Prevention of young infant infections using oral azithromycin in labour in Fiji (Bulabula MaPei): study protocol of a randomised control trial

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ABSTRACT

Introduction Infections are a leading cause of neonatal mortality globally and can be transmitted from mother-to-child vertically or horizontally. Fiji has higher rates of serious neonatal infections and infant skin and soft tissue infections (SSTIs) than high-income countries. Research from the Gambia found that a single dose of oral azithromycin in labour decreased bacterial carriage and infections in mothers and infants, particularly infant skin infections. The Bulabula MaPei clinical trial evaluates the safety and efficacy of a single dose of azithromycin in labour in reducing the incidence of maternal and infant SSTIs and other infections and the impact on bacterial carriage. It will also describe the effect of azithromycin on antimicrobial (AMR) resistance, the maternal and infant microbiome, and infant dysbiosis.

Methods and analysis We are conducting a blinded, placebo-controlled randomised clinical trial administering 2 g of oral azithromycin, or placebo, given to healthy, pregnant women (≥18 years) in labour in Suva, Fiji. The primary outcome is the cumulative incidence of SSTIs in infants by 3 months of age. Secondary outcomes include the incidence of other infant and maternal infections, and safety and tolerability of azithromycin in mother and infant. Following informed consent, 2110 pregnant women will be randomised in a 1:1 ratio, with all study staff and participants masked to group allocation. Mother/infant pairs will be followed up for 12 months over six visits collecting clinical data on infections, antimicrobial use, safety and anthropometrics, in addition to nasopharyngeal, oropharyngeal, rectovaginal and vaginal swabs, maternal breastmilk and infant stool samples, in order to compare bacterial carriage, AMR rates and microbiome. Recruitment for Bulabula MaPei started in June 2019.

Ethics and dissemination This trial was approved and is being conducted according to the protocol approved by The Royal Children’s Hospital Human Research Ethics Committee, Australia, and the Fiji National Health Research and Ethics Review Committee. The findings of this study will be disseminated in peer-reviewed journals and presented at conferences.

Trial registration number NCT03925480.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This blinded, randomised controlled trial is powered to determine a reduction in skin and soft tissue infections (SSTIs) in infants by 3 months of age, born to Fijian women administered a single dose of oral azithromycin during labour.

⇒ The benefits and potential harms of azithromycin on common bacterial pathogens will be determined by comparing carriage, antimicrobial resistance, and infant and maternal microbiome, including potential sequela associated with microbiome dysbiosis, between the azithromycin and placebo groups.

⇒ The primary outcome (SSTIs) is determined on clinical criteria by trained study nurses, which may vary between observers in diagnostic sensitivity and interobserver agreement.

INTRODUCTION

Globally, infections cause approximately 21% of 2.4 million neonatal deaths each year and 52% of all under-five deaths,1 with a disproportionate amount of these deaths occurring in low/middle-income countries (LMICs). Meningitis and sepsis are serious and common causes of neonatal morbidity and mortality.1,2 The bacterial pathogens responsible for these conditions are age-dependent. They vary between LMICs and high-income countries (HICs), with Group B Streptococcus (GBS), Staphylococcus aureus (SA), Streptococcus pneumoniae (SPN), Group A Streptococcus (GAS) and Escherichia coli being common causes in infants in LMICs.3,4,5

Neonatal infections can be transmitted vertically, from mother to newborn through the placenta or birth canal, or horizontally via close contact during breastfeeding or household members. Vertical transmission of microorganisms including GBS and E.
coli are common causes of early-onset neonatal sepsis (NNS) which is associated with a high mortality rate of 10%–15%.6 Intrapartum antibiotic prophylaxis prevents GBS NNS in HICs.7 However, lack of resources means that universal GBS screening with intrapartum prophylaxis in pregnancy is not feasible in many LMICs.7 Additionally, sexually transmitted infections (STIs) such as Chlamydia trachomatis can be transmitted vertically, causing neonatal conjunctivitis and pneumonia,8 and indirectly increase the risk of NNS by increasing the likelihood of prematurity and low birth weight (LBW),9 10 common contributors to neonatal mortality.

Maternal infections are also common, with approximately 5 million cases of pregnancy-related infection occurring each year, resulting in 75 000 maternal deaths.11 12 Sepsis causes a greater proportion of maternal deaths in LMICs than HICs.13 14 Skin infections are a common source of sepsis in both mothers and young infants.15 For mothers, this occurs through postoperative wound infections after caesarean section and following tears and episiotomies during delivery.15 16 For infants, omphalitis may be the initial site of invasive disease.17

There is emerging evidence that azithromycin may prevent vertical transmission of these pathogens during labour in low-resource settings, and a recent systematic review showed that administration during pregnancy reduced the risk of LBW and prematurity.18 Azithromycin has Gram-positive (GBS, SA, SPN, GAS) and some Gram-negative bacterial activity and can also treat organisms associated with STIs. A randomised control trial (RCT) of single-dose oral azithromycin during labour in Gambian women reduced GBS, SA and SPN carriage and reduced maternal and infant infections, including infant skin infections by 50% (3.1% vs 6.4%, p=0.034) and maternal mastitis by 70% (1.4% vs 5.1%, p=0.005) up to 2 months postdelivery.19 20 An RCT of 2013 women in the USA undergoing nonelective caesarean section receiving standard antibiotic prophylaxis with adjunctive azithromycin or standard antibiotic prophylaxis alone decreased the risk of maternal endometritis (3.8% vs 6.1%, p=0.02) and wound infection (2.4% vs 6.6%, p<0.001).21

In Fiji, skin and soft tissue infections (SSTIs), including impetigo, are common and occur in up to 12% of infants.22 23 Moreover, some bacteria commonly associated with impetigo and invasive disease, including SPN, have high carriage rates in Fijian infants. In 2015, SPN carriage was 35% in 5 to 8-week-old infants; 44% in 12–23 months old and 8% in adults caregivers.24 Rates of young infant meningitis of 2.6 per 1000 live births and 2.4 per 1000 live births for 3–11 months old are higher than rates seen in other middle-income countries.25 26 and higher than rates in HICs (0.21 per 1000 live births and 0.09–0.14 per 1000 live births, respectively).27 28 Additionally, the prevalence of 26.8% of C. trachomatis and 2.2% Neisseria gonorrhoeae in pregnant Fijian women is very high.29 This high burden of SSTIs and other serious infections, in conjunction with high carriage rates of potential pathogenic bacteria demonstrate the need for suitable interventions to prevent infections in mothers and infants in Fiji. Evidence of decreased carriage after administration, and known activity against bacteria commonly causing sepsis and specifically SSTIs suggest that azithromycin may be a suitable intervention. We hypothesise that azithromycin administered during labour will reduce the cumulative incidence of SSTIs in infants from birth to 3 months of age, and reduce other maternal and infant infections, antibiotic prescriptions and the bacterial carriage of common pathogens.

