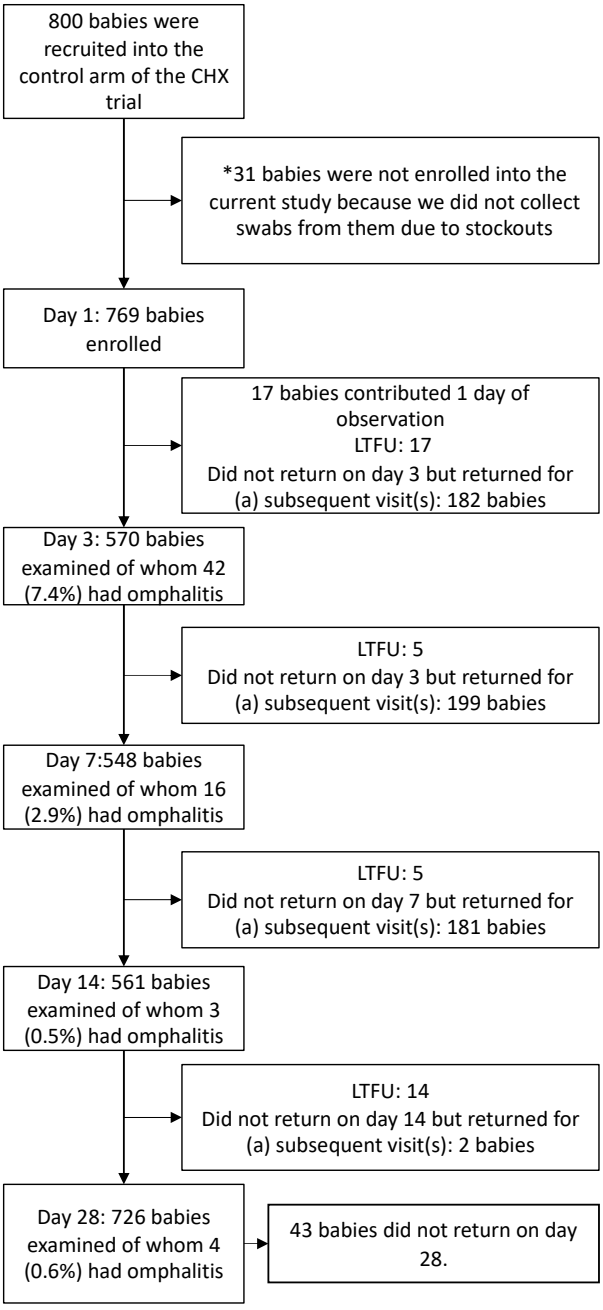

Figure 1. Flow chart of participants included in the study. MLFTU: Lost to follow-up; babies that never returned for the subsequent scheduled visits. * The missing data resulted from stockouts of swabs that were spread out during the course of the study.

Table 1. Distribution of social-demographic characteristics among study participants with and without omphalitis at the three study sites in central Uganda.

Mothers Characteristics	No Omphalitis N = 704 (%)	Omphalitis N = 65 (%)
Age		
19 years or less	94 (13.4)	11 (16.9)
20–24 years	281 (39.9)	21 (32.3)
≥25 years	329 (46.7)	33 (50.8)
Level of education		
Primary	228 (32.4)	24 (36.9)
Secondary and Tertiary	476 (67.6)	41 (63.1)
Socioeconomic status		
Quintile 1	224 (31.8)	17 (26.2)
Quintile 2	80 (11.4)	9 (13.9)
Quintile 3	124 (17.6)	9 (13.9)
Quintile 4	136 (19.3)	15 (23.1)
Quintile 5	140 (19.9)	15 (23.1)
Mode of delivery		
Spontaneous vaginal delivery	637 (90.5)	59 (90.8)
Assisted vaginal delivery	64 (9.1)	6 (9.2)
Caesarian section	3 (0.4)	0 (0)
Gravidity		
One pregnancy	212 (30.1)	18 (27.7)
Two or more pregnancies	492 (69.9)	47 (72.3)
Vaginal colonization		
No	214 (33.9)	19 (31.7)
Yes	418 (66.1)	41 (68.3)
* Missing data	72 (10.2)	5 (7.7)
Health facility		
Kawaala HC III	265 (37.6)	10 (15.4)
Kitebi HC III	248 (35.3)	28 (43.1)
Mukono general hospital	191 (27.1)	27 (41.5)
Initiation of breastfeeding		
Early (within 1 h of birth)	678 (96.3)	58 (89.2)
Late	26 (3.7)	7 (10.8)
First breast milk		
Gave it to baby	699 (99.3)	63 (96.9)
Threw it away	5 (0.7)	2 (3.1)
Birth weight		
Low	25 (3.5)	2 (3.3)
Normal	679 (96.5)	63 (96.9)
Home remedy substance applied on the cord		
No	610 (86.7)	53 (81.5)
Yes	94 (13.3)	12 (18.5)
Sex of baby		
Male	375 (53.3)	30 (46.2)
Female	329 (46.7)	35 (53.8)

* The missing data resulted from stockouts of vaginal swabs that were spread out during the course of the study.

3.3. Bacteriological Profile of Omphalitis

Among the 65 neonates who developed omphalitis, we isolated at least one species of potentially pathogenic bacteria from 41 (63.1%) neonates. Of the 41 neonates, 43 potentially pathogenic bacterial species were isolated, 39 neonates were colonized with one bacterial species each while 2 neonates were colonized with two bacterial species each. All except five of the bacterial isolates were Enterobacteriaceae. The predominant potentially pathogenic bacterial species isolated from the neonates with omphalitis included E. coli 27.7% (18/65), *K. pneumoniae* 15.4% (10/65), and *C. freundii*, 7.7% (5/65). Other bacterial species included *Enterobacter* spp. 6.1% (4/65), *Acinetobacter* spp., 4.6% (3/65), *S. aureus* 3.1% (2/65), and *K. oxytoca* 1.5% (1/65). Twenty-four potentially pathogenic bacteria were isolated from neonates assessed on day 3, thirteen potentially pathogenic bacteria from cases assessed on day 7, while three potentially pathogenic bacteria were isolated from neonates observed on both days 14 and 28 of the follow-up.

3.4. Antimicrobial Resistance Profiles

Among the Enterobacteriaceace isolates, 86.8% (33/38) were resistant to ampicillin, 73.7% (28/38) to amoxicillin-clavulanic acid, and 60.5% (23/38) were resistant to trimethoprim-sulfamethoxazole. There was a relatively low proportion of strains that were resistant to third-generation cephalosporins, such as ceftriaxone in 13.2% (6/38), ceftazidime in 10.5% (4/38), and gentamicin in 10.5% (4/38). All organisms but one were susceptible to imipenem. One of the two *S. aureus* isolates in this study was methicillin-resistant (MRSA) and phenotypically expressed erythromycin inducible clindamycin resistance (D-test positive). Other organism-specific antibiotic resistance patterns are summarized in Table 2. We did not find extended-spectrum beta-lactamase and carbapenem-resistant gram-negative bacteria (Table 2).

Table 2. Resistance patterns of organisms isolated from neonates with omphalitis at three study sites in central Uganda.

	Number (%) of the 65 Neonates Whose Umbilical Cord Stumps Were Colonized with Potentially Pathogenic Bacteria Resistant to One or More Antiobiotics			
Antibiotic	***Escherichia coli* (*n* = 18)**	***Klebsiella pneumoniae* (*n* = 10)**	***Citrobacter species* (*n* = 5)**	***Enterobacter species* (*n* = 4)**
Ampicillin	14 (21.5)	10 (15.4)	5 (7.7)	4 (6.2)
Ampicillin-Clavulanic acid	13 (20.0)	8 (12.3)	4 (6.2)	3 (4.6)
Trimethoprim-Sulfamethoxazole	11 (16.9)	7 (10.8)	4 (6.2)	1 (1.5)
Ciprofloxacin	2 (3.1)	3 (4.6)	1 (1.5)	1 (1.5)
Chloramphenicol	2 (3.1)	3 (4.6)	2 (3.1)	0
Gentamicin	1 (1.5)	3 (4.6)	0	0
Amikacin	2 (3.1)	6 (9.2)	0	1 (1.5)
Ceftriaxone	3 (4.6)	2 (3.1)	0	0
Cefuroxime	5 (7.7)	2 (3.1)	1 (1.5)	0
Ceftazidime	1 (1.5)	2 (3.1)	0	0
Imipenem	0	2 (3.1)	0	0

3.5. Factors Associated with Omphalitis

We found that neonates of women who were vaginally colonized with potentially pathogenic bacteria were almost equally likely to get omphalitis compared to those of women who were not colonized (adjusted hazard ratio (aHR) 1.1; 95% CI 0.63, 1.9) (Figure 2 and Table 3). The analysis revealed that the hazard of omphalitis among neonates initiated late on breastmilk was approximately three times that among neonates who were initiated early (aHR 3.1; 95% CI 1.3, 7.3) (Figure 3 and Table 3). Other maternal and neonatal characteristics were not strongly associated with omphalitis (Table 3).

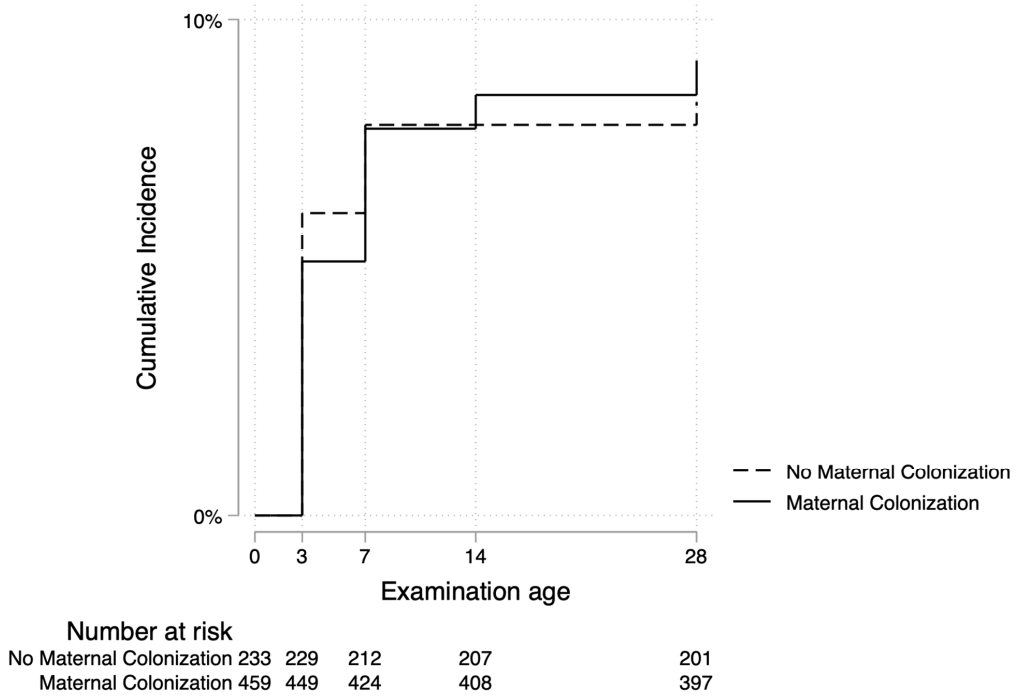

Figure 2. Rate of omphalitis by maternal vaginal colonization with potentially pathogenic bacteria at three study sites in central Uganda.

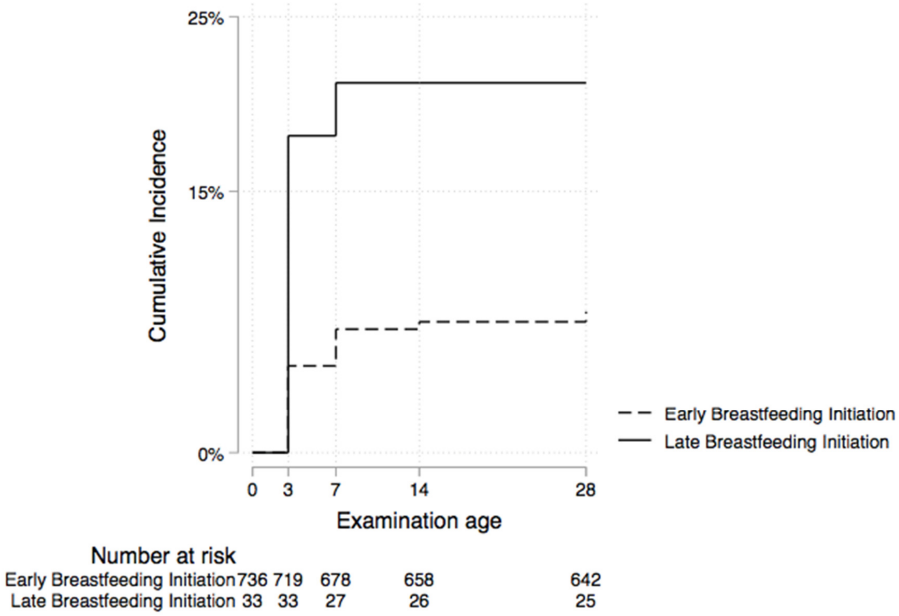

Figure 3. Rate of omphalitis by breastfeeding initiation status at three study sites in central Uganda.

Table 3. Factors associated with omphalitis among neonates at the three study sites in central Uganda.

Mothers Characteristics	Unadjusted HR 95% CI	Adjusted HR 95% CI *N* = 692
Vaginal colonization		
No	1	1
Yes	1.1 (0.63, 1.9)	1.1 (0.63, 1.9)
Age		
19 years or less	1.5 (0.73, 3.1)	1.4 (0.63, 3.0)
20–24 years	1	1
25 years and above	1.3 (0.76, 2.3)	1.1 (0.63, 2.0)
Level of education		
Primary	1	1
Secondary and Tertiary	0.83 (0.50, 1.4)	0.83 (0.49, 1.4)
Socioeconomic status		
Quintile 1	1	1
Quintile 2	1.4 (0.64, 3.2)	1.3 (0.56, 3.1)
Quintile 3	0.96 (0.43, 2.1)	0.91 (0.39, 2.2)
Quintile 4	1.4 (0.71, 2.8)	1.4 (0.66, 2.8)
Quintile 5	1.4 (0.69, 2.8)	1.4 (0.70, 3.0)
Birth weight		
Normal	1	1
Low	0.89 (0.22, 3.7)	1.1 (0.26, 4.4)
Initiation of breastfeeding		
Early	1	1
Late	2.8 (1.3, 6.2)	3.1 (1.3, 7.3)
Child Sex		
Male	1	1
Female	1.3 (0.80, 2.1)	1.4 (0.85, 2.4)

4. Discussion

In this study, we sought to estimate the incidence of omphalitis among HIV unexposed neonates born at three primary healthcare facilities in central Uganda and to describe the bacteriological profile of the cord stump of the omphalitis cases. We found that the omphalitis incidence proportion was almost 10% in the first 28 days of life. This finding is worrying given that Omphalitis is associated with neonatal sepsis in 2 out of every 100 cases [15]. Our findings suggest that interventions such as chlorhexidine that reduce the risk of omphalitis could be useful in our and similar settings [24]. This suggestion contradicts the current World Health Organization guidelines that do not recommend chlorhexidine use for babies born in health facilities [25]. However, our suggestion is in agreement with Hodgkin [26], who questions the limitation of chlorhexidine use to only home births given that the incidence of omphalitis is also high in health facility births. A cohort study conducted in Pakistan found results similar to ours (an incidence proportion of 8%) [13]; however, previous randomized control studies in Africa and Asia estimated the omphalitis incidence proportions to range from 1% to 7.6% [10–13]. The observed differences in the incidence proportions could result from varying case definitions of omphalitis across the studies. In our study, we defined omphalitis as the presence of pus

while other studies (in Tanzania and Bangladesh) defined omphalitis based on redness and/or swelling, with or without pus.

We identified potentially pathogenic bacteria that could have caused omphalitis and characterized their antimicrobial susceptibility patterns. Two-thirds of neonates with omphalitis predominately grew gram-negative bacteria, similar to what was observed in two hospital-based studies in India [9,27]. However, other studies in south Asia identified gram-positive bacteria as the most likely cause of omphalitis [15,28,29]. It is not clear why we predominately isolated gram-negative bacteria rather than the expected gram-positive bacteria such as *S. aureus*. Most (87%) of the isolated gram-negative bacteria in our study exhibited resistance to the first-line antibiotics used for treating neonatal infections. We also observed resistance to third-generation cephalosporin antibiotics. These findings are consistent with those in a cohort study in Pakistan [15]. The World Health Organization recommends ampicillin and gentamicin as first-line treatment for serious neonatal infections but cloxacillin is the first-line treatment for umbilical infections because *S. aureus* is the presumed cause. However, cloxacillin as a choice of treatment becomes problematic when the umbilical cord stumps of neonates with omphalitis are primarily colonized with gram-negative bacteria. Our findings advocate for periodic and context-specific culture and sensitivity results to guide generic treatment options.

We did not observe an association between vaginal colonization of women in labor and omphalitis in this study. However, we found that neonates who were initiated late on breastmilk had an increased risk of omphalitis compared to neonates who started breast-feeding early. These findings are similar to those in a Tanzanian study [16], which found that neonates who were breastfed in the first hour of life had a lower risk of omphalitis. Initiating breastfeeding in the first hour of life has been shown to reduce neonatal morbidity and mortality, possibly through the transfer of protective antibodies in colostrum [30,31]. Our finding that neonates who were initiated late on breastmilk had an increased risk of omphalitis compared to neonates who started breastfeeding early may be due to reverse causality. The babies with sub-clinical omphalitis at birth could have failed to breastfeed in the first hour of life. Alternatively, this finding could have been a spurious result (chance) since it was not the main objective of this study and could also have resulted from the Table 2 fallacy [32]. The Table 2 fallacy suggests that the exposure association we observed could have affected the heterogeneity across levels of other covariates in our model or were confounded by other covariates in our model [32].

One of the limitations of this study was that we did not have data on the outcome among babies that were possibly taken to other health facilities spontaneously between the scheduled visits and this may have resulted in a slight underestimation of the incidence of omphalitis. We assume that the underestimation is very small because the scheduled visits were frequent (days 1, 3, 7, 14, and 28). In this cohort, only two babies died. One died on day 16 after getting omphalitis, and the second died on the 28th day (without omphalitis). Therefore, we do not think death was a substantial competing event to omphalitis in this particular cohort and therefore we did not conduct a competing risk analysis. We also studied babies that were enrolled in a randomized controlled trial and our results are subject to limitations of results from randomized controlled trials such as the possibility of reduced generalizability.

5. Conclusions

In conclusion, the incidence rate of omphalitis among neonates born in three primary healthcare facilities in or close to Kampala in Uganda was 0.095 cases per 28 child-days, corresponding to an estimated incidence risk of almost 10%. Predominantly gram-negative bacteria were isolated from neonates with omphalitis, and most of the bacteria exhibited resistance to commonly used antibiotics. The antimicrobial resistance of the isolated bacteria could complicate the treatment of serious neonatal infections and may continue to represent an impediment to enhancing survival until care for the cord stump is improved.

Author Contributions: Conceptualization, J.T., H.S. (Halvor Sommerfelt), J.K.T., D.M., G.N., O.N., F.B., H.S. (Hans Steinsland) and V.N.; Data curation, J.T., H.S. (Halvor Sommerfelt), J.K.T., D.M., H.S. (Hans Steinsland) and V.N.; Formal analysis, H.S. (Halvor Sommerfelt), J.K.T., D.M., H.S. (Hans Steinsland) and V.N.; Funding acquisition, J.T., H.S. (Halvor Sommerfelt), J.K.T., G.N. and V.N.; Investigation, J.T.; Methodology, J.T., H.S. (Halvor Sommerfelt), D.M., G.N., O.N., F.B. and V.N.; Project administration, J.T.; Supervision, J.K.T.; Visualization, J.T. and F.B.; Writing—original draft, J.T.; Writing—review and editing, J.T., H.S., J.K.T., D.M., G.N., O.N., F.B., H.S. and V.N. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Research Council of Norway through a young investigator grant (project number 234500) and its Centre of Excellence scheme and the University of Bergen (UiB), Norway to the Centre for Intervention Science in Maternal and Child Health (CISMAC; project number 223269) and its funding of the parent Chlorhexidine trial. The Research Council of Norway played no role in design, analysis, or interpretation of the study findings.