METHODS

Aims and objectives

The primary objective of this blinded placebo-controlled randomised trial is to determine the cumulative incidence of SSTI cases in infants from birth to 3 months of age following a single-dose of oral azithromycin administered during labour compared with placebo.

Secondary objectives:

To compare intervention and placebo groups with regard to:
1. Cumulative incidence of infant infection (meningitis, sepsis, pneumonia, SSTI, fever, diarrhoea, urinary tract infection) up to 12 months of age.
2. Cumulative incidence of maternal infection (mastitis, sepsis, postoperative wound infections, SSTI, fever, meningitis, pneumonia, abdominal or pelvic abscess, endometritis, urinary tract infection, pyelonephritis, chorioamnionitis) by 6 weeks postdelivery, and similarly up to 12 months post-delivery.
3. Cumulative incidence of antibiotics prescribed to infants and mothers up to 12 months of age/post-delivery.
4. Prevalence of maternal and infant cases of impetigo with detection of SA and/or GAS.
5. Prevalence of azithromycin non-susceptibility in SA and/or GAS isolates from maternal and infant impetigo cases (up to 3 months and up to 12 months postdelivery).
6. Incidence of maternal cases with chorioamnionitis from placental biopsy histopathology.
7. Prevalence of infants with diagnoses that have been associated with microbiome dysbiosis (eczema, wheeze or adiposity) at 12 months of age.
9. Cumulative incidence of infant and maternal serious adverse events (SAE) throughout the study.
10. Prevalence of maternal and infant bacterial carriage (including GBS, SA, SPN, GAS and E. coli) at key time points, principally 7 days post-delivery.
11. Incidence of common organisms relevant to STIs in maternal vaginal samples.
12. Rate of antimicrobial non-susceptibility among maternal and infant bacterial carriage isolates (including GBS, SA, SPN, GAS and E. coli) at selected time points.

**Design**

This is a blinded, randomised, placebo trial of a single 2 g dose of azithromycin or placebo, administered to women in labour or immediately prior to delivery in the case of caesarean section. There are 2110 mother/infant pairs being recruited and randomised in a 1:1 ratio to each study arm, with six visits over 12 months of follow-up for the mother/infant pair (figure 1).

**Study setting and population**

This study is set in Fiji, where the two principal ethnic groups are indigenous Fijians (iTaukei), who comprise 57% of the population, and Fijians of Indian descent, 38% of the population.30 Recruitment, randomisation and the initial visit occur at Colonial War Memorial Hospital (CWMH), the main tertiary hospital in the capital city Suva where approximately 44% of births occur.31 32 Follow-up visits will occur at various Maternal Child Health (MCH) clinics in the Greater Suva region.

**Patient and public involvement**

There was collaboration with CWMH and MCH clinic staff who were not involved in the study, to ensure study processes were appropriately integrated into these settings. Patients were not involved in the design of the study.

**Recruitment and informed consent**

Study midwives and nurses approach pregnant women at an antenatal clinic. A formal, informed, written and witnessed consent process is conducted if the approached woman is interested in the study. The patient information and consent form is explained to the woman in full and discussed with the family and husband if possible. Recruitment commenced in June 2019 and was completed in February 2022 but was delayed for 42 weeks due to disruptions from the COVID-19 pandemic.

**Eligibility**

Pregnant women are eligible for inclusion if they are at least 18 years old and intend to deliver at CWMH, who have a principal residence in the Greater Suva area and expect to be available with their infant for the duration of the study. Women admitted for delivery at the time...
of final eligibility assessment, prior to randomisation and who gave written informed consent are eligible. Women with cardiac, renal, or hepatic abnormalities, or taking specific drugs that may interact with azithromycin are excluded from the study. For a full list of eligibility criteria, see online supplemental file 1.

Enrolment
After informed consent and the eligibility criteria are met, the pregnant woman is consigned a unique recruitment number. At delivery, eligibility is reconfirmed (including that they were still willing to participate), and the woman is enrolled and randomised (including assigning a randomisation number). Nine hundred and forty mothers will be enrolled in a swab study to assess microbiological outcomes. The first 400 will also be included in the microbiome subset. However, due to COVID-19 disruptions impacting face to face follow-up and the collection of samples, additional participants were enrolled in the microbiome subset to ensure a full set of 400 samples from each participant, as recruitment into this microbiome subset was more substantially impacted by COVID-19.

Interventions
Participants are randomised to receive either 2 g of oral azithromycin (four 500 mg tablets) or a placebo. Those in the placebo group are given four tablets containing rege- latised starch, calcium dibasic phosphate, magnesium stearate and Opadry II White, which look identical to, and are packaged in the same manner as the azithromycin tablets. The azithromycin tablets were manufactured by Laboratorios Cifna (Spain) and then were repackaged into blister packs by Idifarma (Spain). These tablets are administered during labour (or immediately prior to delivery in the case of caesarean section), witnessed by the study nurse/midwife, who records whether participants vomit the following administration. All participants receive routine clinical care deemed necessary as per CWMH treating medical staff, including administration of routine prophylactic antibiotics (ampicillin and gentamicin) as clinically required by caesarean section or suspected chorioamnionitis.

Randomisation and allocation concealment
Participants are randomised 1:1 to the intervention and control arms with stratification by ethnicity (Indigenous Fijian vs other), given established differences in bacterial carriage and infection rates. Blocked randomisation was performed with permuted blocks of variable length. An independent statistician created a computer-generated randomisation list, and then Idifarma labelled the Investigational Product (IP) according to this list. Randomisation numbers are written on blister packs containing the IP, stored separately by stratum and assigned to participants by study staff based on the participant’s self-reported ethnicity consecutively in ascending order (online supplemental file 2). The infant is automatically enrolled and issued a unique identifier linked to the mother’s randomisation number. A list of all randomised women and their corresponding recruitment numbers is kept in a secure room.

Blinding and unblinding
The investigators, participating women, parent(s)/ guardian(s) of infant participants, sponsor, study staff and laboratory personnel are blinded. Sealed envelopes containing the treatment allocation corresponding to each randomisation number are available at the study site at CWMH for medical emergencies. A list is kept by the independent statistician that can be used to provide treatment allocation information if requested by the Data Safety Monitoring Board (DSMB) if concerns are raised through a review of study safety data. Overall study unblinding will occur after all data collection has ceased, the statistical analysis plan is finalised and the database has been locked.

Study procedures
Study procedures are performed according to Standard Operating Procedures and Good Clinical Practice. Baseline demographic and health information is collected from participants prior to delivery. After delivery of the infant, the placenta is collected to perform placental biopsies for histopathological diagnosis of chorioamnionitis (online supplemental file 3). The unique hospital identifier of the mother and infant are recorded to allow the study doctor to search for any subsequent hospitalisations for each participant, and relevant delivery outcomes for mother and infant are extracted by study staff from medical records.