Institutional Review Board Statement: Informed consent was obtained for both the interview and specimen storage. Ethical approval was obtained from the Research and Ethics Review Committee of School of Medicine, SOMREC, Makerere University (REC REF 2015-118) and from Uganda National Council of Science and Technology (HS 1927).

Informed Consent Statement: Written informed consent was obtained from the women for enrolment into the trial and the current cohort study.

Data Availability Statement: Datasets used for this study can be obtained through reasonable request from the principal investigator of the Chlorhexidine trial, Victoria Nankabirwa.

Acknowledgments: We thank all the women who participated in our study and the midwives at Kawaala, H.C., III, Kitebi, H.C., III, and Mukono general hospital for their cooperation. Special thanks to the CISMAC research nurses for their hard work and commitment.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. GBD 2019 Under-5 Mortality Collaborators. Global, regional, and national progress towards Sustainable Development Goal 3.2 for neonatal and child health: All-cause and cause-specific mortality findings from the Global Burden of Disease Study 2019. *Lancet* **2021**, *398*, 870–905. [CrossRef] [PubMed]
2. Uganda Bureau of Statistcs (UBOS) and ICF. *Uganda Demographic and Health Survey 2016: Key Indicators Report*; UBOS: Kampala, Uganda, 2017.
3. Okomo, U.; Akpalu, E.N.K.; Le Doare, K.; Roca, A.; Cousens, S.; Jarde, A.; Sharland, M.; Kampmann, B.; Lawn, J.E. Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: A systematic review and meta-analysis in line with the STROBE-NI reporting guidelines. *Lancet Infect. Dis.* **2019**, *19*, 1219–1234. [CrossRef] [PubMed]
4. Boos, M.D., Sr. *Avery's Diseases of the Newborn*, 10th ed.; UN General Assembly: New York, NY, USA, 2018.
5. Mullany, L.C.; Darmstadt, G.L.; Khatry, S.K.; Katz, J.; LeClerq, S.C.; Shrestha, S.; Adhikari, R.; Tielsch, J.M. Topical applications of chlorhexidine to the umbilical cord for prevention of omphalitis and neonatal mortality in southern Nepal: A community-based, cluster-randomised trial. *Lancet* **2006**, *367*, 910–918. [CrossRef] [PubMed]
6. UN General Assembly, New York USA. Transforming Our World: The 2030 Agenda for Sustainable Development. 21 October 2015. A/RES/70/1. Available online: https://www.refworld.org/docid/57b6e3e44.html (accessed on 6 May 2019).
7. Celebi Celik, F.; Tuzun, F.; Duman, N.; Keskinoglu, P.; Kumral, A.; Ozkan, H. Current factors affecting the risk of omphalitis in newborns: A prospective case-control study. *Int. J. Clin. Pract.* **2021**, *75*, e14071. [CrossRef]
8. Goldenberg, R.L.; McClure, E.M.; Saleem, S. A Review of Studies with Chlorhexidine Applied Directly to the Umbilical Cord. *Am. J. Perinatol.* **2012**, *30*, 699–702. [CrossRef]
9. Faridi, M.; Rattan, A.; Ahmad, S.H. Omphalitis neonatorum. *J. Indian Med. Assoc.* **1993**, *91*, 283–285.
10. Sazawal, S.; Dhingra, U.; Ali, S.M.; Dutta, A.; Deb, S.; Ame, S.M.; Mkasha, M.H.; Yadav, A.; Black, R.E. Efficacy of chlorhexidine application to umbilical cord on neonatal mortality in Pemba, Tanzania: A community-based randomised controlled trial. *Lancet Glob. Health* **2016**, *4*, e837–e844. [CrossRef]
11. El Arifeen, S.; Mullany, L.C.; Shah, R.; Mannan, I.; Rahman, S.M.; Talukder, M.R.R.; Begum, N.; Al-Kabir, A.; Darmstadt, G.L.; Santosham, M.; et al. The effect of cord cleansing with chlorhexidine on neonatal mortality in rural Bangladesh: A community-based, cluster-randomised trial. *Lancet* **2012**, *379*, 1022–1028. [CrossRef]
12. Semrau, K.E.A.; Herlihy, J.; Grogan, C.; Musokotwane, K.; Yeboah-Antwi, K.; Mbewe, R.; Banda, B.; Mpamba, C.; Hamomba, F.; Pilingana, P.; et al. Effectiveness of 4% chlorhexidine umbilical cord care on neonatal mortality in Southern Province, Zambia (ZamCAT): A cluster-randomised controlled trial. *Lancet Glob. Health* **2016**, *4*, e827–e836. [CrossRef]

13. Soofi, S.; Cousens, S.; Imdad, A.; Bhutto, N.; Ali, N.; Bhutta, Z.A. Topical application of chlorhexidine to neonatal umbilical cords for prevention of omphalitis and neonatal mortality in a rural district of Pakistan: A community-based, cluster-randomised trial. *Lancet* **2012**, *379*, 1029–1036. [CrossRef]
14. Mullany, L.C.; El Arifeen, S.; Winch, P.J.; Shah, R.; Mannan, I.; Rahman, S.M.; Rahman, M.R.; Darmstadt, G.L.; Ahmed, S.; Santosham, M.; et al. Impact of 4.0% chlorhexidine cleansing of the umbilical cord on mortality and omphalitis among newborns of Sylhet, Bangladesh: Design of a community-based cluster randomized trial. *BMC Pediatr.* **2009**, *9*, 67. [CrossRef]
15. Mir, F.; Tikmani, S.S.; Shakoor, S.; Warraich, H.J.; Sultana, S.; Ali, S.A.; Zaidi, A.K.M. Incidence and etiology of omphalitis in Pakistan: A community-based cohort study. *J. Infect. Dev. Ctries.* **2011**, *5*, 828–833. [CrossRef] [PubMed]
16. Mullany, L.C.; Faillace, S.; Tielsch, J.M.; Stoltzfus, R.J.; Nygaard, K.E.; Kavle, J.A.; Farag, T.H.; Haji, H.J.; Khalfan, S.S.; Ali, N.S.; et al. Incidence and Risk Factors for Newborn Umbilical Cord Infections on Pemba Island, Zanzibar, Tanzania. *Pediatr. Infect. Dis. J.* **2009**, *28*, 503–509. [CrossRef] [PubMed]
17. Nankabirwa, V.; Tylleskär, T.; Tumuhamye, J.; Tumwine, J.K.; Ndeezi, G.; Martines, J.C.; Sommerfelt, H. Efficacy of umbilical cord cleansing with a single application of 4% chlorhexidine for the prevention of newborn infections in Uganda: Study protocol for a randomized controlled trial. *Trials* **2017**, *18*, 322. [CrossRef]
18. Tumuhamye, J.; Steinsland, H.; Bwanga, F.; Tumwine, J.K.; Ndeezi, G.; Mukunya, D.; Namugga, O.; Kasede, A.N.; Sommerfelt, H.; Nankabirwa, V. Vaginal colonization with antimicrobial-resistant bacteria among women in labor in central Uganda: Prevalence and associated factors. *Antimicrob. Resist. Infect. Control* **2021**, *10*, 37. [CrossRef] [PubMed]
19. Tumuhamye, J.; Steinsland, H.; Tumwine, J.K.; Namugga, O.; Mukunya, D.; Bwanga, F.; Sommerfelt, H.; Nankabirwa, V. Vaginal colonisation of women in labour with potentially pathogenic bacteria: A cross sectional study at three primary health care facilities in Central Uganda. *BMC Infect. Dis.* **2020**, *20*, 98. [CrossRef]
20. Open Data Kit: The Standard for Mobile Data Collection. Available online: https://opendatakit.org (accessed on 16 July 2022).
21. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 10th ed.; Approved Standard; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2009.
22. Rothman, K.J. *Epidemiology: An Introduction*, 2nd ed.; Oxford University Press, Inc.: New York, NY, USA, 2012.
23. Senaviratna NA, M.R.; Cooray TM, J.A. Diagnosing Multicollinearity of Logistic Regression Model. *Asian J. Probab. Stat.* **2019**, *5*, 1–9. [CrossRef]
24. Nangia, S.; Dhingra, U.; Dhingra, P.; Dutta, A.; Menon, V.P.; Black, R.E.; Sazawal, S. Effect of 4 % chlorhexidine on cord colonization among hospital and community births in India: A randomized controlled study. *BMC Pediatr.* **2016**, *16*, 121. [CrossRef]
25. Bin Zaman, S.; Siddique, A.B.; Ruysen, H.; Kc, A.; Peven, K.; Ameen, S.; Thakur, N.; Rahman, Q.S.-U.; Salim, N.; Gurung, R.; et al. Chlorhexidine for facility-based umbilical cord care: EN-BIRTH multi-country validation study. *BMC Pregnancy Childbirth* **2021**, *21*, 239. [CrossRef]
26. Hodgins, S. Chlorhexidine and newborn omphalitis and mortality. *Lancet Glob. Health* **2017**, *5*, e270–e271. [CrossRef]
27. Mason, W.H.; Andrews, R.; Ross, L.A.; Wright, H.T., Jr. Omphalitis in the newborn infant. *Pediatr. Infect. Dis. J.* **1989**, *8*, 521–525. [CrossRef] [PubMed]
28. Sengupta, M.; Banerjee, S.; Banerjee, P.; Guchhait, P. Outstanding Prevalence of Methicillin Resistant Staphylococcus aureus in Neonatal Omphalitis. *J. Clin. Diagn. Res.* **2016**, *10*, Dm01–Dm03. [CrossRef] [PubMed]
29. Sawardekar, K.P. Changing spectrum of neonatal omphalitis. *Pediatr. Infect. Dis. J.* **2004**, *23*, 22–26. [CrossRef] [PubMed]
30. Edmond, K.M.; Zandoh, C.; Quigley, M.A.; Amenga-Etego, S.; Owusu-Agyei, S.; Kirkwood, B.R. Delayed Breastfeeding Initiation Increases Risk of Neonatal Mortality. *Pediatrics* **2006**, *117*, e380–e386. [CrossRef] [PubMed]
31. NEOVITA Study Group. Timing of initiation, patterns of breastfeeding, and infant survival: Prospective analysis of pooled data from three randomised trials. *The Lancet Global health.* **2016**, *4*, e266–e275.
32. Westreich, D.; Greenland, S. The Table 2 Fallacy: Presenting and Interpreting Confounder and Modifier Coefficients. *Am. J. Epidemiol.* **2013**, *177*, 292–298. [CrossRef]