At each study visit, the mother and infant have their axillary temperatures recorded and are examined for SSTI, and if found, the location is recorded to classify SSTI as new or pre-existing since the last visit. Antibiotic use since the last visit, history of AEs (for a full list of AE events) and laboratory personnel are blinded. Sealed envelopes containing the treatment allocation corresponding to each randomisation number are available at the study site at CWMH for medical emergencies. A list is kept by the independent statistician that can be used to provide treatment allocation information if requested by the Data Safety Monitoring Board (DSMB) if concerns are raised through a review of study safety data. Overall study unblinding will occur after all data collection has ceased, the statistical analysis plan is finalised and the database has been locked.

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### Table 1  Timing and details of study visits

<table>
<thead>
<tr>
<th>Visit</th>
<th>Screening</th>
<th>Enrolment/allocation</th>
<th>Postallocation</th>
<th>Close-out</th>
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<tbody>
<tr>
<td></td>
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<td>CWMH admission to</td>
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<td>6 months</td>
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<tr>
<td></td>
<td>contact</td>
<td>discharge</td>
<td>±3 days</td>
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<td>Timing</td>
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<tr>
<td></td>
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<td>±4 days</td>
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<td>3 months</td>
<td>6 months</td>
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<td>prior to enrolment</td>
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<td>±3 days</td>
<td>postnatals7 days</td>
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<td>6 months</td>
<td>12 months</td>
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<tr>
<td></td>
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<td></td>
<td>±7 days</td>
<td>postnatal±14 days</td>
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<td>Confirm eligibility</td>
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<td>Intervention</td>
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<td>2 g oral azithromycin administered</td>
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<td>Oral placebo administered</td>
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<td>Assessment</td>
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<tr>
<td></td>
<td>Demographic/baseline data*</td>
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<tr>
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<td>Placental sample taken</td>
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<tr>
<td></td>
<td>Examination of the infant</td>
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<td>Adiposity measures of the infant</td>
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<td></td>
<td>Examination of the mother</td>
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<td></td>
<td>Feeding review</td>
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<td>History of illness between visits</td>
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<td></td>
<td>Antibiotic use review</td>
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</table>

*Includes demographic and health data (baseline symptoms of sexually transmitted infections and hearing impairment, rural/urban status, marital status, number in household, family income, tobacco exposure, substance use, maternal education, asset index) and delivery outcomes (time and date of membrane rupture, induction of labour, number of vaginal examinations during delivery, mode of delivery, signs of chorioamnionitis, meconium exposure and Apgar scores). CWMH, Colonial War Memorial Hospital.
Microbiological outcomes

Samples for microbiological analyses are being collected at different time points (table 2) and transported to the Fiji Centre for Disease Control laboratory within 6 hours of collection for storage.

Bacterial carriage

Dual flocked swabs (Puritan) are used to collect oropharyngeal, vaginal and rectovaginal samples, with one swab head used for bacterial carriage and the other for microbiome analysis where applicable. Oropharyngeal swabs are collected by study staff from the tonsillar area of the throat; vaginal swabs are collected by inserting a swab 1–2 cm into the vagina, with this process repeated for rectovaginal swabs before inserting the same swab 1 cm into the anus. Nasopharyngeal swabs are collected by study staff from the posterior nasopharynx as per WHO carriage guidelines.38 Swabs are placed into 1 mL skim milk-tryptone-glucose-glycerol, except vaginal swabs, which are placed into universal transport media (Copan). For infants, three small scoops of stool are taken from a soiled nappy and put into a specimen container. Breast milk samples are collected from mothers asked to express their milk manually. All bacterial carriage samples are placed in a cool box immediately after collection.

Microbiome

One head of the dual-headed swabs from the oropharyngeal, vaginal and rectovaginal swabs are placed into DNA/RNA shield collection tubes (Zymo Research). Furthermore, at certain visits, a second infant stool sample is taken and put into a DNA/RNA shield faecal collection tube (Zymo Research). All microbiome samples are kept at room temperature after collection and during transport to the laboratory.

See online supplemental file 6 for further sample collection details.

Primary outcome definitions and assessment

The primary outcome of SSTIs is defined as the occurrence of impetigo, furuncle, omphalitis, abscess, cellulitis and/or staphylococcal scalded skin syndrome in infants up to 3 months of age. SSTIs are assessed at each study visit by study staff, with training and assessment informed by established guidelines and methodology used by other similar published studies (online supplemental file 7).38–41 Further, if SSTIs are identified during visits, staff discuss and send photos of these to the study doctor for confirmation, with the study doctor making the final assessment based on these photos and history provided by staff.

The cumulative incidence of SSTIs will be calculated from SSTI data collected at each study visit and any hospitalisations which document SSTIs as recorded by the treating doctor.

Impetigo is defined as an active bacterial skin infection characterised by sores that start as round or oval pus-filled bumps which progress into blisters, or the sores produce a clear honey-coloured fluid that forms a crust on the skin. When the crusts are removed, the area underneath appears red and eroded.22

Furuncle is defined as pus-filled lesions that are painful and usually firm, occurring when infection around the hair follicles spreads deeper.

Omphalitis is defined as a newborn infection of the umbilical stump, which presents as superficial cellulitis that may involve the entire abdominal wall.

Skin abscess is defined as a collection of pus built up within the body’s tissue with redness, pain, warmth, and swelling of the affected area.41

Cellulitis is defined as a skin infection that is red, painful, swollen, tender and warm to the touch.41

Staphylococcal scalded skin syndrome is defined as an illness characterised by red, blistering skin that looks like a burn or scald.

Secondary clinical outcome definitions

1. Infant infections, a binary composite variable, defined as the occurrence of one or more of: meningitis; sepsis; pneumonia; SSTI; diarrhoea; urinary tract infection; ophthalmia neonatorum or fever up to 12 months of age.

2. Maternal infections, a binary composite variable, defined as the occurrence of one or more of: mastitis; sepsis; postoperative wound infections; SSTI; fever; meningitis; pneumonia; abdominal or pelvic abscess; endometritis; urinary tract infection; pyelonephritis; or chorioamnionitis by 6 weeks postdelivery, and 12 months postdelivery. SSTI in participating mothers is the occurrence per the previous definition, plus mastitis and postoperative wound infection.

See online supplemental file 8 for further definitions.

Trial safety and conduct

Safety outcomes collected in the study include solicited non-serious AE in mother and infant collected at all study visits that are known common adverse drug reactions associated with azithromycin such as nausea in maternal participants, and are solicited through relevant questions in the Case Report Form (CRF). Any concerns regarding these events are discussed with the study doctor and referred as appropriate. SAEs (online supplemental file 4) are identified and reported by the study doctor throughout the study, detected through searching electronic hospitalisation records, solicited at each study visit and as indicated by participants and study staff. Voluntary withdrawals from the study will also be asked for permission to continue to check these hospital records for the normal study follow-up period in order to collect safety and outcome data. Before recruitment, an independent DSMB was established that regularly reviews data related to AE and SAEs.

External study monitoring by a clinical trials monitor is conducted.

Required changes to study procedures during COVID-19

Recruitment started in July 2019 but stopped between 19 March 2020 and 30 June 2020 and from 21 April 2021 and...
<table>
<thead>
<tr>
<th>Participant</th>
<th>Microbiological study subset</th>
<th>Visit timing</th>
<th>Preintervention</th>
<th>Postintervention</th>
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</thead>
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<tr>
<td></td>
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<td>Enrolment/allocation</td>
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<td>Visit 2</td>
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<td>CWMH admission to discharge</td>
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<td>Nasopharyngeal swab</td>
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<td>Oropharyngeal swab</td>
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<td>Stool</td>
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<td></td>
<td>Microbiome</td>
<td>Oropharyngeal swab</td>
<td>x</td>
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<td>Stool</td>
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<tr>
<td>Maternal</td>
<td>Swab study</td>
<td>Nasopharyngeal swab</td>
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<td>Oropharyngeal swab</td>
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<td>Rectovaginal swab</td>
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<td>Vaginal swab</td>
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<td>Breastmilk</td>
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<td>Oropharyngeal swab</td>
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<td>Vaginal swab</td>
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CWMH, Colonial War Memorial Hospital.
6 November 2021 because of COVID-19. During these periods, follow-up visits were performed via phone, but no physical examination or collection of microbiological samples could occur. As physical examinations were not possible, participants were asked about new skin lesions since the last visit and then asked to send photos for verification by the study doctor. For participants recruited from 6 November 2021, there will be no collection of microbiological samples at 6 weeks and 12 months (at visits 3 and 6) due to difficulties with importing specific consumables required in the context of COVID-19.

**Data management**

The data from CRFs are entered into a REDCap (Research Electronic Data Capture) database hosted at the Murdoch Children’s Research Institute, with 100% data verification for all CRF data related to primary outcomes and SAEs to ensure this matches database entries. There is a separate REDCap database for laboratory data. Data are cleaned in an ongoing manner.

Study records are stored so that participants’ confidential information is only accessed within the study team as necessary and protected from unauthorised access.

**Sample size**

To detect a 50% decrease in SSTIs, from 6% in the controls to 3% in the intervention arm, with 90% power and a two-sided alpha of 0.05, a sample size of 1002 participants per arm is required. These assumptions were based on rates of impetigo in infants in Fiji and declines in skin infection in the Gambia following 2 g of oral azithromycin. This sample size was inflated to 1055 participants per arm (total n=2110) to allow for 5% dropout.

For the secondary outcome of bacterial infant carriage at 7 days of age, 940 mother/infant pairs (470 per arm) will provide 95% power at a two-sided 0.05 alpha level to detect an 80% reduction in infant GBS carriage with the intervention, assuming 5% placebo group infant GBS carriage and no loss to follow-up. These assumptions were based on maternal carriage rates in Suva, an assumed 50% likelihood of GBS transfer to neonates from colonised mothers and carriage reduction levels found in the Gambian trial. This sample size will also detect reductions in the infant carriage rate for all relevant bacteria.

**Statistical analysis**

Participant characteristics in each trial arm will be described. Continuous variables will be summarised using means and SDs (or medians and IQRs for non-symmetrical data), and categorical variables reported as frequencies and percentages. The primary analysis will be performed according to the intention-to-treat principle. A secondary analysis conducted with the per-protocol population may exclude those who did not receive complete administration of the IP as allocated or were later found to be ineligible at the time of randomisation. The primary outcome will be expressed as a cumulative incidence: the proportion of infants who develop an SSTI within the first 3 months of life. The comparison between trial arms will be presented as a risk ratio with 95% CIs and p values, estimated using log-binomial regression with the stratification variable (ethnicity) as a covariate. A risk difference with 95% CIs and p values will be presented as a secondary analysis. The same analysis methods will be used for other secondary outcomes. Carriage rates and antibiotic nonsusceptibility will be compared between groups using risk ratios and 95% CI. For the primary outcome, multiple imputation will be used to handle missing data, and sensitivity analyses adjusting for time period will be performed to assess the impact of changes to outcome assessment due to COVID-19-related restrictions across the study duration. SSTIs will be tabulated by visit and mode of assessment to see if there is variation in diagnosis of SSTIs by mode of assessment across these study visits. All statistical analyses will be performed using Stata 16.

**DISCUSSION**

Reducing neonatal mortality is essential for improving child health globally. In 2019, 47% of all under-five deaths occurred in the newborn period, with approximately a third of all neonatal deaths happening on the first day of life, and three-quarters within the first week. As infections are a common cause of neonatal and maternal mortality, developing targeted interventions in these periods is critical for achieving the 2030 Sustainable Development Goal targets. Administering azithromycin during labour should be considered an emerging intervention in this area, having beneficial effects through decreasing carriage of potential pathogens in mothers and infants, thereby reducing the likelihood of transmission and development of serious infections including invasive pneumococcal disease, GBS NNS and neonatal infections caused by STIs such as chlamydia and gonorrhoea.

Despite the broader potential impact on other serious infections, this study will primarily look at the effect on the incidence of SSTIs in infants up to 3 months of age whose mothers received azithromycin during labour. Our study is powered accordingly to investigate this, making it different from the previous Gambian study that was powered to look at their primary outcome of the effect on bacterial carriage. Building on the Gambian study, we will look at potential sequelae of this intervention on microbiome dysbiosis, by assessing conditions of wheeze, eczema and adiposity previously associated with microbiome dysbiosis.

Despite receiving the same training and assessment requirements for the study, staff may have different skill levels, creating some differences in primary outcome reporting from study visit examinations. However, this is mitigated as participants are asked if any hospitalisations have occurred since the last study visit. Further, the electronic medical record system is searched for hospitalisations for infections and other SAEs covering the duration.
of the follow-up period, and these hospital admissions for SSTIs are included in the primary outcome.

Samples were being collected to determine the effects of azithromycin and on AMR up to 12 months of age. The Gambian study reported significantly higher levels of azithromycin resistance associated with the intervention in the neonatal period (at 4 weeks), but this returned to baseline 12 months after administration, with no differences between groups. A systematic review synthesising evidence on the emergence of AMR after mass azithromycin distribution found an increase in macrolide resistance immediately after treatment, which seemed to dissipate with time. Dysbiosis, when antibiotic exposure alters the structure and function of the human microbiome, has been associated with a variety of chronic health conditions in children, including asthma and obesity. Two studies examining the short-term effects of azithromycin administered to infants and young children found reductions in microbial diversity and changes in bacterial composition compared with placebo groups. However, very few studies examine the immediate effect of intrapartum antibiotic prophylaxis on the infant microbiome or the longer-term effects of antibiotic use on the infant microbiome. Our study will provide useful information related to these areas.