Paper IV

PLOS ONE

RESEARCH ARTICLE

Neonatal sepsis at Mulago national referral hospital in Uganda: Etiology, antimicrobial resistance, associated factors and case fatality risk

Josephine Tumuhamye[1]*, Halvor Sommerfelt[1], Freddie Bwanga[2], Grace Ndeezi[3], David Mukunya[1], Agnes Napyo[1,4], Victoria Nankabirwa[1,5], James K. Tumwine[3]

1 Centre for Intervention Science for Maternal and Child Health (CISMAC), Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway, **2** Department of Medical Microbiology, Makerere University, Kampala, Uganda, **3** Department of Pediatrics and Child Health, Makerere University, Kampala, Uganda, **4** Department of Public Health, Busitema University, Mbale, Uganda, **5** Department of Epidemiology and Biostatics School of Public Health, Makerere University, Kampala, Uganda

* tphynne@gmail.com

OPEN ACCESS

Citation: Tumuhamye J, Sommerfelt H, Bwanga F, Ndeezi G, Mukunya D, Napyo A, et al. (2020) Neonatal sepsis at Mulago national referral hospital in Uganda: Etiology, antimicrobial resistance, associated factors and case fatality risk. PLoS ONE 15(8): e0237085. https://doi.org/10.1371/journal.pone.0237085

Editor: Patrick Butaye, Ross University School of Veterinary Medicine, SAINT KITTS AND NEVIS

Received: December 29, 2019

Accepted: July 20, 2020

Published: August 10, 2020

Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: This work was funded by the Norwegian programme for capacity development in higher education and research for development (NORHED) through the Survival Pluss project (Project Number; UGA-13-0030). JKT received the grant. URL: https://norad.no/en/front/funding/norhed/projects/norhed-project-in-south-sudan-

Abstract

Background

Sepsis is the third most common cause of death among neonates, with about 225,000 newborns dying every year globally. Data concerning the microbial etiology of neonatal sepsis and antimicrobial resistance profiles of its causative agents are necessary to inform targeted and effective treatment and prevention strategies.

Objective

To determine the proportion of newborns with symptoms and signs of sepsis who had a positive blood culture, its bacterial etiology, the antimicrobial resistance patterns as well as the factors associated with culture-positivity and case fatality at Mulago national referral hospital in Uganda.

Methods

We conducted a cross-sectional study among 359 neonates with symptoms and signs of sepsis who presented to the pediatric emergency care unit of Mulago national referral hospital from mid-January to end of December 2018. We performed blood culture and antimicrobial susceptibility testing, and conducted polymerase chain reaction to identify methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. We used multivariable logistic regression to estimate the association between potential risk factors and culture-positive neonatal sepsis.

Findings

Of the 359 neonates recruited, 46 (12.8%; 95% CI 9.5%, 16.7%) had a positive blood culture. The predominant isolated bacteria were *Staphylococcus aureus* in 29 (63.0%),

and-uganda/. The NORHED played no role in design, analysis or interpretation of the study findings. The contribution by VN and HS was supported by the Centre for Intervention Science in Maternal and Child Health (CISMAC; project number 223269), which is funded by the Research Council of Norway through its Centres of Excellence scheme and the University of Bergen (UiB), Norway.

Competing interests: The authors have declared that no competing interests exist.

Escherichia coli in seven (15.2%), and *Klebsiella pneumoniae* in five (10.9%). Of the 46 pathogens, 73.9% were resistant to ampicillin, 23.9% to gentamicin and 8.7% to ceftriaxone. We isolated MRSA from the blood specimens of 19 (5.3%) of the 359 neonates, while 3 (0.8%) grew extended spectrum beta lactamase producers. The case fatality risk among neonates with neonatal sepsis was 9.5% (95% CI: 6.6%, 13.0%). Cesarean section delivery was strongly associated with culture-positive sepsis (adjusted odds ratio 3.45, 95% CI: 1.2, 10.1).

Conclusion

One in eight neonates with clinical signs of sepsis grew a likely causative bacterial pathogen. *S. aureus* was the main pathogen isolated and a third of these isolates were MRSA. A significant proportion of the isolated bacterial pathogens were resistant to the first and second line antibiotics used for the treatment of neonatal sepsis. There is need to revisit the current treatment guidelines for neonatal sepsis.

Introduction

The mortality among children less than 5 years of age has declined considerably over the last two decades, but neonatal mortality remains high, accounting for 2.6 million annual deaths[1]. Uganda has a neonatal mortality of 22.3 deaths per 1,000 live births which is one of the highest in the world[2]. Severe infections are the leading cause and accounts for approximately one-third of newborn deaths in sub-Saharan Africa[3]. Among survivors, neonatal sepsis may be accompanied by long-term complications such as neurodevelopmental impairment[4]. To achieve the third sustainable development goal of reducing neonatal mortality to under 12 deaths per 1,000 live births, a better understanding of the etiology of neonatal sepsis is needed.

Important signs and symptoms of neonatal sepsis include inability to breastfeed, convulsions, fast breathing, severe lower chest wall in-drawing, lethargy, fever and hypothermia[5]. Neonatal sepsis is a clinical syndrome including septicemia and meningitis that is classified according to disease onset[6]. Early-onset sepsis (EOS) is defined as disease among neonates aged 72 hours or less while late-onset sepsis occurs from 4 to 28 days[6]. Early-onset sepsis usually results from an infection acquired *in utero* or during the birth process and group B *Streptococcus*(GBS) is the most common pathogen causing EOS in high income countries whereas *Staphylococcus aureus*, *Klebsiella species* and *Escherichia coli* are the most common causes in low and middle income-countries[7–9]. In low and middle income countries, late-onset sepsis is usually a result of infection from the surrounding environment (hospital or community) and the incriminated pathogens are majorly gram negative bacteria including *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter* spp., *Pseudomonas aeruginosa*; as well as *S. aureus* [10–12]. However, the pathogen profile differs depending on the region. There is a predominance of gram negative pathogens and low prevalence of GBS in south Asia and sub-Saharan African compared to the high GBS prevalence in high income countries[10, 11, 13]. Surveillance of the etiology of neonatal sepsis and resistance patterns of the causative bacteria is critically important in informing the empirical treatment of neonatal sepsis and in guiding the development of preventive strategies, including the development and deployment of vaccines. This information is particularly important in resource-limited settings where access to blood cultures is limited and if available, often unaffordable.

In Uganda, neonatal sepsis is managed according to the Uganda Clinical Guidelines, which recommend ampicillin and gentamicin as first-line and third-generation cephalosporins as second-line treatment[14]. Although previous studies[15–17] have reported on neonatal sepsis in Uganda, data on the etiology and antimicrobial resistance patterns of the causative bacteria is limited. There is a need to carefully characterize bacterial pathogens from blood cultures of neonates with clinical signs of sepsis because antibiotic susceptibility profiles of the causative organisms change over time[18]. In this study, we aimed to determine the bacterial etiology of culture-positive sepsis and antimicrobial resistance patterns. We also estimated the case fatality risk of neonatal sepsis and factors associated with culture-positive sepsis at Mulago national referral hospital.

Methods

Study design and setting

We conducted a hospital-based cross-sectional study at the pediatric emergency care unit of Mulago national referral hospital from January to December 2018. Mulago is the largest tertiary hospital in Uganda, has an average of 100 births per day and its pediatric emergency care unit admits an average of 20 newborns per week. It is the teaching hospital for Makerere University College of Health Sciences, located in Kampala, the capital city of Uganda. The pediatric emergency care unit admits critically ill children for up to 24 hours before they are transferred to the general pediatric wards.

Participants

We included neonates, i.e. babies less than 29 days of age, who presented with any of the following symptoms and signs during daytime (i.e. 8:00hrs to 17:00hrs) on weekdays: inability to breastfeed, convulsions, fast breathing (more than 60 breaths per minute), severe lower chest in-drawing, lethargy, fever (axillary temperature $\geq$37.5˚C) and low body temperature (axillary temperature$<$35.5˚C) according to the integrated management of childhood illnesses (IMCI) guidelines[19]. We decided to exclude neonates with severe congenital anomalies from the study and those that reported during the night (after 18:00hrs) and (or) during the weekends (Saturday and Sunday).

Sample size estimation

The sample size was calculated based on the main objective, which was to estimate the proportion of newborns with culture-positive sepsis among children admitted with clinical signs of sepsis. Based on the following assumptions; 95% confidence interval, prevalence of culture proven septicemia 37%[17] and precision 5%, we needed a sample size of 359 newborns with clinical sepsis.

Study procedure and informed consent

We enrolled neonates who presented with symptoms and signs of sepsis upon admission at the emergency pediatric unit. A trained nurse obtained written informed consent and used validated pretested questionnaires to capture information on demographic and clinical characteristics from mothers or other primary caretakers of the study participants. We collected information on the presence of the following symptoms and signs: inability to feed, convulsions, fast breathing, severe lower chest wall in-drawing, lethargy, fever, hypothermia, umbilical cord stump infection and skin pustules. We also recorded data on the mode of delivery, birth weight, sex of the child, gestational length, antibiotic use before admission, prior

hospitalization and its duration. Mothers or caretakers of participants were asked about a history of fever, foul smelling vaginal discharge during their last trimester of pregnancy and premature rupture of membranes. We followed up the participants until they were discharged and collected information which included the length of hospitalization, antibiotics used for treating current illness and immediate clinical outcomes which was defined as either death or recovery.

Blood specimen collection

Three milliliters of venous blood were drawn aseptically from the participants before the administration of antibiotics. Two milliliters of the blood were inoculated into pediatric blood culture bottles (BD Bactec^{TM} Peds Plus^{TM}/F) for culture. The specimens were transported daily to MBN Clinical Laboratories[20].

Laboratory methods

Blood culture. Blood culture bottles were incubated in an automated incubator (BD BACTEC™FX40, USA) at 37˚C for 24 hours. Bottles that indicated absence of bacteria in BACTEC were incubated for five more days before considering them negative. When a bottle was flagged positive, blood culture aliquots were sub cultured on blood, chocolate and MacConkey agar (BioLab Budapest, Hungary) and incubated at 35˚C to 37˚C for up to 96 hours.

Identification of bacterial species. Identification of bacterial species was done based on colony morphology, Gram stain appearance and standard biochemical tests[21]. All isolates were stored in 20% glycerol in Brain Heart Infusion broth at -80˚C for subsequent molecular identification of methicillin resistant *Staphylococcus aureus* (MRSA).

Drug susceptibility testing. Antimicrobial susceptibility testing was performed on the Mueller-Hinton agar using the disk diffusion method as per the Clinical Laboratory Standard Institute (CLSI) guidelines[22]. A lawn culture was made on Muller-Hinton agar plate on which the antibiotic disks were placed and incubated between 35 ^{o}C to 37˚C for 24 hours. The D-test was performed to detect the presence of erythromycin-induced clindamycin resistance among *S. aureus* isolates as previously described[23]. We screened *Enterobacteriaceae* isolates for ESBL resistance using the combination disk method as previously described[24, 25].

Detection of *mecA* gene for the identification of MRSA: Methicillin-resistant *S. aureus* (MRSA) was detected using polymerase chain reaction for the *mecA* gene as previously described[26].

Quality control. Standard aseptic methods of blood collection and processing were adhered to. *S. aureus* ATCC 25923 was used to quality control *S. aureus* isolates, *E. coli* ATCC 25922 for lactose fermenting bacteria and *P. aeruginosa* ATCC 27853 for non-lactose fermenting bacteria. For genotyping, ATCC 25923 (*mecA*-negative) and an in-house *S. aureus mecA*-positive strain were used to control for the *mecA* gene.

Study variables

The main outcome of the study was culture-positive sepsis. A positive blood culture was defined by the isolation of at least one of the following potential pathogens; *S. aureus*, *E. coli*, *K. pneumoniae*, *S. pneumoniae*, *N. meningitides*, Group B *streptococcus*, *Streptococcus pyogenes*, *C. freundii*, *Enterococcus* spp., *Salmonella* spp., *Acinetobacter* spp., *Pseudomonas* spp. and *Enterobacter* spp. A negative blood culture was defined by the absence of bacterial growth or the isolation of possible contaminants such as *Corynebacterium* spp., *Bacillus* spp. and *coagulase-negative Staphylococcus* species. Secondary outcomes included: antibiotic resistance profiles of the isolated pathogens and death of the newborns participating in the study. Other

variables used to describe the participants or adjust our estimates were birth weight (dichotomized into low birth weight, i.e. less than 2.5 kg, and normal birth weight, i.e. 2.5 kg or more), age of the newborn, premature rupture of membranes (PROM), umbilical cord stump infection, lethargy, hypothermia, fever, inability to breastfeed, initiation of breastfeeding, previous antibiotic use, re-admission, length of hospitalization, current treatment and presence of resistant pathogens in blood.

Statistical analysis

We entered data doubly into EPI-Data version 13 and data analysis using STATA 15.0 (StataCorp, College Station, Tx, USA). We summarized categorical variables as proportions and described continuous variables using means and their standard deviations (SD). We performed crude and multivariable logistic regression analysis to assess factors associated with culture-positive sepsis. Crude odds ratios (OR) and 95% confidence intervals (95% CI) were estimated for each exposure variable. Based on previous literature, we decided to include the following variables in our multivariable model; PROM, maternal fever in the last trimester and mode of delivery[27, 28]. Other variables we included were birth weight, umbilical cord infection, prior antibiotic use, foul-smelling vaginal discharge, maternal age and education background. To assess the association between culture-positivity and risk of subsequent death, we estimated the corresponding risk ratio (RR) with a generalized linear model of the binomial family with a log link. Based on existing literature[29, 30], we adjusted the RR for antibiotic treatment before admission, whether the sepsis was of early or late onset, re-admission for the current illness, and mode of delivery.

Ethical approval and consent to participate

We obtained ethical approval from the Mulago National Referral Hospital Research and Ethics Review Committee (MHREC-1069) and written informed consent from parents/primary caretakers of the neonates enrolled in the study.

Results

Description of study participants

None of the neonates considered for inclusion had severe congenital anomalies and, among the 596 babies suspected of having clinical sepsis, we recruited 359 neonates that met our predefined IMCI criteria. Their mean age at admission and recruitment was 8 (SD 7.1) days; about half(53%) of them were admitted within their first, another 27% in their second week of life. Their mean birth weight was 3.1 kg (SD 0.6) and the median duration of hospitalization was 5.4 (IQR 4, 6) days. The mothers of the enrolled neonates had a mean age of 26 years (SD 5.5). Other maternal factors included: fever in the last trimester 105 (29.2%) and foul-smelling vaginal discharge 89 (24.8%) (Table 1).

Bacterial etiology

Of the 359 neonates, 46 (12.8%; 95% CI 9.5%, 16.7%) had a positive blood culture and we did not identify more than one bacterial pathogen in any of them. Of the 46 babies, 15 (32.6%) had early-onset, whereas 31 (67.4%) had late-onset sepsis. Gram-positive bacteria constituted 70% (32/46) of all isolates of which 90.6% (29/32) were *S. aureus*. Other gram-positive organisms included *S. pneumoniae* 2.2% (1/46) and *Enterococcus* spp. 4.3% (2/46). The remaining 30% (14/46) were gram-negative organisms which included seven *E. coli* isolates, five *K. pneumoniae* isolates, and one *Neisseria* spp. and *C. freundii* isolate (Table 2). We isolated likely

Table 1. Demographic and clinical characteristics of neonates with clinical sepsis at Mulago national referral hospital.

	Culture positive sepsis		
	Yes 46 (%)	**No 313 (%)**	**N = 359 (%)**
Neonatal characteristics			
age (days)			
0 to 3 (early onset)	15 (32.6)	110 (35.1)	125 (34.8)
4 to 28 (late onset)	31 (67.4)	203 (64.9)	234 (65.2)
Sex			
Male	23 (50.0)	178 (57.1)	201 (56.2)
Female	23 (50.0)	134 (42.9)	157 (43.8)
Birth weight			
Low	5 (10.9)	43 (13.7)	48 (13.4)
Normal	41 (89.1)	270 (86.3)	311 (86.6)
Inability to breast feed			
No	23 (50.0)	163 (52.1)	186 (51.8)
Yes	23 (50.0)	150 (47.9)	173 (48.2)
Fever			
No	14 (30.4)	112 (35.8)	126 (35.1)
yes	32 (69.6)	201 (64.2)	233 (64.9)
Difficulty in breathing			
No	32 (69.6)	219 (70.0)	251 (70.0)
Yes	14 (30.4)	94 (30.0)	108 (30.1)
Convulsions			
No	36 (78.3)	266 (85.0)	302 (84.1)
Yes	10 (21.7)	47 (15.0)	57 (15.9)
Lethargy			
No	35 (76.1)	268 (85.6)	303(84.4)
Yes	11 (23.9)	45 (14.4)	56 (15.6)
Umbilical cord infection			
No	38 (82.6)	246 (78.6)	284 (79.1)
Yes	8 (17.4)	67 (21.4)	75 (20.9)
Prior antibiotic use			
No	34 (73.9)	221 (71.5)	255 (71.8)
Yes	12 (28.5)	88 (28.5)	100 (28.2)
Maternal characteristics			
Age (Years)			
Less than 20	6 (13.0)	26 (8.3)	32 (8.9)
20–24	17 (37.0)	120 (38.3)	137 (38.2)
25–29	13 (28.7)	90 (28.8)	103 (28.7)
30 or more	10 (21.7)	77 (24.6)	87 (24.2)
Education			
Primary	17 (37.0)	105 (33.8)	122 (34.2)
Secondary	23 (50)	170 (54.7)	193 (54.0)
Tertiary	6 (13.0)	36 (11.6)	42 (11.8)
Mode of delivery			
Spontaneous vaginal delivery	23 (50)	195 (62.7)	218 (61.1)
Assisted vaginal delivery	17 (37.0)	99 (31.8)	116 (32.5)
Caesarean section	6 (13.0)	17 (5.5)	23 (6.4)

(*Continued*)

Table 1. (Continued)

	Culture positive sepsis		
	Yes 46 (%)	**No 313 (%)**	**N = 359 (%)**
Marital status			
Unmarried	6 (13.0)	32 (10.2)	38 (10.6)
Married	40 (87.0)	281 (89.8)	321 (89.4)
Fever in pregnancy			
No	38 (82.6)	216 (69.0)	254 (70.7)
Yes	8 (17.4)	97 (31.0)	105 (29.3)
Foul vaginal discharge			
No	32 (69.6)	238 (76.0)	270 (75.2)
Yes	14 (30.4)	75 (24.0)	89 (24.8)
Prolonged rupture of membranes			
No	34 (73.9)	199 (63.6)	232 (64.9)
Yes	12 (26.1)	114 (36.4)	126 (35.1)

https://doi.org/10.1371/journal.pone.0237085.t001

contaminants from about 2% (7/359) of the neonates. The likely contaminants included 2 *Bacillus* spp., 4 *Corynebacteria* spp., and one isolate of coagulase-negative *Staphylococcus*.