Administration of azithromycin during labour may be a cheap and simple intervention that could improve neonatal morbidity and mortality in LMICs, alongside strengthening maternal-child health services. This study, together with other large clinical trials that are currently being undertaken in Africa and Asia documenting the effectiveness of azithromycin administered during pregnancy or in labour, on stillbirths, maternal and neonatal infections, and mortality, will add to the evidence for consideration at both national levels and by multilateral groups such as WHO involved in international guideline setting.

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Contributors FMR conceived and was responsible for the overall design. CS was responsible for the microbiology component. FMR and EW drafted the protocol. MH-N wrote the manuscript, with contributions to the development of the manuscript by FMR and CDN. EFGN provided database curation and management for the study, and CDN provided statistical oversight. MH-N adapted the protocol and SOPs for field and laboratory work, with significant input regarding laboratory work from CS and CLP. TR and SC ensured that SOPs were appropriate for the setting. TR primarily managed the site in Fiji with coordination from MH-N, and SC was the study doctor responsible for SAE reporting. KB acted as the overall study manager. MH-N initially developed relevant SOPs and partnerships in Fiji required for placental biopsies, which JH then developed further and coordinated. AS and KM supported study development and served as part of the steering committee for the study. JF, ER, KS and IT supported study development and provided support and guidance related to approval processes in Fiji.

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Competing interests CDN is a coinvestigator on a Merck Investigator Studies Programme grant funded by MSD on pneumococcal serotype epidemiology in children with pneumonia and an investigator on a Pfizer-funded clinical research collaboration of PCV vaccination in Mongolia.

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REFERENCES

3 Lawn JE, Blencowe H, Oza S, et al. Every newborn: progress, priorities, and potential beyond survival. Lancet 2014;384:189–205.
8 Adachi K, Nielsen-Saines K, Klausner JD. Chlamydia trachomatis infection in pregnancy: the global challenge of preventing adverse
Open access

30 Fiji Islands, World Directory of Minorities and Indigenous Peoples: Minority rights group international; 2017.
31 Birth rate, crude (per 1,000 people) - Fiji. *World Bank Data: The World Bank, 2021.*
48 Neonatal mortality. UNICEF, 2020
57 Azithromycin-Prevention in labor use study (A-PLUS). *ClinicalTrials.gov*, 2019
SUPPLEMENT 1. FULL ELIGIBILITY CRITERIA

Inclusion criteria:

1. Pregnant women at least 18 years old intending to deliver at Colonial War Memorial Hospital (CWMH)
2. Women who have been admitted to CWMH for delivery at the time of eligibility assessment
3. Women who expect to be available with their infant for the duration of the study and who agree to adhere to all protocol requirements
4. Women who will have a principal place of residence within the Greater Suva area for the follow-up period and within a practical distance of the study site to allow compliance with protocol-required visits and follow-up, including attending follow-up at specified clinics
5. Women who have provided written informed consent prior to study-related procedures being performed

Exclusion criteria:

1. Women who have a known macrolide allergy
2. Women who have taken antibiotics in the week prior to randomisation
3. A woman unable or unwilling to provide informed consent for her participation in the trial or the participation of her infant
4. Women who decide prior to randomisation that they are no longer willing to participate or to have their infant participate
5. Women who have ever received, or who anticipate receiving during the study period, any investigational agent other than the study drug
6. Women who are CWMH, Murdoch Children’s Research Institute (MCRI) or study site employees who work directly with study staff or who are working on the study
7. Women taking warfarin due to the potential for drug interactions with azithromycin
8. Women with any cardiac abnormality
9. Women taking other medications known to prolong the QT interval such as antiarrhythmics; antipsychotic agents; antidepressants; and fluoroquinolones
10. Women with known electrolyte disturbances, including in cases of hypokalaemia and hypomagnesaemia
11. Women who will undergo general anaesthetic for delivery
12. Women carrying a foetus with intrauterine death confirmed before randomisation
13. Women carrying a foetus with a prognosis unlikely to survive
14. Women with known HIV infection and/or taking nelfinavir
15. Women who have participated in the study during a previous pregnancy
16. Women who have been admitted for management of premature labour who have unruptured membranes (this is a temporary exclusion such that the participant may be assessed for eligibility again in the same or a subsequent admission to CWMH).
17. Women with renal impairment
18. Women with hepatic impairment
19. Women with myasthenia gravis
20. Women who are taking any ergot medications
SUPPLEMENT 2. Randomisation and administration of the investigational product

Investigational product information relevant to randomisation

The investigational product will be supplied as four coated 500mg tablets in blister packs.

Azithromycin will be given orally, as a single 2g dose, comprised of four 500mg tablets taken together prior to delivery. Specifically, the tablets are commercially available generic azithromycin manufactured by Laboratorios Cinfa, Spain. The tablets have been de-blistered and repackaged into blister packs (by Idifarma, Spain) and relabelled with randomisation codes for binding of treatment allocation.

Placebo will be given orally, as a single 2g dose, which comprises four 500mg tablets taken together prior to delivery. Placebo tablets do not contain active ingredients and are manufactured by Idifarma, Spain, so that each placebo tablet is matched for size and colour with the azithromycin tablets for binding of treatment allocation. Each tablet contains only pregelatinized starch, calcium dibasic phosphate, magnesium stearate and Opadry II White. The tablets are manufactured under Good Manufacturing Practice (GMP) conditions.

The placebo tablets are packaged into blister packs and labelled, by Idifarma, with randomisation codes for blinding treatment allocation.

The processes at Idifarma are conducted by personnel independent of the trial.

Method for Assigning Participants to Treatment Group

Approximately 2110 participants will be prospectively randomised to one of the two treatment groups (azithromycin arm or placebo arm) in a 1:1 ratio.

Each of the two treatment groups will have approximately 1055 participants.

The randomisation scheme will be computer generated using block randomisation. An independent statistician from Melbourne Children’s Trials Centre, MCRI, without involvement in participant enrolment or treatment administration, will produce and keep secure a computer-generated list of study randomisation codes and treatment allocation. The randomisation code will have permuted blocks of variable length. Given established differences in bacterial carriage and infection rates, randomisation will be stratified by ethnicity (Indigenous Fijian vs other) to minimise imbalance across the treatment arms.

Eligible women will be randomised at Visit 1 following confirmation of eligibility after admission for delivery. The study personnel assign participants the next available randomisation number using the randomisation list and associated tablet blister packs for the appropriate stratum (separate boxes of blister packs for each ethnicity stratum: Indigenous Fijian vs other). The study staff will then select the specific investigational product tablet blister pack. The participant ID number, date and time of dispensing will be documented for each dispensed blister pack.