Antimicrobial resistance profiles of isolated bacteria

We found that among the 46 neonates with a positive blood culture, resistance to first-line antibiotics was identified in ampicillin 39 (84.8%) and gentamicin in 11 (23.9%), while resistance to second-line 3rd generation cephalosporins was identified in 4 (8.7%). Overall, the proportions of babies with likely causative bacteria that were resistant to first-line and to second-line antibiotics were 13.9% (50/359, 95% CI; 10.5, 17.9) and 1.1% (4/359, 95% CI; 0.3, 2.8) respectively. Two thirds of the babies who had blood cultures with *S. aureus* had MRSA, while erythromycin-induced clindamycin-resistant *S. aureus* were observed in31%. All the *S. aureus* isolates were susceptible to vancomycin. Forty-one percent (19/46; 95% CI 27.0%, 56.8%) of the babies with culture-positive sepsis had MRSA. Overall, the proportion of participants with MRSA was 5.3% (19/359; 95% CI 3.2%, 8.1%).

All *K. pneumoniae* and six *E. coli* isolates were resistant to ampicillin. Resistance to gentamicin was observed in two (28.6%) of seven *E. coli* isolates and in two (40%) of five *K. pneumoniae* isolates. Three of the *K. pneumoniae* isolates, one *E. coli* and one *Neisseria* spp. isolates were resistant to third-generation cephalosporins (ceftriaxone and ceftazidime). All gram-negative bacteria isolated in this study were susceptible to the carbapenem class of antibiotics

Table 2. Bacterial pathogens isolated from blood of neonates with symptoms and signs sepsis at Mulago national referral hospital.

Bacteria Isolated	Overall (%)	Early onset (0–3 days)	Late onset (4-28days)
S. aureus	29 (8.1)	13 (7.0)	16 (10.0)
E. coli	7 (2.0)	5 (2.7)	2 (1.2)
K. pneumoniae	5 (1.4)	3 (1.6)	2 (1.2)
Enterococcus spp.	2 (0.6)	2 (1.1)	0
Neisseria spp.	1 (0.3)	0	1 (0.6)
S. pneumoniae	1 (0.3)	0	1 (0.6)
C. freundii	1 (0.3)	0	1 (0.6)
Total	46	23	23

https://doi.org/10.1371/journal.pone.0237085.t002

Table 3. Antimicrobial resistance profile of pathogens isolated from neonates with symptoms and signs of sepsis at Mulago national referral hospital between January and December 2018.

Antibiotics	All participants	Neonates with culture-positive sepsis	*S. aureus*	*E. coli*	*K. pneumoniae*
	N = 359 (%)	n = 46 (%)	n = 29	n = 7	n = 5
Erythromycin	21 (5.8)	21 (45.7)	21(72.4)	NA	NA
Vancomycin	0	0	0	NA	NA
Tetracycline	13 (3.6)	13 (28.3)	13 (44.8)	NA	NA
Penicillin	28 (7.8)	28 (60.9)	28 (96.6)	NA	NA
Gentamicin	11 (3.1)	11 (23.9)	7(24.1)	2 (28.6)	2 (40.0)
Trimethroprim-Sulphamethoxazole	34 (9.5)	34 (73.9)	27 (93.1)	4 (57.1)	3 (60)
Chloramphenicol	8 (2.2)	8 (17.4)	7 (15.2)	0	1 (2.2)
Ampicillin	39 (10.9)	39 (84.8)	28 (96.6)	6 (85.7)	5 (100.0)
Amoxycillin-clavulanic acid	9 (2.5)	9 (19.6)	NA	5 (71.4)	4 (80.0)
Ceftazidime*	4 (1.1)	4 (8.7)	NA	1 (14.3)	3 (60)
Ceftrioxone*	4 (1.1)	4 (8.7)	NA	1 (14.3)	3 (60)
Cefuroxime*	4 (1.1)	4 (8.7)	NA	1 (14.3)	3 (60)
Imipenem*	4 (1.1)	4 (8.7)	NA	1 (14.3)	3 (60)

*The four isolates (resistant to these antibiotics) are the same.

https://doi.org/10.1371/journal.pone.0237085.t003

(Table 3). However, three bacterial pathogens phenotypically exhibited ESBL resistance translating to a proportion of 6.5% (3/46; 95%CI 1.4%, 17.9%) among neonates with positive-culture sepsis. Enterobacteriaceae that exhibited carbapenem resistance included one *E. coli* and three *K. pneumoniae* isolates. Overall, the proportion of the neonates with MRSA was 5.3% (19/359; 95% CI 3.2%, 8.1%), with ESBL producers 0.8% (3/359; 95% CI 0.02%, 2.4%) and with carbapenem resistance 1.1% (95% CI 0.3%, 2.8%).

Factors associated with culture-positive sepsis

The odds of being born by caesarian section among neonates with culture-positive sepsis was three times that among babies born by spontaneous vaginal delivery (AOR 3.45, 95% CI; 1.19,10.05). Subgroup analysis yielded similar findings (Table 4).

Immediate clinical outcomes

Of the 359 neonates with symptoms and signs of sepsis, 34 died, translating to a case fatality risk of 9.5% (95% CI; 6.6%, 13.0%). The case fatality risk for culture-positive sepsis was 15.2% (7/46; 95% CI 6.3%, 28.9%) in comparison to 8.6% (27/313; 95% CI 5.8%, 12.3%) among the culture negative participants. After adjusting for previous antibiotic use, age, re-admission and mode of delivery, the odds of death among neonates with culture-positive sepsis were 1.6 times higher than that among those with culture-negative sepsis (adjusted RR 1.60; 95% CI 0.66, 4.0).

Of the 34 neonates that died, 25 (73.5%) were treated with only first-line antibiotics (ampicillin and gentamicin) while 9 (32.4%) were treated with second-line antibiotics (ceftazidime, ceftriaxone and cefixime). Pathogens isolated from newborns that died included three *S. aureus* isolates (two of which were MRSA), two *E. coli* isolates (one of which was an ESBL producer), one ESBL producing *K. pneumoniae* isolate and one *C. freundii* isolate. Regarding the length of hospitalization among those who died, 30 neonates were hospitalized for $\leq$5 days, two neonates for 14 days, one for 16 and one for 18 days.

Table 4. Neonatal and maternal factors potentially associated with culture-positive sepsis among neonates with symptoms and signs sepsis at Mulago national referral hospital.

Neonatal characteristics	Culture-positive sepsis (0–28 days)		Culture-positive sepsis (0–7 days)*		Culture-positive sepsis (0–14 days)*	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (days)						
0 to 3	1	-	-	-	-	-
4 to 28	1.12 (0.58, 2.16)	-	-	-	-	-
Birth weight						
Normal	1	1	1	1	1	1
Low	0.77 (0.29, 2.05)	0.84 (0.30, 2.31)	1.03 (0.29, 3.71)	1.35 (0.34, 5.31)	1.29 (0.43, 3.88)	0.91 (0.29, 2.89)
Umbilical cord infection						
No	1	-	1	1	1	-
Yes	0.77 (0.34, 1.74)	-	0.59 (0.21, 1.63)	0.55 (0.18, 1.66)	0.62 (0.26, 1.47)	-
Prior antibiotic use						
No	1	-	1	-	1	-
Yes	0.79 (0.46, 1.36)	-	0.80 (0.33, 1.94)	-	0.88 (0.55, 1.43)	-
Maternal characteristics						
Age (Years)						
Less than 20	1.63 (0.59, 4.53)	1.43 (0.49, 4.18)	0.69 (0.14, 3.38)	0.59 (0.11, 3.11)	1.33 (0.44, 4.01)	1.17 (0.38, 3.65)
20–24 (Reference group)	1	1	1	1	1	1
25–29	1.02 (0.47, 2.21)	1.0 (0.45, 2.22)	0.71 (0.25, 2.00)	0.67 (0.22, 2.09)	0.80 (0.34, 1.85)	0.74 (0.31, 1.78)
30 or more	0.92 (0.40, 2.11)	0.74 (0.30, 1.79)	0.78 (0.27, 2.22)	0.52 (0.16, 1.66)	0.85 (0.36, 2.04)	0.74 (0.29, 1.88)
Education						
Primary	1.03 (0.38, 2.79)	1.11 (0.39, 3.19)	1.17 (0.29, 4.67)	1.39 (0.31, 6.21)	1.12 (0.38, 3.34)	1.19 (0.38, 3.76)
Secondary	0.86 (0.33, 2.25)	0.89 (0.30, 1.79)	1. 11(0.30, 4.16)	1.14 (0.27, 4.72)	0.99 (0.35, 2.84)	1.00 (0.33, 3.03)
Tertiary	1	1	1	1	1	1
Mode of delivery						
Spontaneous vaginal delivery	1	1	1	1	1	
Assisted vaginal delivery	1.47 (0.75, 2.88)	1.59 (0.79, 3.18)	1.54 (0.64, 3.71)	1.82 (0.70, 4.76)	1.30 (0.63, 2.66)	-
Caesarean section	3.02 (1.10, 8.43)	3.45 (1.19, 10.05)	3.58 (0.84, 15.17)	4.45 (0.92, 21.52)	2.78 (0.81, 9.61)	-
Fever in pregnancy						
No	1	1	1	1	1	1
Yes	0.47 (0.21, 1.04)	0.47 (0.21, 1.07)	0.28 (0.08, 0.96)	0.20 (0.05, 0.76)	0.44 (0.19, 1.04)	0.37 (0.15, 0.91)
Foul vaginal discharge in pregnancy						
No	1	1	1	1	1	1
Yes	1.18 (0.84, 1.66)	1.30 (0.90, 1.88)	1.27 (0.83, 1.95)	1.53 (0.94, 2.48)	1.18 (0.83, 1.69)	1.43 (0.97, 2.12)
PROM						
No	1	1	1	1	1	1
Yes	0.61 (0.31, 1.22)	0.53 (0.25, 1.11)	0.53 (0.21, 1.31)	0.46 (0.17, 1.25)	0.58 (0.27, 1.26)	0.47 (0.21, 1.04)

*Analysis restricted to 0–7 and 0–14 day old babies.

https://doi.org/10.1371/journal.pone.0237085.t004

Discussion

This study aimed to determine the proportion of neonates admitted with signs and symptoms of clinical sepsis to the pediatric department of the Ugandan national referral hospital in Kampala who had a positive blood culture. It aimed to also describe the etiology of culture-positive sepsis in these newborns and the antimicrobial resistance profiles of isolated bacteria and the factors associated with culture-positive sepsis.