The Randomisation Number will identify the participant throughout the study period and on all study-related documentation. The participant ID allocated to the mother will be five characters long, with the first four characters representing the 4-digit randomisation number and the last character as “M”. The participant ID allocated to the infant will be five characters long, with the first four characters representing the 4-digit randomisation number and the last character as “B”. In the case of twins, the baby delivered second will have the first four characters of the participant ID as the randomisation number and the last character as “T”. Additional letters as a suffix to the participant ID will indicate whether each participant is enrolled in the swab study and/or microbiome subset.
If a participant is found to be ineligible during screening and is not randomised or discontinues from the trial after randomisation, the participant Recruitment Number and Randomisation Number will not be reallocated.

**Investigational product administration and treatment compliance**

For enrolled participants, study staff will get the tablet blister pack labelled with the participant’s randomisation number from the appropriate box based on the participant’s ethnicity. Study staff are blinded to treatment allocation.

Following enrolment, study staff will facilitate and monitor oral administration of the investigational product to the participant. The participant should sit upright and swallow the four tablets with water as early as possible after randomisation (and prior to delivery).

Investigational product administration details will be recorded (including date, time and whether or not the complete set of tablets were administered).

Study staff will record whether or not vomiting occurred after tablet administration and prior to delivery, the time this occurred and whether any tablet was seen in the vomitus. The prescribed dosage, timing and mode of investigational product administration must be as specified in this protocol. Any departures from the intended regimen must be recorded, along with the reason for the departure.

Participants who do not ingest the whole four tablets of the investigational product (due to, for example, refusal or vomiting within the 10 minutes following administration) will undergo all study assessments as per protocol up to 12 months post-delivery.

The incomplete administration of the investigational product dose will be recorded in the data collection form.
SUPPLEMENT 3. PLACENTAL BIOPSY METHODS

- All study staff collecting placental samples receive training to ensure all specimens are collected according to protocol.
- All women enrolled have their placenta collected following delivery to look for chorioamnionitis. The placenta is then biopsied and processed into slides for histopathological reading.

Procedure

After the umbilical cord is cut, staff place the placenta into a specimen container filled with 10% formaldehyde.

The placenta is then biopsied by taking samples from the umbilical cord, amniotic membranes, and central parenchyma - these are important components required for histopathology to diagnose chorioamnionitis.

- **Umbilical cord:** Two slices approximately 3mm thick are taken at different sites of the umbilical cord; one closer to the insertion of the cord and one further away from the insertion of the cord. For each slice, three vessels should be able to be visualised. These slices are then placed into a cassette.

- **Amniotic membrane:** One membrane roll sample is collected by identifying the rupture site of the foetal/amniotic membrane on the maternal side of the placenta. The membrane at the edge of this membrane rupture is rolled around blunt forceps so that the edge of the membrane is at the centre of the roll. Then two slices are cut from this membrane roll, approximately 3mm thick, and placed into cassettes.

- **Central parenchyma:** A sample from the central parenchyma of the placenta is taken by slicing through the centre of the placenta, ensuring that the slice contains the fetal surface of the placenta, including the membranes and at least one blood vessel. This is then further divided to create a central segment with a surface amnion and at least one fetal blood vessel. This is then further cut into an approximately 3mm slice and then placed into a cassette.

At the end of the process, the study staff member will have collected:

- 2 X sections of the umbilical cord (Placed in one cassette)
- 2 X sections of the membrane roll (Placed in one cassette)
- 1 X central parenchymal sample (Placed in one cassette)

Each cassette is placed into a specimen container containing 10% formaldehyde and fixed for at least 24 hours prior to embedding in paraffin for storage and transport. Finally, tissue sections are cut and mounted on slides for histopathology reading.
SUPPLEMENT 4. DEFINITIONS AND FULL LIST OF ADVERSE EVENTS:

i. Definitions

AE Definition
An AE is any untoward, undesired or unexpected clinical event in the form of signs, symptoms, disease or laboratory or physiological observations occurring in a participant exposed to an investigational product, whether or not related to the investigational product.

Pre-existing diseases are not considered AEs unless there is a change in intensity, frequency or quality. Lack of efficacy is also not considered an AE, per se.

For this trial, all AE that fulfil the definition of a Serious Adverse Event (SAE) and/or a solicited non-serious adverse event, as defined below, will be recorded for each maternal and infant participant.

SAE definition
An SAE is any untoward medical occurrence that:

- results in death;
- is life-threatening;
- requires inpatient hospitalisation or prolongs existing hospitalisation;
- results in a persistent or significant disability or incapacity;
- is a congenital anomaly/birth defect in the offspring of a mother who has received the investigational product (Congenital anomaly/birth defects are not relevant for this study, and therefore will not be considered a serious adverse event and will instead be considered as pre-existing conditions).

Additionally, serious medically important events that may not meet a definition above may be considered SAEs when based upon appropriate medical judgement, they jeopardise the participant or require medical or surgical intervention to prevent one of the outcomes listed above. For this trial, serious medically important events may include infant hearing impairment and pyloric stenosis.

ii. List of solicited non-serious adverse events:

Infants
At visits one-three:
Diarrhoea/loose stools, vomiting, feeding difficulty, urticaria, angioedema, eczema, wheeze, and oral candidiasis.

Visits four-six:
Diarrhoea/loose stools, feeding difficulty, eczema, wheeze.

Mothers
At visits one-three:
Diarrhoea/loose stools, nausea, vomiting, abdominal pain, dyspepsia, loss of appetite, urticaria, photosensitivity, angioedema, palpitations, wheeze, vaginitis, candidiasis, dizziness, headache, visual disturbance, taste disturbance, numbness, fatigue, arthralgia, pruritus/itch.

Visits four-six:
Diarrhoea/loose stools, nausea, vomiting, abdominal pain, dyspepsia, palpitations, and wheeze.
SUPPLEMENT 5. IMPETIGO SWABS

- Impetigo swabs are collected if maternal or infant participants have impetigo detected by study staff on examination at visit four (3 months follow-up) and/or visit six (12 months follow-up).
- Only one sore from each participant will be swabbed, preferably open purulent lesions or sores with pus. Alternatively, crusted or pustular lesions or sores are swabbed.