The predominate pathogens isolated from neonates with symptoms and signs of sepsis were *S. aureus*, *E. coli* and *K. pneumoniae*. Our findings were similar to those from previous studies in East Africa[5, 31]. Unlike our study, *Klebsiella* spp. and *K. pneumoniae* were the most commonly isolated bacterial species in blood drawn from neonates with clinical sepsis in a hospital-based Zambian study, possibly explained by a nosocomial outbreak[32]. Generally, many African studies report gram-positive bacteria, especially *S. aureus*, as the main cause of neonatal sepsis; a situation very different from that in South Asia where gram-negative bacteria, especially *Acinetobacter* spp. and *K. pneumoniae* seem to be the main cause[10, 33]. The predominance of *S. aureus* among neonates with culture-positive sepsis in our study may indicate vertical transmission, more so because this pathogen was also common among babies with early onset sepsis.

We observed resistance to antibiotics (ampicillin, gentamicin and ceftriaxone) commonly used to treat neonatal sepsis. Ampicillin and gentamicin were the first-line antibiotics used to treat almost all of the neonates in our study. Among the bacterial pathogens isolated from the blood of participants, we observed almost three quarters were resistant to ampicillin and almost one quarter to gentamicin. This is similar to what other studies in Tanzania[31], Ethiopia[34] and Zambia[32] have reported. The resistance patterns observed in our study are not surprising because the pathogens adapt to selection pressure exerted by the misuse of common antibiotics[35]. *K. pneumoniae* and *E. coli* isolates have intrinsic antimicrobial resistance mechanisms which include chromosomally encoded antibiotic inactivating enzymes or efflux pumps; that enable the many gram-negative bacteria to exhibit such resistance to ampicillin/penicillin antibiotics[36].

Our findings are comparable to those in Kenya[37] and Nigeria[38, 39]. On the contrary, a Ugandan study previously conducted at the Mulago hospital reported resistance among the bacterial pathogens to gentamicin to be lower than that in our study[17]. Since that study was conducted more than 15 years ago from the same hospital as our study, the differences observed between the findings may be explained by a remarkable and worrying acquisition of antimicrobial resistance genes among important invasive bacterial pathogens. Four of 14 (29%) gram-negative pathogens in our study were resistant to third-generation cephalosporins. The proportions of resistance to third-generation cephalosporin in our study were similar to those observed in other African studies[34, 40].

ESBL producers accounted for 6.5% of the isolated pathogens. Other studies in low-income settings reported proportions of ESBL pathogens ranging from 10.5% to 25% among pathogens from neonates with sepsis[41–44]. Although the proportion of ESBL producing bacteria was relatively low in our study, it was imprecisely estimated, and their isolation in the blood of neonates with sepsis is important. This is because ESBL pathogens are associated with long hospitalization stays and death[45, 46]. In-fact, two of the 3 neonates with ESBL in this study died.

Most of the *S. aureus* strains isolated in this study were methicillin resistant. The high proportion of MRSA among *S. aureus* isolates suggests the possibility of transmission from the colonized maternal genital tract or transmission from the labour ward and neonatal units after unhygienic personal or obstetric practices. Susceptible *S. aureus* bacteria adapt to antimicrobial pressure through the acquisition of mobile genetic elements carrying antimicrobial resistance genes from other bacteria encoding modified penicillin binding protein 2a which has very low affinity for beta-lactam drugs[47].

In this study, we found that the odds of developing culture-positive sepsis among neonates born through cesarean section were three times that of those born through vaginal delivery. Although statistically imprecise, our findings are similar to those from other studies[34, 48] that found cesarean-section to be associated with neonatal sepsis. This finding is not

surprising, especially in settings with poor infection control practices. In addition, this association may be explained by underlying indications for cesarean-section such as obstructed labour, premature rapture of membranes and prolonged labor. The immature immune system of neonates puts them at high risk of infection, especially those born prematurely or with low birth weight and who are not breastfeeding[49]. In contrast to other studies[50, 51], we did not find strong associations between low birth weight, premature rupture of membranes and culture-positive neonatal sepsis.

Strengths and limitations

Recruiting neonates from the national referral hospital over a one-year period may be a relatively good representation of neonates with clinical symptoms and signs of sepsis in Kampala and the surrounding areas. However, our study was too small to yield statistically precise estimates for the less common pathogens and for the resistance patterns for the less common ones. Further, it had inadequate power to fully explore the association between the exposures and neonatal sepsis. As most other studies, we will have underestimated the proportion of neonates with neonatal septicemia, both because blood culture in itself has a poor sensitivity[52] and because we did not collect cerebrospinal fluid to diagnose meningitis.

Conclusion

An eighth of neonates admitted to the national referral hospital with clinical signs of sepsis had a positive blood culture. *S. aureus* was the most commonly isolated pathogen, and two-thirds of such isolates were MRSA. Resistance to first-line antibiotics used for the management of sepsis was common. Our neonates with culture-positive sepsis had a high case fatality risk, and there is therefore an urgent need for quick and accurate diagnostic tools for systemic bacterial infections in neonates. Our and others' findings that caesarian section seems to be associated with culture-positive sepsis indicates that health workers need to be alerted if babies born to mothers with such delivery fall sick.

Supporting information

S1 Dataset. Dataset de-identified.
(XLSX)

Acknowledgments

We thank all the women and their newborn infants who participated in our study.

Author Contributions

Conceptualization: Josephine Tumuhamye, Halvor Sommerfelt, Freddie Bwanga, Victoria Nankabirwa, James K. Tumwine.

Data curation: Josephine Tumuhamye, David Mukunya.

Formal analysis: Josephine Tumuhamye, David Mukunya.

Funding acquisition: Halvor Sommerfelt, Grace Ndeezi, James K. Tumwine.

Investigation: Josephine Tumuhamye, David Mukunya, Agnes Napyo.

Methodology: Halvor Sommerfelt, Freddie Bwanga, Grace Ndeezi, David Mukunya, Agnes Napyo, Victoria Nankabirwa, James K. Tumwine.

Project administration: Josephine Tumuhamye.

Validation: Josephine Tumuhamye, Grace Ndeezi, Agnes Napyo.

Writing – original draft: Josephine Tumuhamye.

Writing – review & editing: Josephine Tumuhamye, Halvor Sommerfelt, Freddie Bwanga, Grace Ndeezi, David Mukunya, Agnes Napyo, Victoria Nankabirwa, James K. Tumwine.

References

1. Global, regional, and national under-5 mortality, adult mortality, age-specific mortality, and life expectancy, 1970–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet (London, England). 2017; 390(10100):1084–150.
2. Uganda Bureau of Statistcs (UBOS) and ICF. Uganda Demographic and Health Survey 2016: Key Indicators Report. Kampala, Uganda. UBOS; 2017.
3. Population-based rates, timing, and causes of maternal deaths, stillbirths, and neonatal deaths in south Asia and sub-Saharan Africa: a multi-country prospective cohort study. The Lancet Global health. 2018; 6(12):e1297–e308. https://doi.org/10.1016/S2214-109X(18)30385-1 PMID: 30361107
4. Schiller R, H IJ, Hoskote A, White T, Verhulst F, van Heijst A, et al. Memory deficits following neonatal critical illness: a common neurodevelopmental pathway. The Lancet Child & adolescent health. 2018; 2 (4):281–9.
5. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC pediatrics. 2010; 10:39. https://doi.org/10.1186/1471-2431-10-39 PMID: 20525358
6. Lever A, Mackenzie I. Sepsis: definition, epidemiology, and diagnosis. BMJ (Clinical research ed). 2007; 335(7625):879–83.
7. Khalil N, Blunt HB, Li Z, Hartman T. Neonatal early onset sepsis in Middle Eastern countries: a systematic review. Archives of disease in childhood. 2020.
8. Zaidi AK, Thaver D, Ali SA, Khan TA. Pathogens associated with sepsis in newborns and young infants in developing countries. The Pediatric infectious disease journal. 2009; 28(1 Suppl):S10–8. https://doi.org/10.1097/INF.0b013e3181958769 PMID: 19106757
9. Giannoni E, Agyeman PKA, Stocker M, Posfay-Barbe KM, Heininger U, Spycher BD, et al. Neonatal Sepsis of Early Onset, and Hospital-Acquired and Community-Acquired Late Onset: A Prospective Population-Based Cohort Study. The Journal of pediatrics. 2018; 201:106–14.e4. https://doi.org/10.1016/j.jpeds.2018.05.048 PMID: 30054165
10. colloboration IotDNISD. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. The Lancet Global health. 2016; 4(10):e752–60. https://doi.org/10.1016/S2214-109X(16)30148-6 PMID: 27633433
11. Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. Lancet (London, England). 2005; 365(9465):1175–88.
12. Downie L, Armiento R, Subhi R, Kelly J, Clifford V, Duke T. Community-acquired neonatal and infant sepsis in developing countries: efficacy of WHO's currently recommended antibiotics—systematic review and meta-analysis. Archives of disease in childhood. 2013; 98(2):146–54. https://doi.org/10.1136/archdischild-2012-302033 PMID: 23142784
13. Okomo U, Akpalu ENK, Le Doare K, Roca A, Cousens S, Jarde A, et al. Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines. The Lancet Infectious diseases. 2019.
14. Mo Health. Uganda Clinical Guidelines. Government of Uganda; 2016.
15. John B, David M, Mathias L, Elizabeth N. Risk factors and practices contributing to newborn sepsis in a rural district of Eastern Uganda, August 2013: a cross sectional study. BMC research notes. 2015; 8:339. https://doi.org/10.1186/s13104-015-1308-4 PMID: 26254874
16. Kayom VO, Mugalu J, Kakuru A, Kiguli S, Karamagi C. Burden and factors associated with clinical neonatal sepsis in urban Uganda: a community cohort study. BMC pediatrics. 2018; 18(1):355. https://doi.org/10.1186/s12887-018-1323-4 PMID: 30424740
17. Mugalu J, Nakakeeto MK, Kiguli S, Kaddu-Mulindwa DH. Aetiology, risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. African health sciences. 2006; 6(2):120–6. https://doi.org/10.5555/afhs.2006.6.2.120 PMID: 16916305

18. Edmond K, Zaidi A. New approaches to preventing, diagnosing, and treating neonatal sepsis. PLoS medicine. 2010; 7(3):e1000213. https://doi.org/10.1371/journal.pmed.1000213 PMID: 20231868

19. Geneva; W. Integrated Management of Childhood Illnesses Handbook.http://whqlibdoc.who.int/publications/2005/9241546700.pdf. 2005.

20. MBN Clinical laboratories [Available from: https://mbnlab.com.

21. AS Winn W., Janda W., Koneman E., Procop G., Schreckenberger P., and Woods G. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th Edition, ed. New York.: Lippincott Williams and Wilkins, New York.; 2006.

22. CLSI. Performance standards for Antimicrobial Susceptibility Testing. 26th ed CLSI supplement M 100. 2016; Wayne, PA: Clinical and Laboratory Satandards Institute.

23. Uzun B, Gungor S, Pektas B, Aksoy Gokmen A, Yula E, Kocal F, et al. [Macrolide-lincosamide-streptogramin B (MLSB) resistance phenotypes in clinical Staphylococcus isolates and investigation of telithromycin activity]. Mikrobiyoloji bulteni. 2014; 48(3):469–76. https://doi.org/10.5578/mb.7748 PMID: 25052113

24. Giriyapur RS, Nandihal NW, Krishna BV, Patil AB, Chandrasekhar MR. Comparison of disc diffusion methods for the detection of extended-spectrum Beta lactamase-producing enterobacteriaceae. Journal of laboratory physicians. 2011; 3(1):33–6. https://doi.org/10.4103/0974-2727.78561 PMID: 21701661

25. M'Zali FH, Chanawong A, Kerr KG, Birkenhead D, Hawkey PM. Detection of extended-spectrum beta-lactamases in members of the family enterobacteriaceae: comparison of the MAST DD test, the double disc and the Etest ESBL. The Journal of antimicrobial chemotherapy. 2000; 45(6):881–5. https://doi.org/10.1093/jac/45.6.881 PMID: 10837444

26. McClure JA, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. Journal of clinical microbiology. 2006; 44(3):1141–4. https://doi.org/10.1128/JCM.44.3.1141-1144.2006 PMID: 16517915

27. Young Infants Clinical Signs Study Group. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. Lancet (London, England). 2008; 371(9607):135–42.

28. Masanja PP, Kibusi SM, Mkhoi ML. Predictors of Early Onset Neonatal Sepsis among Neonates in Dodoma, Tanzania: A Case Control Study. Journal of tropical pediatrics. 2019.

29. Palatnik A, Liu LY, Lee A, Yee LM. Predictors of early-onset neonatal sepsis or death among newborns born at <32 weeks of gestation. Journal of perinatology: official journal of the California Perinatal Association. 2019; 39(7):949–55.

30. Liang LD, Kotadia N, English L, Kissoon N, Ansermino JM, Kabakyenga J, et al. Predictors of Mortality in Neonates and Infants Hospitalized With Sepsis or Serious Infections in Developing Countries: A Systematic Review. Frontiers in pediatrics. 2018; 6:277. https://doi.org/10.3389/fped.2018.00277 PMID: 30356806

31. Mhada TV, Fredrick F, Matee MI, Massawe A. Neonatal sepsis at Muhimbili National Hospital, Dar es Salaam, Tanzania; aetiology, antimicrobial sensitivity pattern and clinical outcome. BMC public health. 2012; 12:904. https://doi.org/10.1186/1471-2458-12-904 PMID: 23095365

32. Kabwe M, Tembo J, Chilukutu L, Chilufya M, Ngulube F, Lukwesa C, et al. Etiology, Antibiotic Resistance and Risk Factors for Neonatal Sepsis in a Large Referral Center in Zambia. The Pediatric infectious disease journal. 2016; 35(7):e191–8. https://doi.org/10.1097/INF.0000000000001154 PMID: 27031259

33. Waters D, Jawad I, Ahmad A, Luksic I, Nair H, Zgaga L, et al. Aetiology of community-acquired neonatal sepsis in low and middle income countries. Journal of global health. 2011; 1(2):154–70. PMID: 23198116

34. Tsehaynesh GE, Moges F, Eshetie S, Yeshitela B, Abate E. Bacterial etiologic agents causing neonatal sepsis and associated risk factors in Gondar, Northwest Ethiopia. BMC pediatrics. 2017; 17(1):137. https://doi.org/10.1186/s12887-017-0892-y PMID: 28587631

35. Mukonzo JK, Namuwenge PM, Okure G, Mwesige B, Namusisi OK, Mukanga D. Over-the-counter suboptimal dispensing of antibiotics in Uganda. Journal of multidisciplinary healthcare. 2013; 6:303–10. https://doi.org/10.2147/JMDH.S49075 PMID: 23990728

36. Ruppe E, Woerther PL, Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. Annals of intensive care. 2015; 5(1):61. https://doi.org/10.1186/s13613-015-0061-0 PMID: 26261001

37. Talbert AW, Mwaniki M, Mwarumba S, Newton CR, Berkley JA. Invasive bacterial infections in neonates and young infants born outside hospital admitted to a rural hospital in Kenya. The Pediatric infectious disease journal. 2010; 29(10):945–9. https://doi.org/10.1097/INF.0b013e3181dfca8c PMID: 20418799

38. Nwadioha SI, Kashibu E, Alao OO, Aliyu I. Bacterial isolates in blood cultures of children with suspected septicaemia in Kano: a two-year study. The Nigerian postgraduate medical journal. 2011; 18(2):130–3. PMID: 21670781

39. Pius S, Bello M, Galadima GB, Ibrahim HA, Yerima ST, Ambe JP. Neonatal septicaemia, bacterial isolates and antibiogram sensitivity in Maiduguri North-Eastern Nigeria. The Nigerian postgraduate medical journal. 2016; 23(3):146–51. https://doi.org/10.4103/1117-1936.190340 PMID: 27623727

40. Musoke RN, Revathi G. Emergence of multidrug-resistant gram-negative organisms in a neonatal unit and the therapeutic implications. Journal of tropical pediatrics. 2000; 46(2):86–91. https://doi.org/10.1093/tropej/46.2.86 PMID: 10822934

41. Eibach D, Belmar Campos C, Krumkamp R, Al-Emran HM, Dekker D, Boahen KG, et al. Extended spectrum beta-lactamase producing Enterobacteriaceae causing bloodstream infections in rural Ghana, 2007–2012. International journal of medical microbiology: IJMM. 2016; 306(4):249–54. https://doi.org/10.1016/j.ijmm.2016.05.006 PMID: 27222489

42. Garcia C, Astocondor L, Rojo-Bezares B, Jacobs J, Saenz Y. Molecular Characterization of Extended-Spectrum beta-Lactamase-Producer Klebsiella pneumoniae Isolates Causing Neonatal Sepsis in Peru. The American journal of tropical medicine and hygiene. 2016; 94(2):285–8. https://doi.org/10.4269/ajtmh.15-0373 PMID: 26643537

43. Marando R, Seni J, Mirambo MM, Falgenhauer L, Moremi N, Mushi MF, et al. Predictors of the extended-spectrum-beta lactamases producing Enterobacteriaceae neonatal sepsis at a tertiary hospital, Tanzania. International journal of medical microbiology: IJMM. 2018; 308(7):803–11. https://doi.org/10.1016/j.ijmm.2018.06.012 PMID: 29980372

44. Nelson E, Kayega J, Seni J, Mushi MF, Kidenya BR, Hokororo A, et al. Evaluation of existence and transmission of extended spectrum beta lactamase producing bacteria from post-delivery women to neonates at Bugando Medical Center, Mwanza-Tanzania. BMC research notes. 2014; 7:279. https://doi.org/10.1186/1756-0500-7-279 PMID: 24886506

45. Flokas ME, Karanika S, Alevizakos M, Mylonakis E. Prevalence of ESBL-Producing Enterobacteriaceae in Pediatric Bloodstream Infections: A Systematic Review and Meta-Analysis. PloS one. 2017; 12(1):e0171216. https://doi.org/10.1371/journal.pone.0171216 PMID: 28141845

46. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. The Journal of antimicrobial chemotherapy. 2007; 60(5):913–20. https://doi.org/10.1093/jac/dkm318 PMID: 17848376

47. Stryjewski ME, Corey GR. Methicillin-resistant Staphylococcus aureus: an evolving pathogen. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2014; 58 Suppl 1:S10–9.

48. Shehab El-Din EM, El-Sokkary MM, Bassiouny MR, Hassan R. Epidemiology of Neonatal Sepsis and Implicated Pathogens: A Study from Egypt. BioMed research international. 2015; 2015:509484. https://doi.org/10.1155/2015/509484 PMID: 26146621

49. Vergnano S, Sharland M, Kazembe P, Mwansambo C, Heath PT. Neonatal sepsis: an international perspective. Archives of disease in childhood Fetal and neonatal edition. 2005; 90(3):F220–4. https://doi.org/10.1136/adc.2002.022863 PMID: 15846011

50. Schrag SJ, Cutland CL, Zell ER, Kuwanda L, Buchmann EJ, Velaphi SC, et al. Risk factors for neonatal sepsis and perinatal death among infants enrolled in the prevention of perinatal sepsis trial, Soweto, South Africa. The Pediatric infectious disease journal. 2012; 31(8):821–6. https://doi.org/10.1097/INF.0b013e31825c4b5a PMID: 22565291

51. Murthy S, Godinho MA, Guddattu V, Lewis LES, Nair NS. Risk factors of neonatal sepsis in India: A systematic review and meta-analysis. PloS one. 2019; 14(4):e0215683. https://doi.org/10.1371/journal.pone.0215683 PMID: 31022223

52. Cohen D, Natshe A, Ben Chetrit E, Lebel E, Breuer GS. Synovial fluid culture: agar plates vs. blood culture bottles for microbiological identification. Clinical rheumatology. 2019.

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

uib.no

ISBN: 9788230855256 (print)
9788230848333 (PDF)