Study procedures
Study staff ensure that the sore is clean using sterile gauze moistened with saline. Then with clean hands, they use a sterile regular flocked swab (Copan) to swab the lesion. For open purulent sores with pus, the swab is rolled over the sore and pus. For crusted sores, staff will break the crust with firm pressure from the swab or use gauze soaked in saline to soften the crust. Once the crust is broken, the swab is rolled over the sore from one side to the other. Sores with a pustule are punctured using the broken end of the swab shaft after breaking at the designated breakpoint, and the swab tip is rolled over the sore from one side to another. The swab is then inserted into 1 ml skim milk-tryptone-glucose-glycerol medium (STGG), placed into a cool box with an ice pack and transported to the Fiji Centre for Disease Control (CDC) laboratory within 6 hours of collection. Samples are thoroughly vortexed and aliquoted before storage at ultra-low temperature (ULT), e.g. -70°C.)
SUPPLEMENT 6. BACTERIAL CARRIAGE AND MICROBIOME SAMPLE COLLECTION

The following swabs and samples are collected from study participants to examine bacterial carriage and/or microbiome:

1. **Nasopharyngeal swabs** are collected for bacterial carriage analysis from maternal and infant participants using a single-headed flocked swab (Copan) inserted into the posterior nasopharynx for five seconds and/or rotated 180 degrees saturating the swab tip. This swab is placed into 1ml STGG. Different sized flocked swabs are used for mothers and infants:
   - **Mother:** Single-headed flexible mini tip flocked swab (Copan)
   - **Infant:** Single-headed ultra mini tip flocked swab (Copan)

2. **Oropharyngeal swabs** are collected from maternal and infant participants using a dual-headed flocked swab (Puritan) inserted into the tonsillar area of the throat without touching the sides of the mouth. One swab head is placed into 1 ml STGG for bacterial carriage analysis. If the participant is also enrolled in the microbiome subset, the second swab head is placed into a DNA/RNA shield collection tube (Zymo Research) for microbiome analysis.

3. **Vaginal swabs** are collected from maternal participants using a dual-headed flocked swab (Puritan) inserted 1-2 cm into the vagina and then turned and rotated once. Preferably, vaginal swabs are collected by study staff. However, staff instruct participants how to collect vaginal samples themselves if they refuse collection by study staff. One swab head is placed into 1 ml Universal transport medium (UTM; Copan) for bacterial carriage analysis. If participants are enrolled in the microbiome subset, the second swab head is placed into a DNA/RNA shield collection tube (Zymo Research), at selected time points, for microbiome analysis.

4. **Rectovaginal swabs** are collected from maternal participants using a dual-headed flocked swab (Puritan) inserted 1-2 cm into the vagina (turned and rotated once) and then inserted 1 cm into the anus. This is preferably collected by study staff. However, the study staff instruct participants how to collect rectovaginal samples themselves if they refuse collection by study staff. One swab head is placed into 1 ml STGG for bacterial carriage analysis. If participants are enrolled in the microbiome subset, the second swab head is placed into a DNA/RNA shield collection tube (Zymo Research), at selected time points, for microbiome analysis.

5. **Stool samples** are collected from infants by taking three small scoops of stool, preferably from a soiled nappy, and transferring them into a specimen container for bacterial carriage analysis. A second stool sample is taken and placed into a DNA/RNA shield fecal collection tube (Zymo Research) for microbiome analysis if participants are enrolled in the microbiome subset.

6. **Breastmilk samples** are collected from maternal participants. Milk is manually expressed, with the first 0.5 ml of breastmilk discarded. The next 1-2 ml of breastmilk is collected in a specimen container.

All bacterial carriage samples are immediately placed into a cool box with ice packs and transported to the CDC laboratory within 6 hours of collection. All samples, except vaginal swabs, are thoroughly mixed and aliquoted prior to storage at ULT. Vaginal swabs are stored at ULT without aliquoting. All microbiome samples are transported and stored at ambient temperature.
SUPPLEMENT 7. TRAINING ON SKIN AND SOFT TISSUE INFECTION DIAGNOSIS

Staff training on skin and soft tissue infections (primary outcome assessment) included multiple components in different settings. This included multiple lectures, skin clinic/dermatology attachments, and assessments in clinical and classroom settings.

Initial training package
Study staff were all qualified nurses and/or midwives and therefore had a clinical background prior to specific study training on skin and soft tissue infections (SSTIs).

As part of the initial package of training, they completed relevant sections of online Integrated Management of Childhood Illness (IMCI) Training Player (known as ICATT), including IMCI algorithm for managing common skin conditions in Fiji that has been validated,[1] and sections on bacterial infections. They had a two-hour lecture on SSTIs which included the study definition of primary outcomes (impetigo, furuncle, omphalitis, abscess, cellulitis, and staphylococcal scalded skin syndrome). Furthermore, they were provided with written references, including the study protocol and Standard Operating Procedure for assessing skin and soft tissue infections that contained definitions, information about conditions/pathology, and pictures of each condition.

Clinical attachment and assessment at Skin Clinic
Following this initial training, they did an attachment at Twomey hospital skin clinic in Suva, which had been used as part of training in previous studies looking at scabies and impetigo.[2] This involved the following:

1. Logbook completion confirmed that study staff had been exposed to all SSTIs included in the primary outcome during their attachment. This included listing all patients seen in the clinic and their presenting conditions, confirmed by attending local dermatologists. The study coordinator or doctor reviewed these logbooks to ensure that staff had seen the key infections of interest.

2. An assessment where study staff were present at ten patient consultations with a dermatologist. For each consultation, study staff had to identify if patients presented with one of the SSTIs of interest, and if so, identify this condition. If the patient presented with an alternative skin condition, e.g. eczema, study staff were not required to identify what this skin condition was, only that it was not one of the SSTIs that was part of this study’s primary outcome. The dermatologist did this for each consultation - this assessment was the gold standard against which the study staff member’s answer was assessed.

Desk-based assessment
All study staff also undertook a desk-based assessment where there was a slideshow of 50 pictures designed by site investigator Dr Maeve Hume-Nixon that showed a mixture of both SSTIs, other skin conditions, and photos of non-pathology in both adults and infants. This was informed by assessments performed for previous studies where staff had been trained in scabies and impetigo detection.[2, 3] Study staff had to identify whether the photo shown was one of the study SSTIs of interest and, if so, the name of condition of interest, with a pass mark of 80%.

Some conditions of interest, such as omphalitis, were not seen commonly at Twomey hospital skin clinic and were more commonly seen at MCH clinics. Therefore staff also attended half a day at these MCH clinics in the Greater Suva area to further expose them to conditions such as omphalitis and impetigo, conditions commonly seen in these clinics.[1]

Ongoing learning
In addition to these formal training components and assessments, the study staff had ongoing training sessions throughout the study presented by the study doctor, and this included demonstration and discussion of relevant photos (with permission of study participants).

Periodically staff visited patients with conditions of interest admitted to hospital wards, for example, infected caesarean section wounds, a learning experience facilitated and led by the study doctor with permission from relevant Colonial War Memorial Hospital (where the study was based) head of...
departments and the patients. The study doctor regularly attended follow-up visits to monitor this SSTI assessment and utilised this opportunity for ongoing teaching of study staff.
SUPPLEMENT 8. SECONDARY OUTCOME DEFINITIONS – CLINICAL INFECTIONS

i. Other infection outcome definitions for infants.

**Diarrhoea**

Diarrhoea will be captured in three different ways in this study:

1) *Hospitalisation for diarrhoea*: a discharge diagnosis of acute diarrhoea or acute gastroenteritis by treating medical staff during hospitalisation for diarrhoea, and/or as categorised as ‘acute diarrhoea’ (as opposed to ‘chronic diarrhoea’ of ≥14 days duration) based on admission notes by the study doctor.

2) *Reported outpatient treatment for diarrhoea*: participating mothers will be asked if their infants have been treated for diarrhoea as an outpatient, without hospitalisation.

3) *Diarrhoea as a symptom reported during study visit*: study staff will ask about ‘diarrhoea’ as a solicited non-serious adverse event at each study visit. The definition of diarrhoea is as per IMCI guidance and differs based on the infant’s age.[4]
   - In visits 1-3, when the infant is aged less than two months, this will be defined as: ‘*If the stools have changed from the usual pattern and are many and watery (more water than faecal matter)*’. The usually frequent or semi-solid stools of a breastfed baby are not diarrhoea.
   - In visits 4-6, when the infant is aged more than two months, this will be defined as ‘*three or more loose or watery stools in a 24-hour-period*’.

**Fever**

Fever in infants will be captured in three different ways in this study, which are defined as follows:

1) *Self-report* (at each study visit: any maternal report of fever in the infant since the previous study visit)

2) *Objective fever during a study visit* (defined as documented temperature ≥37.5°C by staff)

3) *Documented fever during hospital admission* (defined as a documented temperature ≥37.5°C in hospital admission records)

**Pneumonia**

Pneumonia will be captured in two different ways:

1) *Reported outpatient treatment for pneumonia*: participating mothers will be asked if their infants have been treated for pneumonia as an outpatient, without hospitalisation.

2) *Hospitalisation for pneumonia*: Admissions records reviewed, and then classified according to the WHO pneumonia definition:[5] If the presence of both signs of fast breathing (respiratory rate equal or greater than 50 breaths/min in a child aged 2-11 months or greater or equal to 40 breaths/min in a child aged 1-5 years) and chest indrawing present. For those younger than two months, medical staff diagnose pneumonia or congenital pneumonia.

**Meningitis**

All-cause meningitis cases will be a hospital admission with a clinical diagnosis of meningitis.

All-cause meningitis will be divided into categories based on lab investigations.

- *Laboratory confirmed meningitis* would include *probable bacterial meningitis* and *pathogen-specific meningitis cases* as defined below:
• **Probable bacterial meningitis cases** will be classified based on WHO criteria definitions:[6] a suspected meningitis case with CSF examination showing abnormality associated with bacterial meningitis including the following:
  a. A turbid macroscopic appearance;
  b. Increased opening pressure (>180mm water), if available;
  c. Pleocytosis (usually of polymorphonuclear (PMN) leukocytes); WBC counts >10 cells/mm³.
  d. Increased protein concentration (>45mg/dl); or
  e. Decreased glucose concentration (<45 mg/dl).

• **Pathogen-specific meningitis cases** will be a hospital admission for suspected meningitis case that is laboratory-confirmed by culturing or identifying (e.g. by Gram stain, antigen detection or qPCR) the bacterial pathogen GBS, SPN, GAS, SA or *E. coli* in the CSF or blood culture.

• **Clinically suspected and treated meningitis** will be a hospital admission with a clinical diagnosis of meningitis and subsequent treatment that excludes lab-confirmed meningitis.

**Sepsis**

*All-cause sepsis cases* are a hospital admission that meet the diagnosis of sepsis, according to International Pediatric Sepsis Consensus Conference Definition (Goldstein et al. 2005) ‘SIRS in the presence of or as a result of suspected or proven infection’. This is based on the criteria for SIRS described in this definition, based on temperature, tachycardia, respiratory rate, and leukocyte count as per age-specific vital signs and laboratory variables (Goldstein et al. 2005).[7]

Infection according to this definition is:[7]

“A suspected or proven (by positive culture, tissue stain, or PCR test) infection caused by any pathogen OR a clinical syndrome associated with a high probability of infection. Evidence of infection includes positive findings on clinical exam, imaging, or laboratory tests (e.g. white blood cells in normally sterile body fluid, perforated viscus, chest radiograph consistent with pneumonia, petechial or purpuric rash, or purpura fulminans).”

If an infant’s illness (all children participating less than one year of age) met this definition of sepsis (International Pediatric Sepsis Consensus Conference Definition) but had clinical presentations with features meeting the definitions of pneumonia or meningitis (described above), the illness will be classified as pneumonia or meningitis, rather than as sepsis.

**Urinary Tract Infections**

Based on the WHO Hospital care for Children.[5] Urinary tract infections will be classified based on microscopy of a ‘clean’ urine sample (‘clean-catch’ urine specimen, or in-out urinary catheter, or suprapubic bladder aspiration) with more than five white cells per high-power field, or a dipstick showing a positive result for leukocytes or culture positive.

**Ophthalmia neonatorum**

Ophthalmia neonatorum as diagnosed by hospital staff.
ii. **Maternal infection outcome definitions**

**Pneumonia, urinary tract infection, and pyelonephritis**

Pneumonia, urinary tract infection, and pyelonephritis will be all captured in two ways:

1) Reported outpatient treatment.
2) Hospitalisation for condition.

**Mastitis and post-operative wound infection**

Mastitis and post-operative wound infection will be captured in three ways:

1) Through clinical examination and diagnosis by study staff at scheduled study visits.
2) Hospitalisation for condition.
3) Self-reported outpatient treatment for these conditions.

*Mastitis* is defined as part of the breast (usually unilateral) becoming red, painful, swollen and hard, which may be accompanied by general symptoms of fever and malaise.[8]

*Post-operative wound infection* is defined as the presence of either superficial or deep incisional surgical site (for caesarean section) infection characterized by cellulitis or erythema and induration around the incision or purulent discharge from the incision site with or without fever and includes necrotizing fasciitis.

**Fever**

Fever in mothers will be captured in three different ways:

1) *Self-report* (at each study visit: any self-reported maternal fever, since the previous study visit)
2) *Objective fever during a study visit* (defined as documented temperature ≥38°C by staff)
3) *Documented fever during hospital admission* (defined as a documented temperature ≥38°C in hospital admission records).

**Abdominal or pelvic abscess, endometritis, and puerperal sepsis**

Based on a clinical discharge diagnosis during hospital admission by treating medical staff.

**Meningitis**

All-cause meningitis cases in mothers will be a hospital admission with a clinical diagnosis of meningitis. This will then be further divided into categories based on laboratory investigations, as per definition of infant meningitis.

**Maternal sepsis**

*All-cause maternal sepsis* cases are a mother's hospital admission with a clinical diagnosis of sepsis, excluding diagnoses of puerperal sepsis. This includes microbiologically confirmed sepsis.

*Microbiologically confirmed sepsis* will be defined as hospital admission for suspected sepsis case that is microbiologically confirmed by growing (i.e. culturing) the pathogen by blood culture or from a normally sterile site (excluding cases of puerperal sepsis).
References: