Chlorhexidine cleansing of the umbilical cord stump and risk of omphalitis and neonatal death

Usha Dhingra

Thesis for the degree of Philosophiae Doctor (PhD)
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Dedication

This thesis is dedicated to the scientists and researchers striving to save lives of moms and babies.

Contributors

The Pemba chlorhexidine (CHX) trial was a collaborative effort between Johns Hopkins Bloomberg School of Public Health, USA; Public Health Laboratory-Ivo de Carneri (PHL-IdC), Pemba; Ministry of Health, Zanzibar; Center for Public Health Kinetics, India; and Annamalai University, India. Dr. Sunil Sazawal led this collaborative work and was the Principal Investigator for this trial. Bill and Melinda Gates Foundation funded the trial.

This PhD work was undertaken under the supervision of Professor Sunil Sazawal (while the studies were conceptualized and undertaken in India and Tanzania), Professor Halvor Sommerfelt, Associate Professor Victoria Nankabirwa, and Professor David Murdoch.

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Abbreviations

AMANHI	Alliance for maternal and newborn health improvement
CHWs	Community health workers
CHX	Chlorhexidine
CI	Confidence interval
CISMAC	Centre for intervention science in maternal and child health
DSMB	Data safety monitoring board
FGDs	Focus group discussions
IDIs	In-depth interviews
IRB	Institutional review board
KAP	Knowledge, attitude and practices
LMICs	Low-income and middle-income countries
MCHWs	Maternal child health workers
MNCH	Maternal, newborn and child health
NMR	Neonatal mortality rate
PHCC	Primary healthcare centers
PHCU	Primary healthcare units
PHL-IdC	Public health laboratory- Ivo de Carneri
RCT	Randomized control trial
RR	Relative risk
SOPs	Standard operating procedures
TBAs	Traditional birth attendants
TIPS	Trials of improved practices
ULCR	Upper-to-lower confidence limit ratio
WHO	World Health Organization

Abstract

Background: Sub-Saharan Africa and South Asia account for 80% of all neonatal deaths in the world. In these two regions, infectious diseases account for one third of neonatal deaths. Early neonatal sepsis is a major contributor to the high neonatal morbidity and mortality and umbilical cord stump infection (omphalitis) is postulated to be a potential precursor to neonatal sepsis. There has been evidence from Asia to suggest that CHX application to the umbilical cord stump can reduce the risk of omphalitis as well as neonatal death. No data on the efficacy of CHX cord care in Africa were available before we embarked on the studies described in this thesis. The studies in this thesis address four main topics: i) understanding childbirth and newborn care knowledge, attitudes and practices (KAP); ii) feasibility, acceptance and compliance with different modes of CHX application on the umbilical cord stump; iii) effect of the proposed CHX formulation on bacterial colonization of the cord stump; and iv) efficacy of CHX application on the risk of omphalitis and neonatal death.

Methods: Two qualitative studies were conducted in Pemba, a part of the Zanzibar archipelago of Tanzania. The first study explored the KAP regarding childbirth, newborn and umbilical cord care and probed barriers and facilitators to introduction of CHX cord care. A total of 80 in-depth interviews (IDIs) and 11 focus group discussions (FGDs) involving mothers, grandmothers, fathers, traditional birth attendants (TBAs) and health service providers were undertaken. A second study, using the Trials of Improved Practices (TIPs) methodology investigated the feasibility, acceptance, and compliance with three possible modes of CHX application. The third study tested the effect of a commercially prepared 4% CHX solution on bacterial colonization in New Delhi. The fourth study was a large community-based randomized control trial (RCT) in Pemba to estimate the efficacy of CHX cleansing of the umbilical cord stump on the risk of omphalitis and neonatal death. The RCT was implemented in two phases. During Phase 1, the study participants were randomly allocated to one of three intervention arms: the treatment arm using CHX, one control arm using a placebo solution and a second control arm practicing dry cord care. During Phase 2, the placebo arm of the trial was dropped, and the participants were randomized to receive either CHX or dry cord care. For babies allocated to the CHX and the placebo arms, mothers/caregivers were advised to apply the solution to the cord stump every day until 3 days after the cord stump had fallen off. For babies assigned to the dry cord care arm, TBAs and hospital staff instructed the mothers/caregivers not to cleanse the umbilical cord stump and to keep it dry. In all trial arms, the cord was examined for redness, pus, swelling and/or foul smell on days 0, 1, 4, 10 and 28.

Findings: The qualitative study showed that the awareness among the community members regarding the importance of facility-based births was high. However, impediments such as lack of transportation; cost of medicines and quality of care at the facilities seemed to be a reason for many women choosing to give birth at home. Some of the reported good practices included use of clean delivery instruments, TBAs' emotional and physical support to mothers, newborn warming and feeding of colostrum. Some behaviors that could undermine newborn health, such as immediate bathing of newborns, poor hand hygiene of TBAs, exposing the newborn to smoke for warming, application of some traditional substances on the cord stump were also reported by some participants.

CHX cord stump cleansing was an acceptable and feasible intervention and could be easily applied by family members after some initial training. The second study revealed that mothers and health service providers preferred a single use dropper bottle for CHX application as compared to either a 100 ml multiple use bottle with cotton swabs or a gel tube. The third study showed that cord stump cleansing with 4% CHX soon after birth substantially reduced bacterial colonization. The large efficacy trial found that CHX cleansing resulted in a large reduction in the risk of omphalitis (Relative risk (RR): 0.65; 95% Confidence interval (CI) 0.61 to 0.70). The mortality in the dry cord care arm was lower than anticipated, and any effect of CHX on neonatal mortality was small (RR: 0.90; 95% CI 0.74 to 1.09). Any effect of CHX application on the risk of death did not differ by whether the babies were born at home (RR: 0.92; 95% CI 0.67 to 1.27) or in a health facility (RR: 0.89; 95% CI 0.70 to 1.14).

Conclusion: CHX cleansing of the cord stump, while acceptable to mothers and families markedly reduced the risk of omphalitis but this reduction did not translate into a substantial decrease in the risk of neonatal death in this East African setting.

Original papers

The thesis is based on the following papers:

I. Dhingra U, Gittelsohn J, Suleiman AM, Suleiman SM, Dutta A, Ali SM, Gupta S, Black RE, Sazawal S. Delivery, immediate newborn and cord care practices in Pemba Tanzania: a qualitative study of community, hospital staff and community level care providers for knowledge, attitudes, belief systems and practices. BMC Pregnancy Childbirth. 2014;14: 173[1].

II. Dhingra U, Sazawal S, Dhingra P, Dutta A, Ali SM, Ame SM, Deb S, Suleiman AM, Black RE. Trial of improved practices approach to explore the acceptability and feasibility of different modes of chlorhexidine application for neonatal cord care in Pemba, Tanzania. BMC Pregnancy Childbirth. 2015;15: 354[2].

III. Nangia S, Dhingra U, Dhingra P, Dutta A, Menon VP, Black RE, 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[3].

IV. Sazawal S, Dhingra U, Ali SM, Dutta A, Deb S, Ame SM, Mkasha MH, Yadav A, Black RE. 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[4].

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I would like to express my sincere gratitude to the Pemba study team. Pemba has limited educational and career opportunities and most of the people with secondary education migrate to Tanzania mainland in pursuit of better career options. This became our biggest challenge when we started the CHX trial, as we could not find formally qualified people, but I got very impressed with people's willingness to learn and work hard. The CHX trial was a very large study wherein we enrolled ~50,000 newborns. We had to run three shifts a

day to get all the data entered into the computers on the same day. It would not have been possible to successfully implement the trial without the hard work and commitment of the study team and support from the Ministry of Health, Zanzibar. I would also like to thank the mothers and babies for being part of this study and welcoming us into their homes.

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I learnt the value of hard work and honesty from my father. As a child, I could not understand why we did not have the same luxuries as some of my dad's friends had, but now, I know the reason. Thanks, Dad for teaching us to succeed against all odds with our hard work and integrity. My mother's teachings during my childhood made me realize the value of humility and selflessness - I know Mom that you are always watching us from heaven and keeping us safe. I am thankful to my siblings, adorable nephews and nieces for their unconditional love and support.

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I pray to God to bless us all in our future endeavors to save mothers and babies.

Introduction

Neonatal mortality in the context of child survival

Global

Babies are most vulnerable and prone to dying in the first 28 days of life[5,6]. Neonatal mortality (death of live born infants within the first completed 28 days of life) remain a global health concern, accounting for almost three fourth of the deaths in the first year of life and nearly half of the total under-five mortality[7,8]. There is a substantial disparity in the neonatal mortality rate (NMR) between low-income countries (27/1,000 live births) and high-income countries (3/1,000 live births)[9]. Sub-Saharan Africa and South Asia have the highest neonatal mortality estimates in the world (~28 /1,000 live births)[10], about half of these deaths occurring on the first day of life[11]. Globally, in the last decade, post-neonatal mortality has declined substantially, but the number of deaths in the neonatal period has not declined at the same rate.

Sub-Saharan Africa

Until recently, neonatal mortality estimates were based on sparse and low-quality data from surveys; had methodological restraints and lack of standardized protocols and definitions. They were accordingly inaccurate. A recently published prospective study from eleven community settings in South Asia and sub-Saharan Africa[12] showed that the pooled NMR in sub-Saharan Africa was 20/1,000 live births with substantial variations between countries. Almost 39% of all newborn deaths occur in sub-Saharan Africa[9], accounting for 8.2% of the burden of disease or 27 million years of life lost in sub-Saharan Africa[13]. The decline in number of neonatal deaths in sub-Saharan Africa has stagnated, partly due to an increasing number of births[14].

Tanzania mainland and Zanzibar

In Tanzania, although the NMR in 2015-2016 fell to an estimated 25/1,000 live births[15] compared to 40/1,000 live births in 1990[16], it is still twice that of the Sustainable Development Goal 3 target 2 of reducing neonatal mortality to 12/1,000 live births by 2030. In 2015, Tanzania was among the ten countries with the highest number of newborn deaths and stillbirths[17]. In Zanzibar, neonatal mortality constitutes almost two thirds of infant mortality[15], so reducing the risk of dying in the first month of life has a high priority.

Causes of neonatal deaths

Global

Leading Causes

The three leading causes of neonatal deaths are complications of preterm birth, intrapartum related complications (previously known as birth asphyxia) and infections[5]. More than half of the neonatal deaths occur in the first 3 days of life and two thirds with in 24-hours of birth[18]. Epidemiological data from six LMICs showed that 36% to 66% of all neonatal deaths in the study populations occurred within 24-hours of birth[19].

Until recently, global estimates for causes of neonatal deaths have been derived through statistical modeling, based on data available from demographic health surveys and vital registration systems from several countries at different levels of development and NMRs[9,10]. According to these estimates, neonates primarily die due to complications of preterm birth; and the proportion of deaths from infections and intrapartum related complications are decreasing. However, the Alliance for maternal and newborn health improvement (AMANHI) [12] study using standardized training and harmonized data collection instruments showed that intrapartum related complications and severe neonatal infections are still two most common causes of neonatal deaths.

Infections including sepsis, pneumonia, meningitis, tetanus and diarrhea account for nearly one third of the total burden of neonatal deaths globally and up to 50% in high mortality settings[7,20,21]. In LMICs, neonatal deaths attributable to infection acquired after birth usually occur in the first 7 days of life[22], while those caused by acquisition of pathogens from the environment usually occur in the late neonatal period[22]. About half of all sepsis-related deaths occur after the first week[18]. The risk of death among newborns with infections depends on many factors such as time of onset, severity of the illness, care seeking patterns and case management[23-25]. Most of these deaths can be averted with appropriate prevention and management[26,27].

Underlying Causes

Most of the neonatal deaths in LMICs occur in children of poor families, where mothers are at high risk of pregnancy complications and newborn care practices are suboptimal[28]. Poor care seeking, poorly functioning health systems, lack of access to quality care, low coverage of MNCH interventions across continuum of care, inadequate care of the small and sick babies contribute to this high and avertable death toll. Underlying causes of neonatal deaths in LMICs are difficult to ascertain, as critically ill neonates often present with non-specific signs and symptoms of disease[22]. Therefore, there is a dearth of data on risk factors, derived from high-quality population-based studies, on care seeking patterns for neonatal illness in LMICs[29,30].

Sub-Saharan Africa

Leading causes

The three most important causes of neonatal deaths in sub-Saharan Africa are infections/sepsis (37%), intrapartum related complications (34%) and complications of preterm birth (24%)[12]. Neonatal sepsis contributed to an estimated 270,000 deaths and 6 million disability-adjusted life years in sub-Saharan Africa in 2019[31]. A review summarizing 32 community-based studies reported infection-specific mortality to range from 2.7/1,000 live births in South Africa to 38.6/1,000 live births in Somalia[32].

Underlying causes

Underlying causes of neonatal deaths in sub-Saharan Africa include weak health systems, inadequate antenatal care, negative effects of certain cultural beliefs and traditions, poor living conditions, lack of education, unplanned pregnancies, girls below 15 years or women over 35 years giving birth, social deprivation and inequity[33].

Tanzania mainland and Zanzibar

Leading causes

The leading causes of neonatal deaths in Tanzania as of 2012 were intrapartum related complications (31%), preterm birth complications (25%), and sepsis (20%)[34]. Recently, the AMANHI study[12] quantified the causes of neonatal deaths in mainland Tanzania and Pemba, Zanzibar and reported that even in 2017, intrapartum related complications (mainland: 39%; Pemba: 47%) and neonatal infections (mainland: 45%; Pemba: 31%) contributed significantly to neonatal deaths.

Underlying causes

In Tanzania, some of the underlying causes of newborn deaths are home births, deliveries by unskilled attendants, lack of appropriate knowledge to handle sick babies, failure to feed newborns with colostrum, late initiation of breastfeeding, lack of exclusive breastfeeding and poor care seeking practices[35]. Other underlying factors include lack of well-defined national MNCH policies and community involvement[36].

Umbilical cord infections

The studies forming the basis for this thesis are centered on the role of CHX for umbilical cord care and its effect on the risk of umbilical cord stump infections and neonatal death. Since 99% of newborn deaths occur in LMICs, the rest of this review is focused on umbilical cord stump infections and neonatal deaths in LMICs. In this thesis, the terms "umbilical cord stump infections", "umbilical cord infections", "omphalitis", "cord infections" and "cord stump infections" have been used interchangeably.

The umbilical cord and cord stump

The umbilical cord connects the fetus to the placenta. Following delivery, the cord is cut, and the remaining stump quickly starts to dry out, harden and turn black. Colonization of the cord stump begins within hours of birth as a result of non-pathogenic microorganisms passing from mother to baby via skin-to-skin contact. Separation of the umbilical cord stump continues at its junction at the skin of the abdomen, with leucocyte infiltration and subsequent absorption of the stump[37]. Colonization with commensals is a normal physiological process and does not cause disease. The cord stump usually falls off between 5 and 15 days, but there is considerable variability in the timing of this separation[38].

Pathway to umbilical cord infections

The newly cut umbilical cord stump can be a pathway to umbilical cord infections due to colonization with pathogenic bacteria, and its compromised epithelial barrier[39]. Due to patency of umbilical vessels in the first few days after birth, pathogenic bacteria can reach the bloodstream leading to systemic infection[39] with or without clinical signs of omphalitis[40]. Umbilical cord infections can be important precursors of neonatal bacterial infections/sepis[6,37,41]. Potentially pathogenic bacteria that colonize the umbilical cord stump include those from the mother's birth canal, at the place of childbirth, and from birth attendants hands[42].

Omphalitis

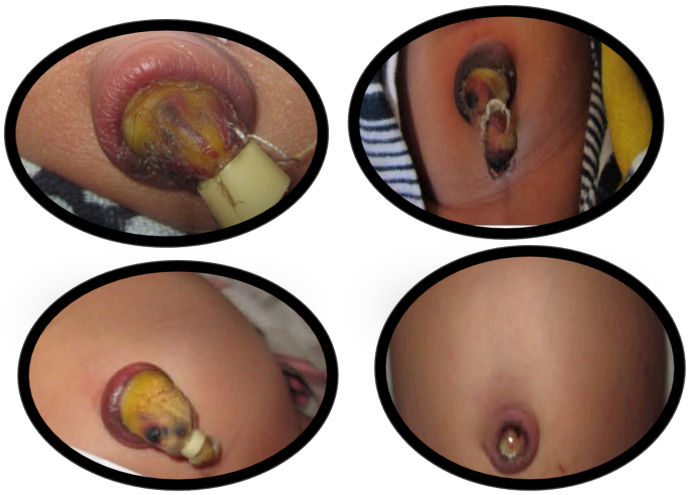
Definition, identification and epidemiology

Omphalitis is an infection of the umbilicus and umbilical cord stump or surrounding tissues characterized by purulent discharge from the umbilical cord stump (or from the navel after the cord has separated), with surrounding erythema, induration, and tenderness[43]. The discharge may have a foul smell, suggestive of infection with anaerobic bacteria. Given this variability, a sign-based algorithm was developed for community-based identification of omphalitis[44]. Omphalitis has been categorized into four severity grades (Fig 1).

Complications of omphalitis include necrotizing fasciitis, infections of the urachal remnant, abscesses, septicemia, and portal vein obstruction[45]. Neonates with severe infection usually have fever, lethargy, poor feeding, and irritability.

There is a dearth of data on the incidence of omphalitis in LMICs. Approximately 2 to 7% of infants in LMICs develop such an infection and nearly 10% of these are considered severe[46], characterized by presence of pus and redness extending more than 2 cm from the stump[47]. The case fatality risk varies based on the definition of omphalitis and ranges from 1% to 15% in hospital settings[48]. Community-based studies from Pakistan (217/1,000) and India (197/1,000) [48] have reported higher incidences. In Pemba, an omphalitis incidence of 120/1,000 newborns has been reported[49], while a study in Tanzania mainland[50] showed incidence of 17/1,000 newborns.

Fig 1: Grades of omphalitis



Risk factors

Risk factors for omphalitis include prolonged rupture of membranes, preterm labor, maternal infection and umbilical cord catheterization[45]. Intermediate or "behavioral" determinants of omphalitis are unhygienic practices around the time of childbirth that increase exposure of the umbilical cord stump to harmful pathogens[42]. These practices include lack of hand washing by birth attendants, unclean birthing surface[51,52], methods and/or materials used to cut and tie the cord[51,53-55], cord cleansing practices and application of harmful materials to the cord stump[42].

Underlying or distal determinants such as illiteracy and low education levels among caregivers may increase the chances of harmful behavioral practices[42] as illiterate mothers/caregivers may not understand educational messages regarding hygienic cord care practices. In most LMICs, caregivers may not have the decision-making power to choose good newborn care practices[56]. Other determinants can also operate at the community or regional level[57]. Social norms and pressures may prevent modification of harmful traditional care practices.

Beside these, other risk factors for omphalitis include low birth weight[58,59], male gender[60,61], and weakened or deficient immunity[62].

Causal agents of omphalitis and neonatal sepsis

There is very little information available on the microbiologic etiology of omphalitis in LMICs with substantial variation in isolates identified in hospital-based studies. Aerobic bacteria were isolated from 70% and anaerobic from 30% of omphalitis cases in Nigerian study[63]. Gram-negative organisms were more frequently identified than Gram-positive organisms (57% vs. 43%) in India[60], while 60% of the aerobic isolates were Gram-positive in Nigeria[63]. *Staphylococcus aureus* was the single most frequent isolate (28%) identified in India[60]; among 57% of cultures from newborns with signs of omphalitis in Oman[59] and among 39% of cultures performed in young infants with signs of omphalitis

in Papua New Guinea[64]. *Escherichia coli* was identified in 14% and *Klebsiella* spp. in 10% of cultures in Oman[59]; while in Papua New Guinea, *E. coli* and *Klebsiella* spp. were independently isolated in 17% of the cultures[64].

Community-based data on the etiology of omphalitis is scarce. A study in Bangladesh showed that Gram-negative organisms were commonly causing colonization of the umbilical stump. The overall proportion of Gram-negative organisms was 43%, 34%, 25% and 24% for *E. coli, K. pneumoniae, Pseudomonas* spp. and *Acinetobacter* spp., respectively[65]. In Pakistan, Mir and colleagues[48], identified *S. aureus* as the most common pathogen (52%), followed by *Streptococcus pyogenes* (18%), GBS (10%), *Pseudomonas* spp. (8.9%), *Aeromonas* spp. (3.2%), and *Klebsiella* spp. (2%). Furthermore, a review of 63 studies, including 13 that focused on community-acquired infections (sepsis, pneumonia and meningitis) in LMICs, found that *Klebsiella* spp., *E. coli* and *S. aureus* were the most common organisms isolated in the first week of life[66]. These three organisms accounted for 55% of culture positive community-acquired neonatal sepsis in developing countries[67].

In the African region, approximately half of potential pathogens causing community-acquired neonatal sepsis were Gram-positive[68]. A meta-analysis including hospital-based data from 84000 neonates in sub-Saharan Africa identified *S. aureus, Klebsiella* spp., and *E. coli* in 25%, 21% and 10% of culture positive sepsis cases respectively[69].

Association of omphalitis, sepsis and neonatal death

Trans-umbilical vein invasion of pathogens from umbilical cord infections to blood stream has been considered to be a major cause of neonatal sepsis[43]. Approximately 2 to 15% of neonates with signs of omphalitis die of a systemic infection or neonatal sepsis, if left untreated[46]. In India, among infants hospitalized for sepsis, 47% had cord infection as compared to 21% among those admitted for other reasons[60]. In Nepal[70], omphalitis defined as redness of the umbilical cord stump extending to abdominal skin was associated with 43% higher odds of sepsis mortality.

Preventive strategies for reducing the risk of omphalitis and associated neonatal sepsis deaths in LMICs

In LMICs, appropriate interventions are needed to reduce the risk of umbilical cord stump infections and associated neonatal sepsis deaths. Traditionally, a wide range of substances are applied to the cord to promote healing and prevent cord infections[71]. In developed countries, various antibiotics and antiseptics, including alcohol, silver sulphadiazine, iodine, CHX, triple dye, and gentian violet[72] had been applied on the umbilical cord stump in the past. Due to low prevalence of infections and hygienic childbirth and newborn care practices in these countries, the preventive association of antiseptics and sepsis/mortality could not be substantiated. Based on lack of evidence of antiseptic use, WHO recommended dry cord care[37]; soap and water in case of visibly soiled cord. In LMICs, however, the incidence of umbilical cord infections is high, as many babies are born at home under unhygienic conditions and in many cultures, harmful substances are applied to the cord. In these countries, umbilical cord care using topical antiseptics could potentially reduce the incidence of omphalitis and newborn sepsis by preventing or reducing umbilical cord stump colonization by pathogenic bacteria. CHX, a broad

spectrum and inexpensive topical antiseptic with strong residual activity and proven safety record has been proposed as an acceptable, feasible, and cost effective intervention for reducing the risk of omphalitis and neonatal deaths in LMICs[47,73,74].

Evidence for CHX cord care until publication of studies from sub-Saharan Africa

Three community-based cluster RCTs in Asia[75-77] evaluated the impact of 4% CHX applied to the umbilical cord stump. These studies estimated the effect of multiple CHX applications (for 7-14 days daily) or single CHX application soon after birth on the risk of omphalitis and neonatal death. The first large cluster RCT in Nepal[78] showed a 75% relative reduction in the risk of severe omphalitis (incidence rate ratio: 0.25; 95% CI 0.12 to 0.53) and a 24% relative reduction in the risk of neonatal death (RR: 0.76; 95% CI 0.55 to 1.04). A second trial in Pakistan[76] used a factorial design to evaluate daily CHX umbilical cord cleansing over 14 days and hand washing[76]. There was no impact of hand washing on either the incidence of omphalitis or on neonatal mortality, but there was a substantial reduction in both the incidence of omphalitis (RR: 0.58; 95% CI 0.41 to 0.82) and in neonatal mortality (RR: 0.62; 95% CI 0.45 to 0.85) in the CHX arm. In a three-arm cluster RCT in Bangladesh[77], the investigators compared dry cord care, a single application of 4% CHX (soon after birth), and multiple applications (up to seven) of CHX to the cord stump. In comparison to dry cord care, a single application of CHX reduced the risk of neonatal death by 20% (RR: 0.80; 95% CI 0.65 to 0.98), but the effect on severe omphalitis (RR: 0.77; 95% CI 0.49 to 1.48) was imprecise. On the other hand, daily application of CHX for 7 days reduced the risk of severe omphalitis by 65% (RR: 0.35; 95% CI 0.15 to 0.81), but did not have an impact on neonatal mortality (RR: 0.94; 95% CI 0.78 to 1.14). Based on the evidence from these three community-based cluster RCTs, WHO updated its cord care guidelines and recommend CHX application to the umbilical cord stump for home births in high NMR settings (≥30 neonatal deaths/1,000 live births)[79].

A systematic review[80] on topical umbilical cord care in 2013 pooled data from these three community-based cluster RCTs[75-77] and concluded that CHX application on the cord reduces neonatal mortality by 17% (pooled RR: 0.83; 95% CI 0.74 to 0.94). A cochrane review[72] later in the same year reported that CHX cord care reduced neonatal mortality by 23% (pooled RR: 0.77; 95% CI 0.63 to 0.94) and omphalitis by 27–56%, depending upon the severity. In 2015, Sinha *et al*[81] updated the earlier cochrane review and included two additional studies in which CHX was applied in a hospital setting[82,83]. Of the 5 studies included in the review for estimating the effect of newborn cord cleansing on neonatal mortality and omphalitis in hospital and community settings, 4 were from high NMR settings from LMICs[75-77,84] while a hospital-based study in Europe had lower NMR and omphalitis incidence[82]. The authors in their review concluded that CHX skin or cord care in community settings could lower the incidence of omphalitis by 50% and neonatal mortality by 12%. The review also concluded that CHX may possibly reduce the risk of omphalitis or infections in hospital settings (RR: 0.48; 95% CI 0.28 to 0.84). All these systematic reviews failed to address post-randomization exclusion of some neonates in community-based cluster RCTs.

In 2016, Sankar *et al*[85] carried out a meta-analysis to provide updated estimates using

intention-to-treat analysis. They included four community-based cluster RCTs and two hospital-based trials[75-77,82,83,86] in their analysis. Of these six studies, only four provided data on NMR; three community-based and one hospital-based study from South Asia. Pooled estimates from three community-based trials[75-77] using intention-to-treat analysis showed a significant reduction in NMR after CHX application (pooled RR: 0.86; 95% CI 0.77 to 0.95) while the small hospital-based study[83] showed a statistically very imprecise effect (RR: 0.11; 95% CI 0.01 to 2.03). Omphalitis incidence data was provided by 5 studies and the review showed an overall substantial (almost 30%) and precisely estimated beneficial effect.

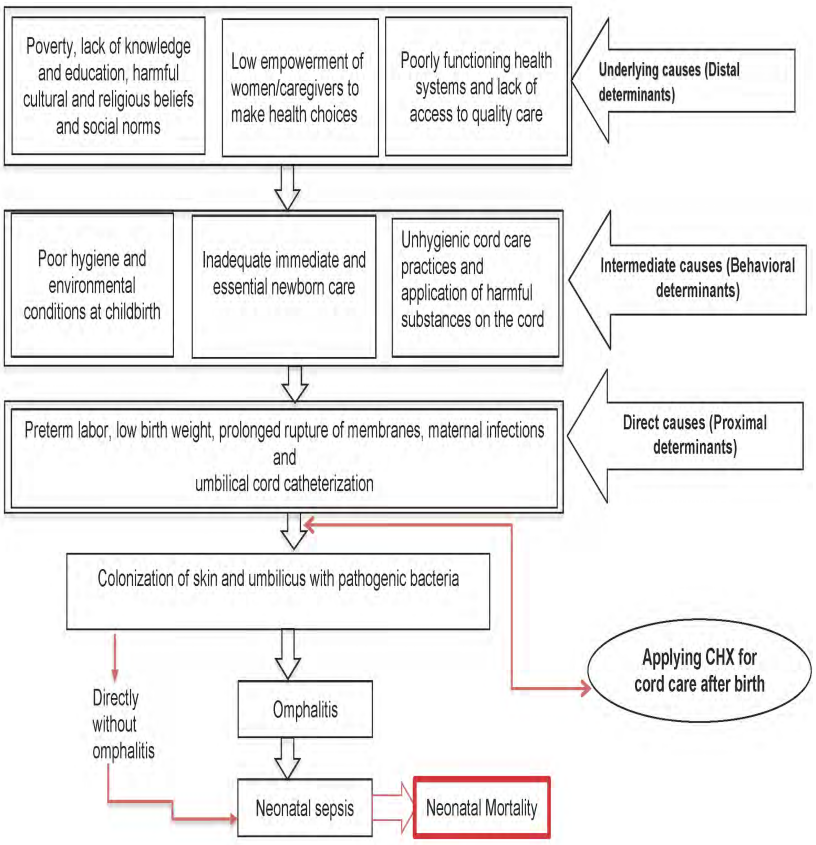
Evidence for CHX cord care from sub-Saharan Africa

There was no evidence on the effect of cord cleansing with 4% CHX on omphalitis or neonatal death in sub-Saharan Africa until 2016. All three studies in Asia[75-77] had several methodological weaknesses and questions were raised regarding the analyses of the data they generated[85]. Given these limitations and the lack of data on the effect of CHX cord care on the risk of omphalitis and neonatal death in Africa, we undertook an individually randomized controlled trial in Pemba, Tanzania. The purpose of this trial was to provide empiric data on the efficacy of CHX cleansing of the umbilical cord stump on the risk of omphalitis and neonatal death to inform the national, regional and global policy on cord care.

A cluster RCT was undertaken in parallel with the Pemba CHX trial in the southern province of Zambia[87]. The Zambia[87] study did not find any difference in NMR between the CHX arm (15.2/1,000 live births) and the dry cord care arm (13.6/1,000 live births; RR: 1.12; 95% CI 0.88 to 1.44). The Zambia trial collected data only on severe omphalitis and reported statistically imprecise and non-significant (i.e. $p \geq 0.05$) effect (RR 0.73; 95% CI 0.47 to 1.13). An individually randomized controlled trial is currently underway in Uganda[88] to evaluate the effect of umbilical cord cleansing with a single application of 4% CHX at birth on the risk of probable serious bacterial infection and omphalitis.

Conceptual Framework

Fig 2: Conceptual framework based on United Nations Children's Fund model for identifying the causes of maternal and newborn illness and death



This thesis builds on the conceptual model proposed by United Nations Children's Fund[89] for identifying the causes of maternal and newborn illness and death (Fig 2). This framework highlights that maternal and newborn health outcomes are largely determined by a number of interconnected causes, including direct causes (proximal determinants) operating at the individual level; underlying causes (distal determinants) operating at the household, community and district levels and intermediary causes (behavioral determinants). Factors at one level may have cascading effect on the other level. Preventable neonatal deaths result from poor maternal health, inadequate antenatal care, inappropriate management of complications during pregnancy and childbirth, poor hygiene during delivery, and lack of or inadequate immediate and essential newborn care[41]. We adapted the United Nations Children's Fund model to conceptualize the individual, underlying and intermediary behavioral factors related to childbirth, newborn and cord care beliefs and practices that could contribute to increased risk of cord infections and hence neonatal death[75-77]. Based on this evidence, we hypothesized that identification of existing beliefs and practices at the individual, household and community level will help facilitate introduction of CHX for the care of the umbilical cord stump, CHX would reduce the risk of omphalitis and thus neonatal sepsis, reduction in risk of omphalitis and sepsis would translate into a lower neonatal mortality.

Study aim and objectives

Overall aim

To evaluate the acceptability and estimate the efficacy of cleaning the umbilical cord stump with 4% CHX solution on the risk of omphalitis and neonatal death. This overall aim was addressed in four phased studies.

Specific Objectives

Study I

To describe the knowledge, attitudes and practices around childbirth, immediate newborn and umbilical cord care and understand facilitators and barriers to the introduction of CHX cord stump cleansing in Pemba, Tanzania.

Study II

To evaluate the feasibility, acceptability and compliance to the various modes of CHX application on the umbilical cord stump in Pemba, Tanzania.

Study III

To evaluate the effect of 4% CHX cord cleansing solution on bacterial colonization of the umbilical cord stump in a hospital and a community setting in New Delhi, India.

Study IV

To evaluate the efficacy of daily 4% CHX cord stump cleansing until 3 days after cord fall on the risk of omphalitis and neonatal death in Pemba, Tanzania.

Study subjects and methods

Study area

Study Population - Pemba

The CHX trial was carried out in Pemba, the smaller of the two main islands of the Zanzibar archipelago, lying off the east coast of Africa in the Indian Ocean (Fig 3). Pemba is divided into 117 Shehias, each made up of 3-5 small villages under a common leader, or Sheha. There are 4 districts: Chake Chake, Pemba's administrative capital; Wete, in the north, is the largest town on the island; and Mkoani, in the south, is the main port; Micheweni has mainly a rural and poor population. The main language is Swahili.

The islands are ruled by the Revolutionary Government of Zanzibar, and are a semi-autonomous region of the Republic of Tanzania. The Pemba Island is ~1360 square kilometers. Over 99% of the population are Shirazi Muslims and reside in an estimated 70,000 households on the island. The literacy rate of the population is 52%; there are 8,500 to 9,000 births each year, about 40% of these births take place at home. NMR in Pemba has declined from 25/1,000 live births in 2010[16] to 18/1,000 in 2017[15]. It is estimated that 12% of the children are acutely malnourished and ~ 69% of children between 6-59 months are anemic[16]. On Pemba Island, spices like cloves, pepper, cinnamon and others are grown in large quantities and are a key source of income. Other important cash sources are seaweed farming, aquaculture and remittances from abroad. Tourism is limited to a few hotels catering mainly to scuba divers. Farming and fishing are vital for most peoples' survival.

Fig 3: Map of Pemba and Zanzibar

Healthcare infrastructure

Ministry of Health, Zanzibar has the sole authority over healthcare service provision to the Pemba population. Pemba is divided into four health districts (same as the political administrative districts): Chake Chake, Wete, Mkoani and Micheweni. Healthcare services are organized into three levels; primary, secondary and tertiary healthcare (Fig 4).

Primary health care: The lowest level of healthcare services includes level 1 primary healthcare units (PHCU), level 2 PHCU+ and primary healthcare centers (PHCC). Level 1 PHCU services focus on basic health care, maternal and childcare, outreach and health education services, immunization, water and sanitation efforts. Level 2 PHCU+ include laboratory facilities and dental services in addition to services offered by level 1 PHCU. The PHCCs are small hospitals (also known as cottage hospitals) serving as the immediate referral centers in the rural areas that are far from referral facilities. PHCCs provide in-patient basic medical and surgical care, emergency obstetric services, psychiatric and ambulance services. On Pemba Island, there are 58 PHCUs and one PHCC.

Secondary health care: This is the second referral level including four district hospitals (Wete, Chake Chake, Mkoani, Micheweni) with 8-120 beds approximately.

Tertiary health care: This comprises Mnazi Mmoja Referral and Teaching Hospital, in Unguja Island. The hospital has approximately 580 beds and provides referral services to the entire population of Zanzibar.

The four district hospitals and a cottage hospital provide outpatient and inpatient health services in Pemba. At the community level, many government health centers provide basic MNCH services. Other community-based health care providers include trained or untrained TBAs at the village level and a number of over-the-counter drug outlets and traditional healers.

Our research group has been conducting research on Pemba Island since year 2000. During these years, a number of census sweeps have been conducted; each household in the island has been given a unique identification number, digitized and the information has been incorporated into an electronic database.

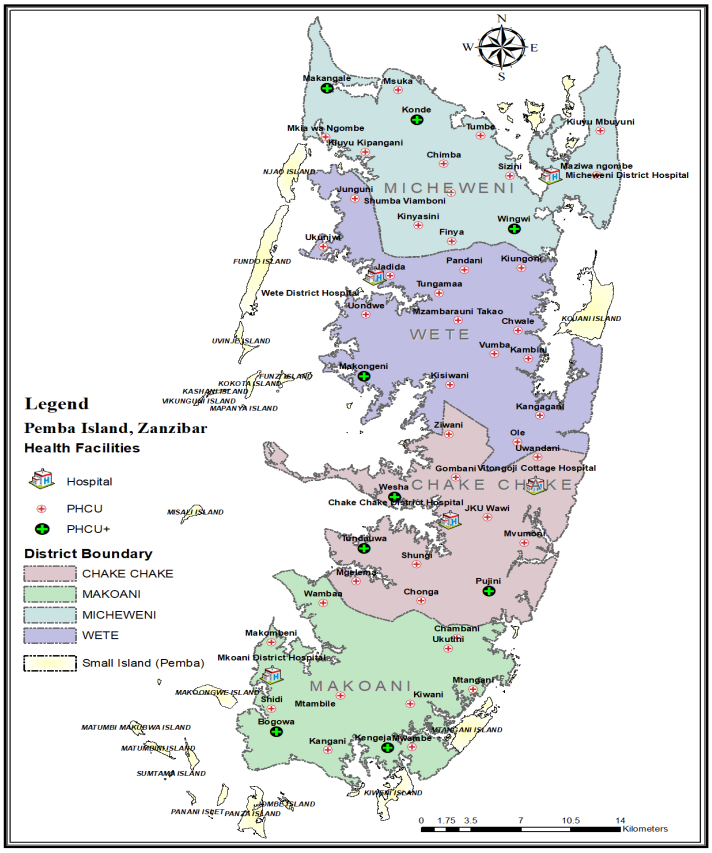
Study Population - New Delhi

Kalawati Saran Children's Hospital in New Delhi is one of the largest tertiary childcare hospitals in India and provides free pediatric services to poor families. There are approximately 375 beds and 40-60 women deliver at the hospital every day**.**

Sangam Vihar is a peri-urban resettlement colony in south Delhi mainly inhabited by migrant families from the neighboring states. There are two Delhi government dispensaries and five primary urban health centers in Sangam Vihar providing reproductive and child health services. Our research team has been working in Sangam Vihar since 1995 and provide free outpatient services to the community. Research team maintains strong linkages with frontline health workers (accredited social health activists, aanganwadi workers, auxiliary nurse midwives) implementing Government

sponsored MNCH policies and local TBAs providing informal MNCH services to the community.

Fig 4: Hospitals and Health facilities on Pemba Island



Methods

Detailed study procedures and implementation strategies for each study contributing to this thesis are described in the 4 published papers[1-4]. A brief description of the methods and implementation of the studies is provided below.

Study I

This was a qualitative study involving 80 IDIs with mothers, grandmothers, and TBAs. The aim of this study was to understand people's experiences, perceptions, opinions, knowledge and practices[90] regarding childbirth, immediate newborn and umbilical cord care with a focus on introduction of CHX cord stump cleansing. In addition, we undertook 11 FGDs with mothers, grandmothers, fathers, maternal child health workers (MCHWs), TBAs and staff from the four hospitals, PHCUs to complement and triangulate the information collected during IDIs.

Tabular Description

Study site	Pemba, Tanzania
Study design	Qualitative
Study participants	Hospital staff, PHCU staff, MCHWs, registered and unregistered TBAs, mothers, fathers and grandmothers
Sampling	Mixed method sampling[91] (combination of purposive and random sampling) to select participants
Qualitative research methods	IDIs and FGDs
Analysis	Thematic approach to identify patterns of meaning across text and coding into broad themes. Triangulation of data using multiple data collection methods and categories of informants

Study Procedures and data collection

Before the start of the study, I organized a ten-day training session for health workers who had earlier participated in data collection for a qualitative study[92] at PHL-IdC. During the training session, health workers were introduced to the purpose of the research, and given demonstrations on field and interviewing procedures. This was followed by mock interviews. To reinforce the training, interviewers conducted practice field interviews; each of the workers interviewed 3 women using the local language. These pilot interviews were transcribed and translated into English by independent language translators. Based on the feedback, the IDI and FGD guides were finalized.

Data collection

The IDI and FGD processes are explained in detail in Paper I[1]. All interviews were held in the interviewee's household at a convenient time in the local language. All the participants contacted by the study team participated in the study. IDIs and FGDs involved open-ended questions and focused on topics like i) preparations for childbirth, ii) factors influencing decision to give birth at home or in a health facility, iii) childbirth and immediate newborn care practices, iv) traditional practices of umbilical cord care and v) suggestions and concerns for introduction of CHX for cleansing the cord stump.

We audio recorded all the IDIs and FGDs; transcribed and translated the recordings and field notes on the same day. I went through the transcripts every evening and interacted with the interviewers and the field team daily to share their experiences in the field. After the data collection was complete, I read and reread all transcripts to familiarize myself with the data. I made a separate summary of each transcript, outlining the key points participants made in response to the questions. Based on initial participant's responses, we made few changes to the IDI and FGD guides in the beginning, but it was not necessary to make continuous changes.

Qualitative data analysis

Once I had reviewed all the transcripts and field notes and developed a general understanding of the scope and context of key experiences, all the interview transcripts were exported to Atlas.ti qualitative data analysis software (ATLAS.ti: Scientific Software Development Version 6.2, Berlin, Germany), where the data was both deductively and inductively coded. Keeping the research questions in mind and after a thorough review of the literature, I developed a codebook under the supervision of Joel Gittelsohn (expert in qualitative research from Johns Hopkins University), re-familiarized myself with the dataset, read the transcripts and notes several times and added more codes to the codebook. Two investigators (me and Shilpi Gupta) independently applied codes to all the transcripts; grouped similar meaning codes into categories. We examined these categories for a significant or interesting pattern[93] to identify broad themes. These broad themes were further refined into more specific themes. Both Shilpi and I are public health researchers involved in maternal and neonatal health and I have been working in Pemba for a long time. My prior interactions and experience with the community guided the design and analysis of this study.

Study II

Study II used TIPs to evaluate the feasibility, acceptability and compliance to the various possible modes of CHX application.

TIPs represent a participatory formative research approach used to evaluate and refine potential health interventions using a piloting approach before introducing them in an intervention study[94]. For TIPs, health researchers select a small number of individuals or households from the target population to try out several practices, and then discuss the pros and cons of each practice. In the present study, we adapted TIPs to explore the feasibility, acceptance and compliance to the three possible modes of CHX application for cleansing of the umbilical cord stump, A: 100 ml bottle with cotton swabs, B: 10 ml single use dropper bottle and C: 3 g single application squeeze tube containing gel.

Tabular Description

Study site	Pemba, Tanzania
Study design	Randomized three period cross-over design
Study participants	Mother-newborn pairs
Enrollment	Both hospitals and community
Modes of intervention	A: 100 ml bottle with cotton swabs, B: 10 ml single use dropper bottle, C: 3 g single application gel tube
Randomization	Separate randomization schedule for hospitals and for the community. Mother-newborn pair randomized to one of the six possible randomization sequences (ABC, ACB, BAC, BCA, CAB, CBA)
Intervention application	Each delivery mode was used for 3 consecutive days
Intervention provider	Trained TBAs/ hospital staff/MCHWs /community health workers (CHWs) demonstrated the cleansing method of application at enrollment, day 4 and day 7 and mothers/caregivers used this approach on days 2, 3, 5, 6, 8 and 9.
Visit schedule	4 Visits – enrollment, days 4 and 7 and final assessment visit on day 10
Analysis	Descriptive statistics (frequencies, percentages, means and standard deviation). Convenience and preference scores were calculated to assess feasibility, acceptance and compliance for each method. Analysis excluded 27 mother-newborn pairs not available for final assessment visit

Study Procedures and data collection

The study team held training sessions at PHL-IdC to train the MCHWs, TBAs, hospital staff and CHWs on the three different modes of applying the CHX preparation. Paper II provides study procedures for hospitals and community enrollment, randomization, follow-up and the final assessment visit[2]. We enrolled 204 eligible mother-newborn pairs from the maternity ward of the four main hospitals and the community in Pemba. We requested mothers/caregivers, TBAs, hospital staff, MCHWs and CHWs to try the 3 different modes of CHX application. Each delivery mode was used for 3 days and the enrolled mothers had an opportunity to use all the three modes of CHX application. The order in which each mode was used was random. Mothers were interviewed on day 10 to record their overall experience with the different modes of CHX application using structured interviews. They were asked to rate each application method in terms of convenience and preference. MCHWs, TBAs, hospital staff and CHWs were also interviewed and their experience and feedback recorded.

Study III

This study was an RCT among babies born at Kalawati Saran Children's Hospital and babies born at home in a community in Sangam Vihar, New Delhi, India to evaluate the effect of the 4% CHX solution formulated for the RCT described in Study IV on the bacterial colonization of the umbilical cord stump.

Tabular Description

Study site	New Delhi, India
Study design	RCT
Study participants	Newborns enrolled within 24-hours of birth
Intervention	CHX
Controls	Placebo Dry cord care
Frequency of intervention application	Twice: Soon after birth following collection of baseline swab and 24-hours after first application
Intervention provider	Trained hospital and study team members
Outcome	Bacterial cord colonization and colony counts
Outcome assessor	Dr Dang's medical diagnostic center analyzed swab cultures and provided microbiology reports
Frequency of outcome assessment	Enrollment, 2- and 48-hours thereafter

Training and reliability

In two full and 3 half-day training sessions I apprised the CHWs, supervisors and hospital staff of the study protocol and the data collection tools. In these training sessions, I used a doll to demonstrate the swab sample collection from the umbilical cord stump as well as how to clean the stump using the assigned method. We undertook two pilot sessions to ensure an effective implementation of the study procedures.

Study procedures and data collection

Details of recruitment, enrollment, randomization, masking, umbilical cord stump swab collection and swab culture analysis are provided in Paper III[3]. We enrolled 247 newborns from the hospital and 79 from the community within 24-hours of birth. We randomized the babies to one of the three arms: CHX, placebo or dry cord care. Hospital doctors/study team members collected umbilical cord swabs at baseline, 2-hours and 48-hours after the first application of CHX/placebo. In the dry cord care arm, no intervention was applied, but swab sample collection timing was same as in the CHX and placebo arms. Immediately after collection, umbilical cord swabs, which had been inserted in Amies charcoal transport medium, were put in a cool box. The cool box was transported to Dr Dang's medical diagnostic center in New Delhi where they were received within 4-hours for bacterial culture. The box was packed with frozen gel packs to maintain an inner temperature of 2-8°C. Dr Dang's lab implemented the following standard operating procedures (SOPs) for swab culture analysis.

Sterile disposable loops were used to streak the specimen on McConkey Agar, Blood Agar and Chocolate Agar plates. The plates were incubated at 37°C for 24-hours and checked for bacterial growth. Plates without growth were further incubated for one more day. Recommended American Type Culture Collection strains were used for quality control for each batch of plates. Smears were prepared from distinct colonies obtained from each of the media plates and were heat-fixed before Gram staining. Bacteria were classified into Gram-negatives and Gram-positives using microscopy-based evaluation of color and morphology of bacteria. Gram-negative and Gram-positive identification cards were used for species identification using Vitek 2 compact system (BioMerieux, Craponne, France) according to the manufacturer's instructions. For colony counts, the lab scientists picked up the neat specimen with a sterile loop and first diluted it in 1 ml of sterile saline, followed by three 10-fold consecutive dilutions and sub cultured them using standard techniques[95]. Microbiology reports were collected from the lab and entered into the computer using in-house data management system.

Study IV

Between May 2011 and Aug 2014, we carried out an individually randomized control trial among 47,545 babies born in hospitals and in the community on Pemba Island to evaluate the efficacy of daily CHX cord stump cleansing until 3 days after cord fall on the risk of omphalitis and neonatal death.

Tabular Description

Study site	Pemba, Tanzania
Study design	Individually randomized control trial
Study participants	Newborns contacted within 48-hours of birth
Intervention	CHX
Control	Dry cord care
Frequency of intervention application	Daily until 3 days after cord fall
Intervention provider	Hospital staff (for births in hospitals) and MCHWs (for births in community) on days 0, 1, 4, 10 and mothers/caregivers on days 2, 3, 5, 6, 7, 8 and 9
Outcome	Omphalitis and neonatal death
Outcome assessor	Study team
Frequency of outcome assessment	Days 0, 1, 4, 10 and 28

Training and reliability

The investigator team held three full day training sessions at PHL-IdC to explain the purpose of the study and the study protocol to the study team members and MCHWs. All data collection instruments were discussed, and the swab collection procedure was demonstrated on a doll. Separate training sessions were organized for omphalitis case detection. The MCHWs and study team members were shown slides with pictures of umbilical cord stumps with varying degrees of redness, swelling, presence or absence of pus discharge. They were trained to recognize the signs and score each sign based on severity. Exercise sets showing photographs with various signs were given to the trainees and they were asked to score each of the photographs. These exercises were

repeated until the correspondence between the master trainer and the individual trainees with a kappa of at least 0.90 was established.

Study Procedures and data collection

We published details of study methods and procedures in Paper IV[4]. Babies were screened for eligibility and enrolled after obtaining consent from the parents (in the presence of a literate witness if the parents were illiterate). This study had two phases. During the first phase of the trial (May 19, 2011 to February 19, 2013), babies were randomized to one of three arms: treatment arm using CHX, a control arm where the cleansing was done with a placebo solution, and a second control arm in which the babies received dry cord care. Based on the recommendations of the Data safety monitoring board (DSMB), during the second phase of the trial (Feb 20, 2013 to 31 August, 2014), the placebo arm of the trial was stopped and participants were randomly assigned to receive either CHX or dry cord care; participants were no longer randomized to receive the placebo solution. Baseline data and demographic characteristics were collected at enrollment. Study workers examined the cord for redness, pus, swelling, and foul odor on days 0, 1, 4, and 10. On day 28, MCHWs and study supervisors collected information on illness, hospitalizations and death.

Statistical Analyses

We designed in-house data management software to manage the data with stringent range, consistency, and logical checks. We used Statistical Package of Social Sciences (SPSS Inc., Chicago, Illinois, USA) and Stata (Stata Corp., College Station, Texas, USA) for statistical analyses.

Study II: We calculated convenience and preference scores based on mothers, hospital staff's, MCHWs, CHWs, and TBAs feedback for each of the three CHX application modes. If the participant chose an application mode as most convenient to use then the application mode was given a convenience score of '2'; if they chose it as convenient, a score of '1' was given and a score of '0' was selected if it was found to be difficult to use. The participants were also asked about their preference for each CHX application mode. A score of "2" was given to the application mode for first preference; "1" for second preference, and the non-preferred mode received a score of '0'. We presented frequencies, percentages, means and standard deviations of these scores.

Study III: The primary outcome was culture growth positivity at 2-hours and 48-hours after the first application of the solution being tested and the secondary outcome was bacterial colony counts from the umbilical and peri-umbilical region to estimate the concentration of viable bacteria. We defined culture growth positivity (proportion of swabs with positive culture) as the growth of any organism from the sample. We assessed the proportion of neonates with positive cultures 2-hours and 48-hours after first application with CHX/placebo or post enrollment in babies assigned to dry cord care. We compared culture growth positivity among babies in the CHX arm to those in the dry cord care and placebo arms. For the secondary analysis, we limited the analysis to those samples that grew bacteria and compared the density of bacterial colonization (2-hours and 48-hours of first application of solution) in the CHX arm to dry cord care and placebo arms. We also compared total colony counts (irrespective of bacterial growth) among the

three arms at baseline, 2-hours and 48-hours after first application/enrollment using boxplots.

We ran descriptive statistics (frequencies, mean, percentages) and drew histograms to understand the data distribution before running the statistical estimations. As colony counts had a right-skewed distribution, they were log-transformed. Culture growth positivity at 2-hours and 48-hours was compared (CHX vs placebo, CHX vs dry cord care) using binary logistic regression models. The independent sample t-test was used to compare the log transformed colony counts at 2-hours and 48-hours of the first application of solution/enrollment.

Study IV: The primary outcome of this study was death until day 28 after birth. The secondary outcome was omphalitis occurring any time during the first 10 days after birth. We combined the cord infection signs recorded at multiple visits and categorized omphalitis into four grades based on severity. We used intention-to-treat analysis to assess the effect of CHX on the risk of omphalitis and neonatal death. All enrolled babies were included in the analysis; in case the family moved out of the area or withdrew from the study; data available until the time baby left the study was included.

We compared individual survival times in babies allocated to the CHX arm with those of babies allocated to the dry cord care arm using Cox proportional hazards regression. We also evaluated the death rates (total number of deaths divided by the total number of participant time at risk) between the CHX and dry cord care arms using Poisson regression, the results of which were similar to those from the Cox regression model. Since it is easier to interpret rate ratios than the hazard ratios, we presented the results of Poisson regression model in Paper IV[4]. In addition to estimating overall effect on neonatal mortality, we also evaluated the effect on neonatal mortality in subgroups: births taking place in hospitals or home, boys or girls, and first application of CHX within 12-hours or later. These analyses were designed with the *a priori* objective of comparing our study results with the previously reported Asian studies. There were two separate randomization lists for hospitals-based and community-based births; therefore, the subgroup analysis by place of birth (community vs hospitals) still maintained balanced random allocations. Generalized linear model of the binomial family with a log link was used to assess the effect of CHX cleansing. This being a very large RCT with successful randomization (Paper IV: Table 1), we did not adjust for baseline characteristics.

Summary of results

Study I

We identified 4 broad themes for this study, i.e. preparations for childbirth, childbirth and immediate newborn care practices, cord care perceptions and practices, and perceptions about introduction of CHX cord stump cleansing.

Preparations for childbirth

Most of the women did not make a decision about the place of childbirth in advance. Although both mothers and TBAs considered it safer to give birth in a health facility, most of the women in rural areas preferred to give birth at home. Women's decision to give birth at home was driven by many factors like poor transportation, previous experience of safe delivery at home, fear of verbal harassment and abuse at the hospital, lack of beds and cost of medical supplies.

In preparation for childbirth, pregnant women were advised to keep gloves, a rubber sheet, plain cloth, thread and a boiled razor blade, new cloth for wrapping the baby, soap, coconut oil and some food items ready. Most of the pregnant women packed these items in a basket so that these were available at the time of childbirth. Usually families informed TBAs after initiation of labor pain; some TBAs gave pregnant women herbs (roots of rose and henna tree, bush herbs, leaves of beans tree), honey and holy water prepared by Muslim leader and massage to help them deliver quickly and easily.

Childbirth and immediate newborn care practices

In homes, women gave birth on beds covered with mackintosh (rubber sheet), mattresses or on the floor. In hospitals, women delivered on beds covered with rubber sheets. Most of the TBAs did not wear gloves or washed their hands before conducting the delivery. Whether the babies were bathed immediately after birth varied according to the training of health care providers. Hospital personnel and trained TBAs delayed babies' first bath, wiped and wrapped the baby in new cloth while untrained TBAs immediately after cleaning the baby, bathed him or her with warm water and sometimes used soap. After bathing, some of the babies were warmed using charcoal and were given coconut oil massage. Only a few trained TBAs and hospital staff advised the mother to practice kangaroo mother care and immediately put the baby on the mother's belly after birth. After discharge from the hospital, very few women practiced kangaroo mother care at home. Breastfeeding, including feeding of colostrum, was a common practice. Most of the mothers followed the TBAs advice to immediately feed colostrum after birth and to exclusively breastfeed their babies.

Cord care perceptions and practices

Most of the TBAs used sterile scissors or a new razor blade to cut the cord. They disinfected these delivery instruments by boiling them in water. The umbilical cord was usually tied with normal tailoring thread; a few of the TBAs also reported use of special thread provided by the hospitals. In the hospitals, mothers were advised not to apply anything on the cord, nearly all TBAs counseled the mothers to cover the cord with cloth to protect it from dust, flies and mosquitoes to prevent infections. TBAs usually provided home remedies when mothers sought their advice after noticing swelling, redness, pus or

bleeding around the baby's cord stump. Very few mothers reported that they applied a variety of substances like saliva, dirty door powder from old doors, hot knives, charcoal powder, pyriproxyfen powder, banana steam, fish bones etc. after the cord stump had fallen off. The mothers reported that cord stump usually fell between 3-10 days after the baby was born and was often buried thereafter.

Perceptions about introduction of CHX cord stump cleansing

Hospital staff, TBAs, MCHWs and community members emphasized that creating awareness in the community about the usefulness of CHX in cord stump cleansing was an important step before implementing CHX cord stump care. Most of the respondents were confident that, with proper training, mothers could themselves apply the CHX on babies' cord. Very few participating mothers, TBAs and health care providers reported that lack of understanding of the usefulness of CHX, its cost and religious beliefs related to umbilical cord care could be some of the potential constraints in the community for implementation of CHX cord stump cleansing.

Study II

The TIPs study enrolled 204 mother-newborn pairs; 177 pairs (87%) completed the 10 days follow-up period. Randomization of babies to three application modes (A: 100 ml bottle, B: 10 ml dropper bottle and C: gel tube) using one of the 6 possible delivery sequences was successfully balanced (Table 1).

Table 1: Randomization of Intervention sequence

Intervention Sequence	(%)
ABC (100ml 10ml gel)	17.6
ACB (100ml gel 10ml)	18.1
BAC (10ml 100ml gel)	17.2
BCA (10ml gel 100ml)	16.7
CAB (gel 100ml 10ml)	15.2
CBA (gel 10ml 100ml)	15.2

Of the 204 mother-newborn pairs, the study team was able to contact most (81%) of the mother-newborn pairs within 12-hours of birth and in 72% of the babies, the first application of CHX occurred within 8-hours. The remaining 39 babies (19%) were contacted within 24-hours after birth. The study achieved high compliance; almost all the enrolled mothers used all the three application modes. Although mothers reported little difficulty in using any of the methods, some of them thought that gel application required more effort (15.8%) and took more time to dry (7.3%). Mothers and families rated the 10 ml dropper bottle (44.6%) as their most preferred choice over the gel tube (33.9%) and the 100 ml bottle (20.9%) for cleansing the umbilical cord stump. Hospital staff, MCHWs, TBAs and CHWs also preferred the 10 ml dropper bottle (43.3%) over the gel tube (38.8%) and the 100 ml bottle (17.9%). These percentages have been incorrectly interchanged between gel and 10 ml (for MCH workers) in Paper II: Table 2.

Study III

Participants

Of the 247 newborns enrolled in Kalawati Saran hospital, 86 were randomized to cleansing of the cord stump with CHX solution, 86 to placebo, and 75 to dry cord care. In the community; 36 newborns were assigned to the CHX cord stump cleansing, 24 to placebo and 19 to dry cord care.

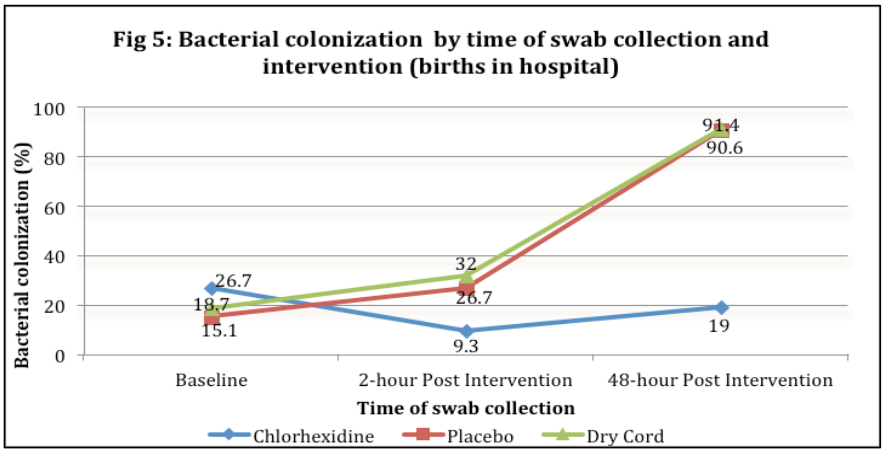
Swab collection

Of the scheduled 978 umbilical swabs, we collected 679 (69%) umbilical swabs from the hospital and 237 (24%) from the community. All the enrolled babies contributed baseline (0-hour) and 2-hours follow-up swab samples. We could not collect 48-hours swab samples from 62 neonates (6% of the scheduled) in the hospital as their families chose to leave the hospital early.

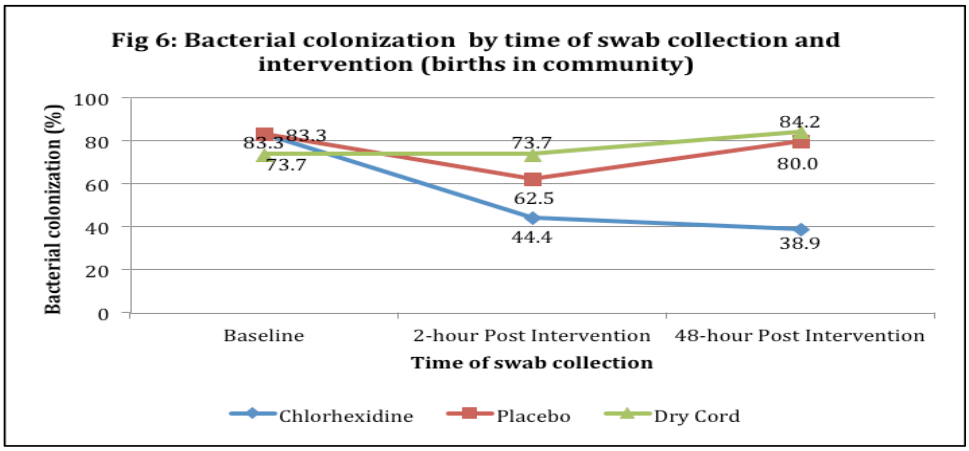
Bacterial colonization

Culture growth positivity

Hospital: A higher proportion of umbilical culture swabs collected from babies randomized to the CHX arm yielded bacterial growth at baseline (n=23; 26.7%) compared to swabs collected from babies randomized to dry cord care (n=14; 18.7%) or placebo (n=13; 15.1%). After 48-hours of the first application of the solution/ enrollment, we observed an increase in the percent of cultures with any bacterial growth from swabs collected in dry cord care arm (n=53; 91.4%) and placebo arm (n=58; 90.6%). CHX substantially reduced the proportion of swabs with bacterial growth at 48-hours follow-up (n=12; 19.0%) (Fig 5).



Community: In the community, 81% (64 of 79 swabs) of the umbilical swabs had any bacterial growth at baseline. The percent of swabs with any bacterial growth remained high in the placebo (n=16; 80%) and the dry cord care arms (n=16; 84.2%) at 48-hours follow-up (Fig 6).



Change in growth positivity

Hospital: We observed 80% lower odds (95% CI 48% to 92% decrease in odds) of any bacterial growth at 2-hours follow-up in the CHX arm compared to the placebo arm, adjusted for the growth positivity at baseline. These odds were 81% (95% CI 53% to 92% decrease in odds) lower in comparison to dry cord care arm. At 48-hours follow-up, CHX had 98% lower odds of bacterial growth (95% CI 93% to 99.8% decrease in odds) compared to both placebo and dry cord care arms.

Community: In the community, we found 53% (95% CI 84% decrease to 37% increase in odds) and 86% lower odds (95% CI 29% to 97% decrease in odds) of any bacterial growth at 2-hours follow-up in the CHX arm compared to placebo and dry cord care arms, respectively, adjusted for the growth positivity at baseline. The CHX arm had a substantial 83% lower odds of growth positivity at 48-hours follow-up compared to the placebo arm; 90% compared to dry cord care arm (Paper III: Table 4).

Colony counts

We estimated colony counts among all the swab samples and from swab samples with positive culture results to assess if CHX reduced density of colonization. Comparison of total colony counts (cfu/ml) among the three arms showed that CHX reduced the colony counts substantially at 2-hours and 48-hours follow-up (Fig 7 and Fig 8). CHX arm compared to placebo and dry cord care had lower mean colony counts (log transformed) at the 2-hours and 48-hours follow-up among swab samples with positive culture results in both the hospital and community settings (Fig 9 and Fig 10).

In the hospital, the mean bacterial colony counts (among swab samples with positive cultures) at 48-hours follow-up in CHX arm was lower in comparison to placebo [difference in log mean: −1.01; 95% CI -1.72 to -0.30] and dry cord care arms [difference in log mean: −1.16; 95% CI -1.93 to -0.39]. In the community, CHX arm compared to placebo and dry cord care arms had lower bacterial colony counts at 48-hours follow-up.

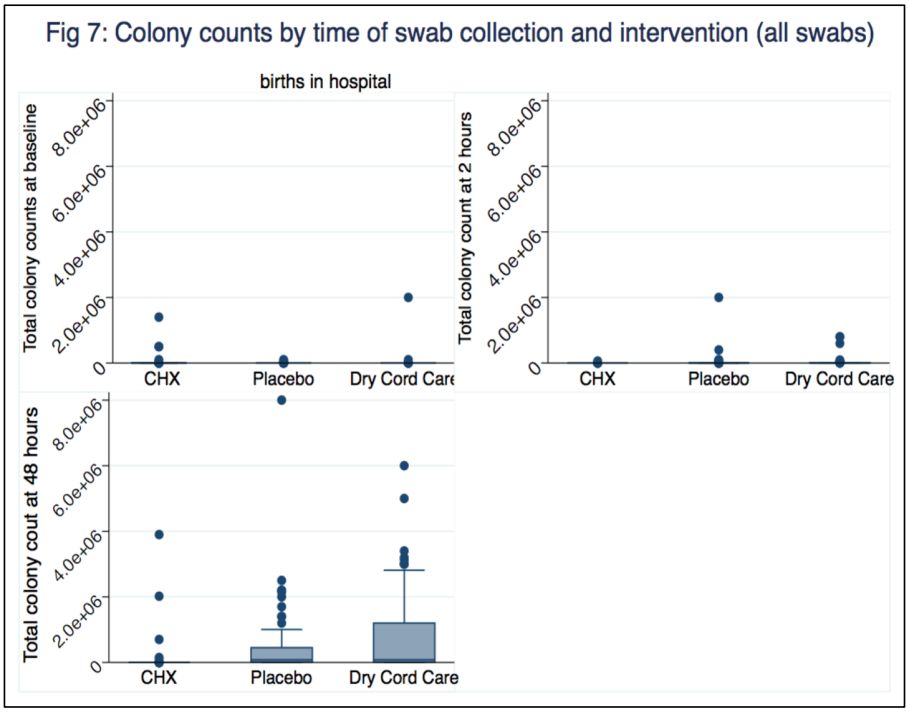


Fig 7: Colony counts by time of swab collection and intervention (all swabs)
births in hospital
Total colony counts at baseline
Total colony count at 2 hours
Total colony cout at 48 hours
0
2.0e+06
4.0e+06
6.0e+06
8.0e+06
CHX
Placebo
Dry Cord Care

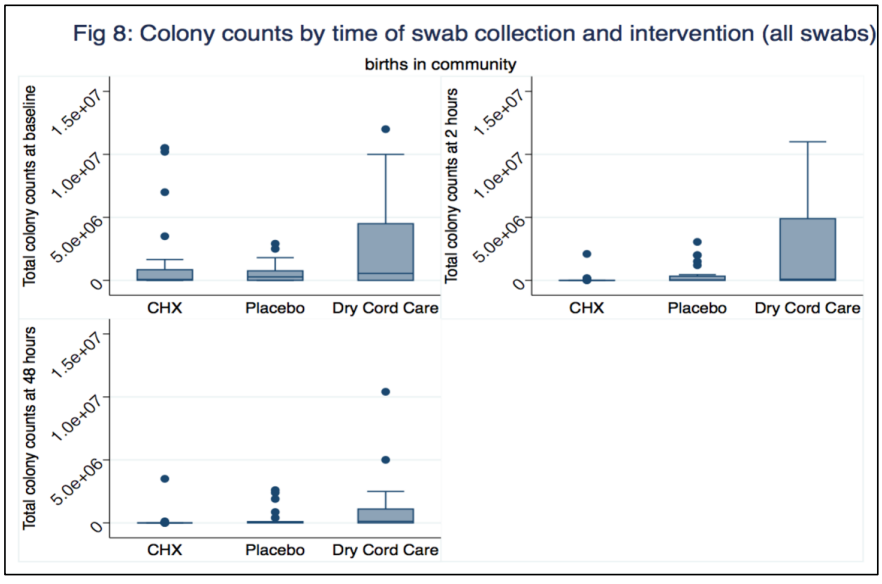
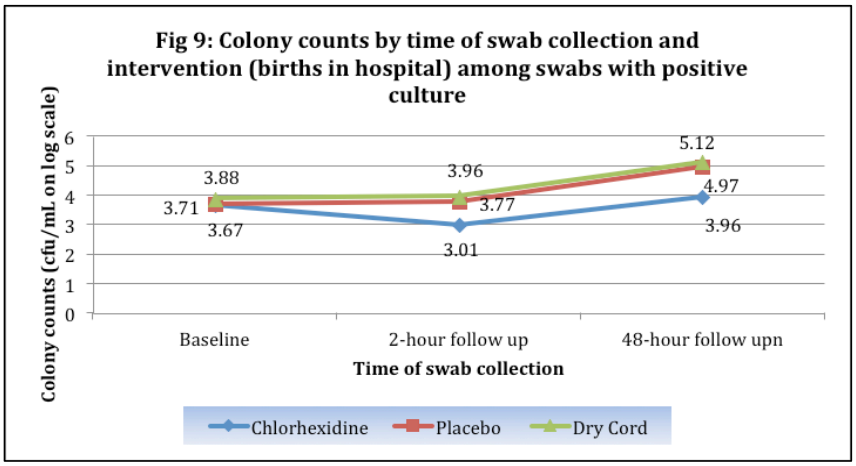
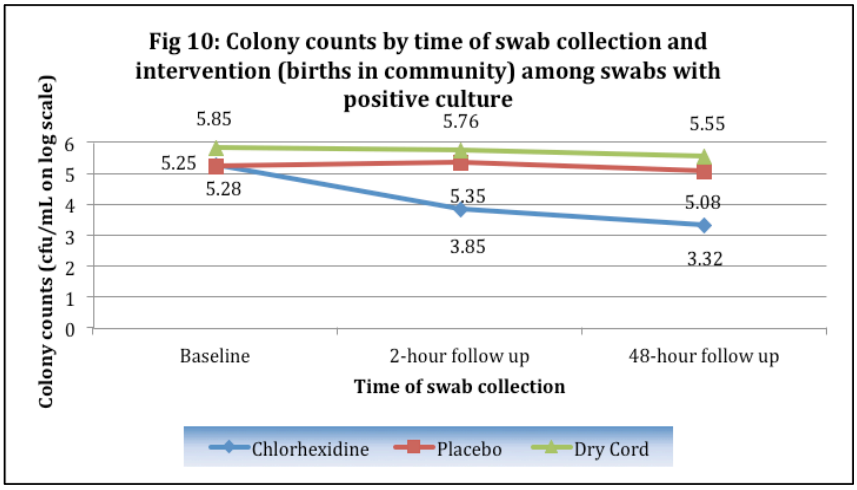


Fig 8: Colony counts by time of swab collection and intervention (all swabs)
births in community
Total colony counts at baseline
Total colony counts at 2 hours
Total colony counts at 48 hours
0
5.0e+06
1.0e+07
1.5e+07
CHX
Placebo
Dry Cord Care





Gram staining and pathogens identified

In the hospital setting, both Gram-positive and Gram-negative bacteria were identified on the umbilical cord base and stump as well as on the peri-umbilical area. Commonly identified bacteria were *Acinetobacter* spp., *Citrobacter diversus*, *Citrobacter* spp., coagulase-negative staphylococcus, *E. coli, Klebsiella* spp., *Pseudomonas aeruginosa*, *Pseudomonas* spp., *S. aureus* and *Streptococcus viridans.*

In the community, *Acinetobacter* spp., *Aeromonas spp.*, *Cedecea davisae*, *Citrobacter* spp., coagulase-negative staphylococcus, *E. coli*, *Enterobacter cloacae*, *K. pneumoniae*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *S. aureus*, *Staphylococcus lentus* and *Staphylococcus sciuri* were the most common bacteria identified.

CHX inhibited growth of both Gram-positive and Gram-negative bacteria (Fig 11).

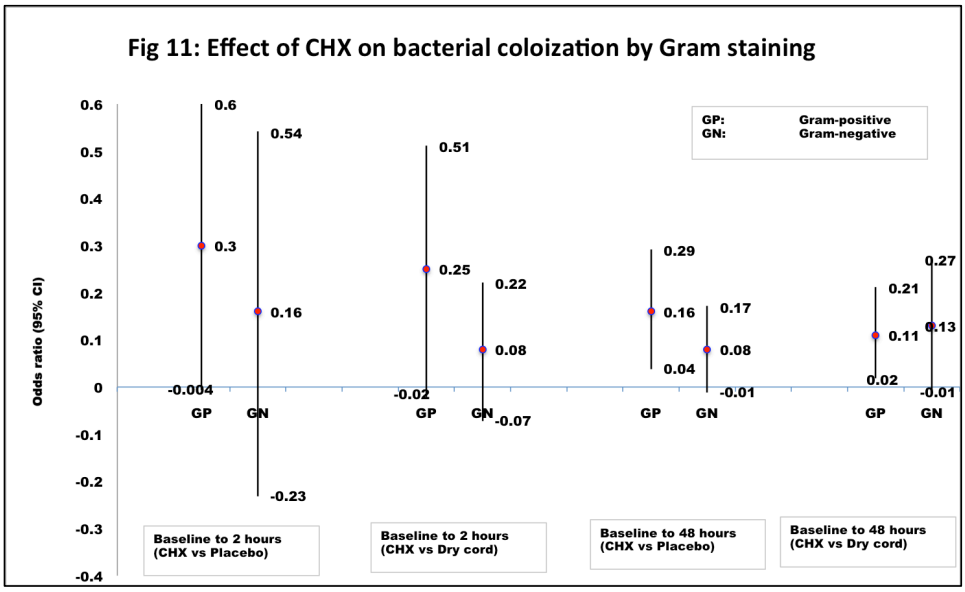


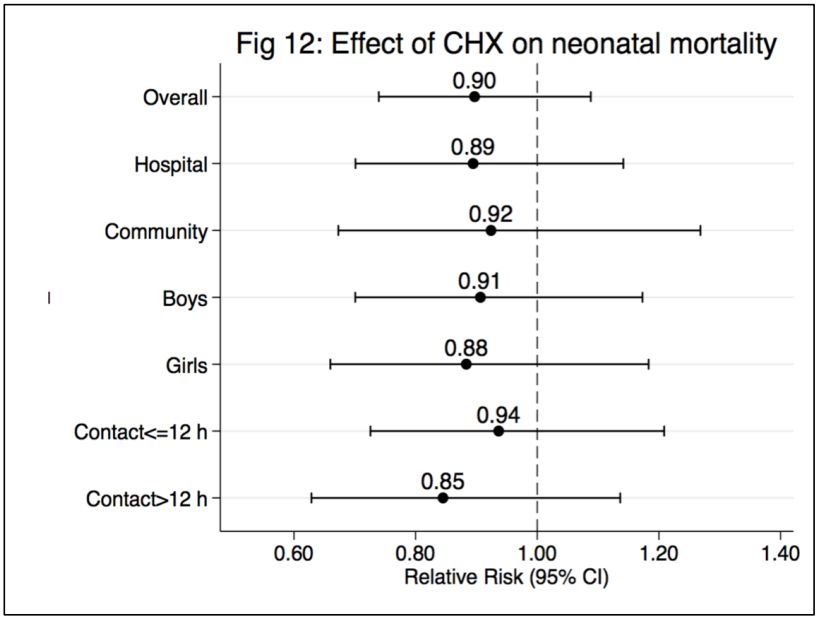
Fig 11: Effect of CHX on bacterial coloization by Gram staining
Odds ratio (95% CI)
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
GP: Gram-positive
GN: Gram-negative
0.6
0.3
-0.004
0.54
0.16
-0.23
0.51
0.25
-0.02
0.22
0.08
-0.07
0.29
0.16
0.04
0.17
0.08
-0.01
0.21
0.11
0.02
0.27
0.13
-0.01
GP
GN
Baseline to 2 hours (CHX vs Placebo)
Baseline to 2 hours (CHX vs Dry cord)
Baseline to 48 hours (CHX vs Placebo)
Baseline to 48 hours (CHX vs Dry cord)

Study IV

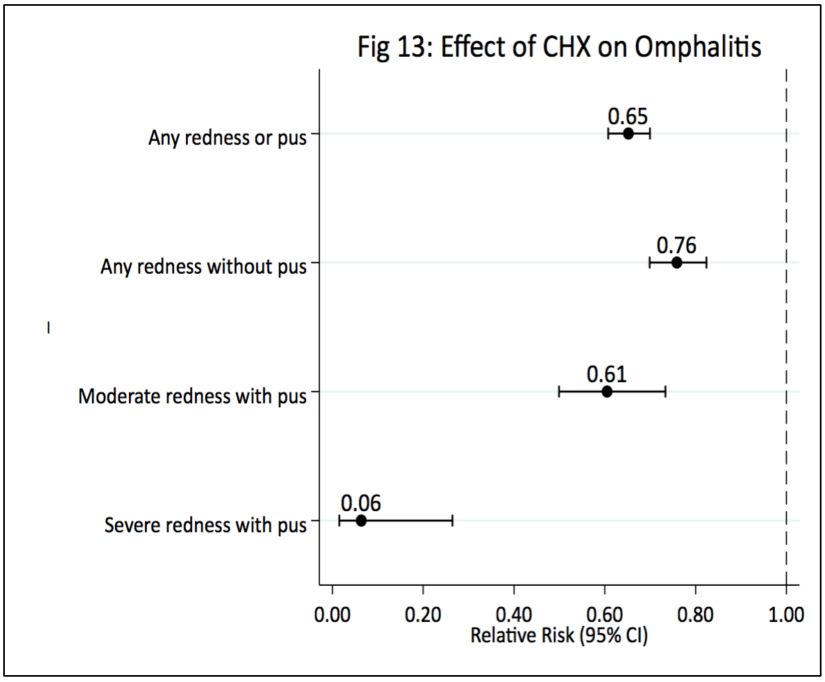
We identified 46,232 eligible babies; families of 2,000 babies did not consent, and we finally enrolled and randomized 44,232 babies into the study (Paper IV: Figure). During Phase I of the study, 22,097 babies were randomized to 3 arms (CHX: 7,292; placebo: 7,321; dry cord care: 7,484) while during phase II, 22,135 babies were randomized to either CHX cleansing (10,723) or dry cord care (11,412). Babies randomized to the placebo arm were not included in present analysis. More than half of the enrolled women (52.6%) gave birth at a health facility.

Almost 94% of babies were contacted within 24-hours of delivery and 97% completed 28 days follow-up. Baseline neonatal, maternal and household characteristics were very similar between CHX and dry cord care arms (Paper IV: Table 1). Intervention coverage was high among the enrolled babies; 98% had been contacted on all planned visitation days (Days 0, 1, 4 and 10).

Overall, we observed 221 neonatal deaths in the dry cord care arm and 189 in the CHX arm, translating to a 10% relative reduction in the risk of death among newborns allocated to the CHX arm as compared to those allocated to the dry cord care arm (RR: 0.90; 95% CI 0.74 to 1.09; p = 0.27). The effect of CHX on the risk of death was not lower in babies born in health facilities compared to those born at home; boys compared to girls, or among babies in whom CHX was applied after as compared to within12-hours of birth (Fig 12). In Paper IV, although the number of deaths when the first contact <=12-hours is reported correctly, but there was a typographical error during publication showing RR: 0.90; (95% CI 0.69 to 1.16) as against actual RR of 0.94 (95% CI 0.72 to 1.20). These estimates are updated in Fig 12.



Compared with babies in the dry cord care arm, babies in the CHX arm had 35% (95% CI 30% to 39%) lower risk of any redness in the umbilical stump or pus and a 94% (95% CI 74% to 98%) lower risk of severe redness with pus (Fig 13).



Discussion

Dry umbilical cord care has been the established recommendation for most newborns[37]. Based on the evidence from the 3 above-mentioned Asian trials[75-77], WHO updated its guidelines and recommends so that in high neonatal mortality settings, CHX should be applied to the umbilical cord stump immediately after home birth[79]. At the time of undertaking the studies described in this thesis, there was no data available from sub-Saharan Africa. This thesis work culminated in a large RCT to estimate the effect of CHX cord care on the risk of omphalitis and neonatal death in Pemba, Tanzania. We found a substantial effect on the risk of omphalitis, but not on the risk of neonatal death as seen in the Asian studies[75-77]. The findings from our study[4] and from another very large trial in Zambia [87], both in populations with low NMR, support current WHO guidelines. I concur with Osrin and Colbourn[96] in that there is no need to change current umbilical cord cleansing guidelines[79] and CHX should not be applied to umbilical cord stump of babies born in low NMR settings.

Discussion of main findings

All the evidence for CHX cord care on the risk of omphalitis and neonatal death had come from cluster RCTs undertaken in South Asia[75-77] until the findings of the two African trials[4,87] were published. The trials in Pemba[4] (Paper IV) and Zambia[87] reported efficacy and effectiveness, respectively, of CHX on the risk of omphalitis and neonatal death in sub-Saharan Africa, where the effect of CHX cord care had yet to be evaluated. In the following sections, I will discuss the findings of the Pemba trial in light of existing literature and to complement the current evidence for i) knowledge, attitude and practices around childbirth, newborn and umbilical cord care, ii) feasibility, acceptability and compliance of the various modes of CHX application, iii) effect of CHX on bacterial colonization of the cord stump and iv) the effect of CHX cord care on the risk of omphalitis and neonatal death.

Knowledge, attitude and practices around childbirth, newborn and umbilical cord care

In the study reported upon in Paper I, we wanted to understand the current practices around childbirth, newborn and umbilical cord care in the community in Pemba before introducing cleansing of the umbilical cord stump with CHX. Most of the women in the community had not heard about an antiseptic and it was important to understand the barriers and facilitators for CHX use before starting the large CHX trial.

In Pemba, a high proportion of women in urban areas preferred to give birth in health facilities while most women in rural areas still preferred to deliver the baby at home with TBAs[1]. Consistent with findings in other developing countries[97-99], participants in our study mentioned lack and high cost of transportation, poverty, high cost of medications or long distances to health facilities as main reasons for women choosing to give birth at home.

There was good compliance to some of WHO's essential newborn care recommendations[100] in that most of the babies were immediately wrapped in a new clean cloth (*Kanga)* and most of the mothers immediately fed colostrum to their babies; but

similar to other sub-Saharan African[101] and South Asian[102,103] settings, immediate bathing of newborns was a common practice in Pemba[1].

Almost all TBAs reported using a sterilized blade for cutting the cord in Pemba; this practice is different from the use of bamboo shoots in Bangladesh[56,104] and India[105] and hasiya, sickle, or a razor blade in Nepal[102,106]. In Pemba, sterilized thread was used to tie the cord. This was in contrast to findings in Zambia[107] where it was most often tied with white or black cotton knitting wool.

Umbilical cord care practices vary by country and by region; studies in Africa[108] and South East Asia[80] have reported the application of a variety of substances like shea butter, cooking oil, herbs, cow dung, ghee or saliva on the cord. Two studies in West Africa[109] showed that over 90% of the babies had something applied to the cord. Our findings are contrary to reports from other sub-Saharan and South Asian settings[71]; most of the women in Pemba practiced dry cord care, very few reported use of dirty door powder, hot knife, charcoal powder, shells, burning wood, banana steam, fish bone etc. on the cord stump immediately after cord separation[1]. Families usually want that the cord stump falls off quickly; and it seems that the application of substances is intended to shorten the time till cord stump separation.

In Pemba[1], TBAs advised mothers to keep the cord dry but when mothers sought advice for cord infections, some of the TBAs provided home remedies such as dust from the door, charcoal powder, sandalwood powder, ground seashell, polypropylene fumarate and/or talcum powder. In Zambia, bleeding of the umbilical cord or delayed cord separation worries mothers and they seek care from traditional healers[107].

In Pemba, we found that mothers and other family members were willing to accept umbilical cord stump cleansing with a CHX solution. Participating mothers, TBAs and MCHWs suggested that appropriate training and education on the use of CHX should be provided[1]. Similarly, in a study in Uganda[110], there was acceptance in the community for the application of a CHX solution on the umbilical cord stump at birth.

Feasibility, acceptability and compliance of the various modes of CHX application

The study teams in the trials in South Asia[75-77] used liquid formulations when evaluating the efficacy of CHX cleansing on omphalitis incidence and neonatal death. In Nepal and Pakistan, the solutions were packaged in plastic bottles and the study worker used a cotton ball to apply the solution[75,76]. In Bangladesh[77], the CHX solution, also packaged in plastic bottles was applied only once (soon after birth) in single cleansing areas and once daily for 7 days in multiple cleansing areas.

In an effort to enhance compliance in our trial in Pemba, we decided to find an appropriate way of applying CHX that was feasible and acceptable to the community. We provided the opportunity to participating mothers and health care providers to evaluate three different ways of applying the CHX solution and explain to us disadvantages and benefits of each. Paper II shows that mothers preferred a 10 ml single use dropper bottle compared to 100 ml bottle and a gel tube[2]. Hodgkins *et al*. reported from a study in Nepal that gel formulation was more acceptable to families than the

liquid formulation[111,112]. Contrary to these findings, mothers in our study (Paper II) reported that application of the gel on the umbilical cord stump was less convenient as an additional effort was required to apply the gel and it took more time to dry. One possible reason for this discrepancy could be that participants self-reported CHX use in the study in Nepal, which may not be reliable, while in our study, mothers were provided with log sheets to record CHX application on daily basis by simply putting a tick mark. MCHWs/study team members made follow-up visitations on days 4, 7 and 10 and recorded mother's usage of CHX by checking log sheets and counting number of unused containers.

Evaluating the effect of CHX preparation on bacterial colonization

The skin of the baby soon after birth is colonized by bacteria which can reach the blood stream through patent umbilical vessels causing systemic infection[113]. The umbilical cord stump and umbilicus is recognized as the first site of bacterial colonization in many babies after birth[114]. Most of the earlier hospital-based studies used alcohol and triple dye to reduce the risk of bacterial colonization but could not substantiate that it resulted in prevention of omphalitis, tetanus, sepsis or death[72].

In our study (Paper III), umbilical cord stump cleansing with 4% CHX substantially reduced the risk of umbilical cord colonization and the density of bacteria[3] among babies born in a hospital as well as among those born at home. Our findings are consistent with earlier findings from studies in Bangladesh[115] and Turkey[116]. In line with earlier reports from Bangladesh[65], we found that 4% CHX had immediate (2-hours after first application), extended and sustained effects (48-hours after first application) on the risk of bacterial colonization by both Gram-negative and Gram-positive organisms[3]. The mild soap and water solution (placebo) did not reduce bacterial colonization effectively, indicating that the effect of CHX is due to its antiseptic action rather than physical cord cleansing[75].

CHX cord care and risk of omphalitis and neonatal death

Our CHX trial in Pemba (Paper IV) revealed a substantial reduction in the risk of omphalitis[4], a reduction which was most pronounced for severe (94%) than for milder forms (ranging from 24% to 39%) of omphalitis. These findings are consistent with those of the earlier Asian[75-77] studies. The relative reduction in the risk of severe omphalitis in the trial in Nepal[75] was 75%; 49% in Pakistan[76] and 65% in Bangladesh[77]. The trial in Zambia[87] also reported a reduction in the risk of omphalitis, but it was very rarely observed even in the control arm, resulting in very wide 95% CI.

In our Pemba trial[4], we also found a small 10% effect against neonatal death (RR: 0.90; 95% CI 0.74 to 1.09). The comcomitant Zambia trial[87] reported 12% increased risk (RR: 1.12; 95% CI 0.88 to 1.44). Both effects were statistically non-significant ($p \geq 0.05$) but estimated with a higher statistical precision in the Pemba trial. The observed NMRs in the control arms in these two African trials were much lower than the NMRs used for sample size estimations, and they were therefore both underpowered to find the envisaged 25% effect against neonatal death.

In our trial in Pemba[4], contrary to the Asian trials[75-77], we observed a substantial prolongation in time till cord stump detachment (manuscript in preparation), and we are

in the process of estimating the association between delayed cord separation and the risk of neonatal death. There is a possibility that the delay in cord separation induced by daily CHX cleansing prolongs the exposure to contamination with pathogenic bacteria[117] and that this may increase the possibility of transmission via patent umbilical vessels without causing omphalitis. Therefore, there is a need for further analysis to fully understand the role that delayed cord falling may play in causation of omphalitis and risk of neonatal death. There is even a possibility that multiple CHX applications over time may select for resistant and more pathogenic organisms[62].

In the following paragraphs, I discuss some broader contextual and analytic issues that could potentially explain the disparity between the findings of the Asian[75-77] and the African trials[4,87].

The Asian studies were done 8 to 14 years ago and during this period, health care has improved, the risk of neonatal death due to severe infections has decreased[5]. Since CHX can only affect the risk of death due to infections, the effect of CHX on overall neonatal mortality we saw in our African trials may be lower than in the earlier Asian trials, if the lower neonatal mortality in African trials to a large extent is caused by lower risk of serious infections. However, severe infections still contribute to approximately one third of neonatal deaths in several African and Asian countries[12]. Such an interpretation is supported by our observations of substantial effects of CHX on the risk of, especially severe omphalitis.

Some of the other contextual differences between the Asian and the African trials are: i) all the three Asian studies were undertaken in populations where more than 90% of the women gave birth at home, while approximately half of babies in the African trials were born in health facilities. It has been suggested that the CHX application had a substantial effect among babies born in potentially unclean home environments. However, our study reported a similarly low efficacy of CHX cleansing among babies born at home and those born in health facilities, ii) there were differences within the Asian trials in terms of sample selection; the trials in Nepal[75] and Bangladesh[77] used a non-selective approach for enrollment while the trial in Pakistan[76] used a very selective approach restricting enrollment to home births attended by TBAs raising concerns about the generalizability of its study findings, iii) the proportion of babies born with low birth weight is higher in Asia than in sub-Saharan Africa[118]. However, it is unlikely that CHX cleansing selectively reduced the risk of death in the subset of preterm and small for gestational age babies that are at an increased risk of umbilical cord stump infections[58,59,119], which could otherwise have contributed to explain differences in effects between the Asian and the African trial findings.

The disparity between the findings could partly be explained by the choice of comparator between the Asian and the African trials. The comparator “dry cord care” is more appropriate in the African trials[4,87] (because it represents current WHO guidelines and umbilical cord care practices). The Asian studies[75-77] implemented “dry cord care” in addition to other prevalent cord care practices, thus the babies in the control arm of the Asian trials would be expected to have a higher risk of omphalitis and thus serious infection and death than the babies in the control arm of the African trials.

All the three Asian trials reported the post-intervention mortality effect as effect on NMR. The Nepal study[75] included only those newborns in the analysis that health workers were able to contact within the first 10 days of life; while the study in Bangladesh[77] included newborns contacted within 7 days of life. Attrition in two of the Asian trials[76,77] was high; ~21% in Bangladesh and ~18% in Pakistan and even if it was not differential between the intervention and control arms, it may result in a selection bias and could potentially have inflated observed effects.

There is a need to scrutinize the findings of the Asian studies with more granularity and compare these findings with those of the two African trials. Daily CHX cleansing for a week in the two Asian trials[75,76] led to moderate but statistically rather imprecise effects. Thus, the Nepal trial[75] indicated a relative mortality risk reduction of 24% with the 95% CI spanning -4% to 45%. In the trial in Pakistan[76], the effect was 26% with the 95% CI ranging from -8% to 50%. Neither the Bangladeshi[77] nor our trial in Pemba[4] found substantial effects, yet they yielded very similar effect point estimates, 6% and 10%, respectively, with 95% CI spanning -14% to 22% ($p=0.5$[120]) and -9% to 24% ($p=0.27$[4]), respectively. Provided the estimates were devoid of biases, these 95% CI depict the ranges that with at least 95% confidence capture the true population effects of daily CHX cleansing for a week. Both of these estimates were far from statistically significant but, among the 5 trials discussed here, they were the most precise, both with upper-to-lower confidence limit ratios (ULCR) of less than 1.5. In addition, taking into account the selective eligibility criteria for participation in Pakistan; and application of non-study substances to the cord stump in Pakistan (90%), Nepal (50%) and Zambia (12%), the interpretation of the findings from the remaining three trials should take into account the fact that their estimates were less precise; the trials in Nepal[4] and Pakistan[3] yielding a ULCR of 1.9, that in Zambia[87] a ULCR of 1.6.

The Nepali trial[75] revealed a 34% relative reduction in the risk of neonatal death when CHX was applied within 24-hours of birth. It regrettably seems that the investigators in the trial in Pakistan[76] overlooked the interaction effect of hand washing and combined the data from the trial arm with hand washing with that from the CHX arm, which questions the reported 38% relative reduction in risk of neonatal death compared to the combination of the data from the hand washing and control trial arms.

A series of meta-analyses[72,80,81,85] have been undertaken to review the findings of the Asian trials. Although, these Asian trials did not provide evidence for CHX cord care for babies born in health facilities, the authors of two[72,80] meta-analyses recommended scaling up of CHX cord care for all babies born in a health facility and at home. Sankar *et al*[85] were more cautious in their interpretation and concluded that there is insufficient evidence for recommending CHX intervention in infants born in health facilities and/or low-NMR settings.

Contextual and analytic differences between the CHX cord care studies in Asia and in Africa are highlighted in Table 2. This discussion seems to point to that the Asian trials[75-77] may have issues related to internal and external validity. There is a need for independent reanalysis of all the data available from the 5 trials[4,75-77,87] to correctly interpret and predict global estimates of reduction in the risk of neonatal death from CHX cord cleansing. In this context, WHO convened a workshop in Geneva and invited

investigators of all 5 community-based trials in Asia and Africa to discuss the way forward. All the participants agreed to send study data to WHO to create a combined data set and analyze data in a meaningful way. We hope that the data, review and discussion of this mantle in conjunction with the detailed meta-analysis undertaken by an independent team from the WHO, will provide an important resource to the scientific community at large and specifically to groups responsible for drafting global and national policy for care of the umbilical cord stump of neonates in LMICs.

Table 2: Current Evidence from the CHX trials in newborns –Summary of Methods and Findings					
Study Characteristics	**Nepal (2006)**	**Bangladesh (2012)**	**Pakistan (2012)**	**Zambia (2014)**	**Pemba (2014)**
Years of Study	2002-2005	2007-2009	2008-2009	2010-2013	2011-2014
Study Design	Cluster RCT	Cluster RCT	Factorial 2x2 (hand washing and CHX)	Cluster RCT	Individually randomized control trial
NMR in the comparator arm	30/1,000	36/1,000	30/1,000	14.4/1,000	15.4/1,000
Total sample size	15,123	29,790	9,741	37,856	36,911
Comparison arm(s)	Dry cord care or soap/water	Dry cord care (1 day, 7 day)	Dry cord care, hand washing factorial design	Dry cord care	Placebo or dry cord care
Frequency of CHX application	Days 1, 2, 3, 4, 6, 8, 10	Once at birth or once daily for 7 days	Daily for 14 days	Daily until 3 days after cord fall	Daily until 3 days after cord fall
Intervention provider	Project staff	Village health worker	TBA and caregiver	Mother/caregiver	MCHW on days 0, 1, 4, 10 and mother/caregiver on rest of the days
Outcome assessor	Field worker	CHW	CHW	Field worker	MCHW/study staff
Low birth weight	30%	~32%	Not reported	7%	Not reported
Preterm	Not reported	21%	Not reported	20.6%	Not reported
% home births	92%	88%	80%	35%	47%
Clean delivery kit	Yes	Yes	No	Yes	No
Home substance applied to cords	50%	6%	80-90%	7-12%	< 1%
Loss to follow-up	~4%	~21%	~18%	~4%	~3%
Intention-to-treat analysis	No Per protocol	No Per protocol	No Per protocol	Yes	Yes

Table 2: Current Evidence from the CHX trials in newborns –Summary of Methods and Findings					
Study Characteristics	**Nepal (2006)**	**Bangladesh (2012)**	**Pakistan (2012)**	**Zambia (2014)**	**Pemba (2014)**
Omphalitis definition*	Three grades: moderate or severe redness; moderate redness with pus/severe redness; severe redness with pus	Four grades: any redness; any redness or pus; moderate redness with pus/severe redness; severe redness with pus	Three grades: mild/ moderate/severe redness; moderate/severe redness; severe redness with pus	One grade: umbilical stump erythema or purulent discharge	Four grades: any redness; any redness or pus; moderate redness with pus/severe redness; severe redness with pus
Relative risk reduction in severe omphalitis (95%CI)	75% reduction (47% to 88% reduction)	Once: 23% reduction (60% reduction to 48% increase) Multiple: 65% reduction (19% to 85% reduction)	49% reduction (82% reduction to 48% increase)	27% reduction (53% reduction to 13% increase)	94% reduction (74% to 98% reduction)
Relative risk reduction in neonatal death (95%CI)	24% reduction (45% reduction to 4% increase)	Once: 20% reduction (2% to 35% reduction) Multiple: 6% reduction (22% reduction to 14% increase)	38% reduction (15% to 55% reduction) factorial analysis	12% increase (12% reduction to 44% increase)	10% reduction (24% reduction to 9% increase)
*Mild/any redness was defined as limited to the cord stump only; moderate redness was defined as redness less than 2 cm extension onto the abdominal skin at the base of the cord stump; and severe redness was defined as redness spreading noticeably (> 2 cm) outward from the base of the stump.					

Methodological considerations

In this section I discuss methodological considerations related to the design, data collection, and data analysis of the studies presented in this thesis, aspects that may have influenced the validity of our findings.

Study design

In preparation for the large randomized efficacy trial, we carried out three preparatory studies to inform us about how to best deliver the CHX intervention and implement the trial in Pemba. The KAP study[1] used a qualitative design to obtain a deeper understanding of the determinants of newborn care[121]. In the TIPs study[2], we observed the actual targeted behavior change based on real experience of mothers and health care providers with the intervention. This study provided data on actual compliance and triangulated the findings from the KAP study. We used the pilot RCT presented in Paper III to evaluate the effect of the chosen CHX formulation for the efficacy study on bacterial colonization of the umbilical cord stump.

Internal validity

Qualitative studies (KAP and TIPs)

A high level of validity or trustworthiness in qualitative research means that the tools, processes, and data[122] are appropriate for answering the research question; while credibility indicates confidence that can be placed in the research findings. Qualitative studies should describe the process of data collection and analysis in detail to enable the reader to assess the validity and credibility. This description needs to be sensitive to the context and possible bias[123]. Some of the potential sources of bias in qualitative research[124] are i) selection bias- when we use purposive and convenience sampling to select the participants based on certain common characteristics; ii) interviewer or interviewee bias- when the participants' response depends on the way the interviewer asks the question and iii) reflexivity- the choice of design, implementation and interpretation of study findings gets influenced by researcher's personal experiences, beliefs and perceptions[123]. We can improve the validity of qualitative studies[123,125] by using strategies such as triangulation, prolonged contact, member checks, saturation and detailed description of researcher's own experiences and thinking. In this subsection, I will discuss some of these strategies that we used during design, implementation and analysis of our qualitative studies:

- Contrary to the tradition in qualitative research where selection of participants based on their representativeness of the target population is not considered important; we used mixed method sampling (both purposive and random sampling) to select participants such that they were likely to represent different demographic and socio-economic characteristics of the target community. Our goal was to make the intervention more appropriate for the efficacy study (which drew its participants from the underlying population).
- We undertook interviews in participants' home and FGDs in sub-district offices to ensure that they were conducted at a time and place convenient for the participants[126].

- We worded the questions in IDI and FGD guides so that they were well understood by the participants and they could share their experiences in detail with the interviewer[127]. The guides were translated into local "Swahili" and pre-tested to ensure proper understanding of the questions.
- We used data from IDIs and FGDs to triangulate the responses, to enhance their representation of reality and were not so much affected by interviewer or interviewee bias. Triangulation helps our understanding of complex phenomena; agreement among different sources indicates high validity[123]. We interviewed both health care providers and mothers/caregivers to improve the credibility of our results.
- We provided extensive training to the interviewers and iteratively refined the IDI and FGD guides based on participant feedback. This enabled us to collect detailed and rich information from the participants.
- Saturation contributes to data adequacy and can be "operationalized as collecting data until no new information is obtained" [128]. In the KAP study, we continued to collect data until such data saturation within each of FGD groups and IDIs was achieved.
- We used both deductive and inductive thematic analysis to interpret our data. This approach allowed available literature to be integral to the process of deductive thematic analysis while allowing for themes to emerge directly from the data using new inductive coding[129].
- Participant's direct experience with the intervention in the TIPs study helped control for possible observer subjectivity.

Randomized control trials (bacterial colonization and efficacy study)

RCTs yield high internal validity of effect measures when the differences observed in the occurrence or distribution of the outcomes between the intervention and in the comparison arms are attributable only to the exposure under study. There are three main sources of bias in RCTs: information bias, selection bias and uncontrolled confounding[130].

Information bias

Information or misclassification bias in RCTs occurs when the information collected on the outcome is incorrect, leading to an error in the effect estimate[130]. *Differential misclassification* of an outcome occurs when the outcome is misclassified differentially according to a participant's intervention status e.g. when trial participants fail to adequately remember (recall bias) or report (reporting bias) the outcome to a larger or lesser extent in the intervention than in the control arm of a trial. Differential misclassification can overestimate or underestimate the true effect of the intervention. *Nondifferential misclassification* of an outcome occurs when the misclassification occurs to the same degree in the trial arms and it tends to produce bias towards the null (compromised specificity or compromised sensitivity combined with compromised specificity) or no change in the observed effect (compromised sensitivity). Nondifferential misclassification obscures the true association but does not exaggerate the effect of an intervention. We took the following steps to reduce misclassification bias:

- The scheduled number of visits and day of visitation was the same in the trial arms to reduce the likelihood of differential recall between arms.
- The investigators, study participants, study personnel and those analyzing the data were kept unaware of whether a baby was allocated to the CHX or the placebo arm of the trials. The CHX and the placebo preparations were identical in packaging, appearance, colour, consistency and smell, so it was impossible for the study team or participants to differentiate between CHX and placebo. It was, however, not possible to maintain such blinding for babies who were allocated to the dry cord care trial arm.
- Our main outcomes of the large RCT were neonatal death and omphalitis. Death, being a hard outcome, is virtually never misclassified unless a mother refuses to report on it or reports a child who lives as having died. If such misreporting is influenced by whether her child got CHX or dry cord care, the misclassification would be differential. We have no reasons to believe that even nondifferential misclassification of death occurred in our trial because our study team members were so well trained. Omphalitis reporting by health workers could be misclassified. They were, however, extensively trained to ensure high reliability and validity. Additionally, a senior team member visited all babies with suspected cord infections to confirm omphalitis and if in doubt, a digital photograph of the possibly inflamed/infected cord stump was sent to the senior lab scientist specially trained to identify cord stump infections based on presence of redness, pus and swelling.

Selection bias

In RCTs, selection bias can occur if the investigators knowingly alter the assignment of subjects to treatment or control arm or if there is a stretch of allocations to one trial arm during a period in which the risk of the outcome is enhanced or reduced. Selection bias can threaten the randomization process and lead to biased treatment effects and misleading conclusions. Selection bias can also result from substantial loss to follow-up in the trial arms. We took steps during design, implementation and analysis to minimize selection bias:

- Intervention allocation was concealed until assignment to prevent selection bias. It was not possible for the study personnel to skip or change the allocation sequence.
- Enrollment could at times be slow; we used block randomization to reduce the likelihood that time trends in illness risk by chance made us enroll more (or fewer) vulnerable babies in the CHX as compared to babies in the dry cord care arm.

An important type of selection bias is attrition bias. Attrition bias may occur when there is substantial loss to follow-up in the trial arms, and is more likely when this loss is differential between the arms. The reasons for differential loss to follow-up could be due to participants moving from the study area, withdrawing their consent to participate in the study, or dying from a disease that is not one of the study end-points and that such loss to follow-up is differential between the trial arms. We had negligible loss to follow-up in the efficacy study; 97% of enrolled babies completed 28 days follow-up and there was no difference in attrition between trial arms. In the pilot study, 62 mothers left the hospital earlier than we had planned for and we could

not collect 48–hours swab samples from their babies; these were distributed similarly between the three trial arms, making a serious selection bias unlikely despite relatively high (62 of 247, i.e. 25%) attrition for that investigation. We took the following steps to minimize the selection bias that could ensue from attrition:

- Our study team had been working in the study areas for a long time and had a very good rapport with the communities. This helped us achieve minimal loss to follow-up in the main trial and among the babies enrolled in the community in the pilot study.
- We followed an intention-to-treat[131] approach by including all followed up babies in the analysis, irrespective of whether they received the intended management of the cord stump.

Confounding bias

Confounding is the distortion in the estimated measure of association between an exposure and an outcome explained by a third factor that is independently associated with both the exposure and the outcome and is not in the causal pathway between them[130]. Confounding and selection bias often overlap and arise from imbalances between the exposed and the unexposed arms with respect to the prevalence of a risk factor other than that of the exposure under study. Confounding can lead to biased effect estimates as the treatment effect gets mixed with the differential underlying risk for the outcome in the different trial arms. Confounding can be controlled for by design or, if the confounder has been captured as a study variable, during analysis, while selection bias arises from differential selection affected by the exposure under study, and it is often not possible to adjust for because variables that represent the imbalance are not captured and can thereby not be included as covariates in regression models.

Large RCTs (such as that reported upon in Paper IV) with appropriate implementation of the randomization procedure are much less prone to confounding than observational studies because randomization is likely to ensure that predictors of the outcome other than the exposure (which is assigned by the investigators) are equally distributed between the trial arms. For smaller RCTs, (such as that reported upon in Paper III), there is a greater chance of imbalance between trial arms and thereby selection (if the predictor is not captured) or confounding (if the predictor is captured) bias.

External validity and transferability

Qualitative studies

"Transferability" is a term frequently used in qualitative research and describes a situation when the findings can be applied to other contexts, situations, times, and populations. Lincoln and Guba[132] recommend providing a detailed account of study setting, people, and data collection experience to the readers. We have provided substantial contextual background information and details of the interviewing process in Paper I[1] such that our findings may have wider relevance beyond the study area and population. Although qualitative studies are generally time- and context-specific and generalizibility is not the desired goal; we believe that our findings are important in

understanding newborn and cord care practices in many similar settings in sub-Saharan Africa and other parts of the developing world.

Randomized control trials

In RCTs, as in other epidemiological studies, external validity refers to the extent to which the research findings based on a given study, which represents a sample of individuals in the target population, can be generalized to people outside that population. We can improve the external validity of a study by keeping the inclusion criteria less restrictive. In our efficacy study (Paper IV), we used wide inclusion criteria and the sample size was very large; the number of omphalitis cases and deaths (events) were substantial, which resulted in effect measures with a high statistical precision. Both wide inclusion criteria and large sample size[133] increases the external validity of the trial findings. Our study findings are generalizable to other low NMR settings with similar access to and utilization of health care and newborn care practices.

High internal validity of our large RCT is a prerequisite for its generalizability. Thus, a biased effect measure emerging from a trial with compromised internal validity, especially when the effect is measured with high statistical precision, could lead to the development of an intervention which, when applied to another population, would not have the intended effect. Since it is often not possible to rigorously estimate the effect of interventions after they have been scaled up, this, often overseen, challenge should prompt us to carefully look into the internal validity of trials when the intervention they examine shows different effects when evaluated elsewhere. The small effect of CHX on the risk of neonatal death, as we observed in our trial in Pemba[4], very similar to that of the trial in Bangladesh[77], should prompt a re-examination of the internal validity of the two other earlier Asian trials[75,76] before concluding that the differences are a result of differences in the population characteristics.

Effect measure modification

Effect measure modification occurs when the association between exposure and outcome depends on the level of another variable. One common way of evaluating effect measure modification is to examine the association separately for each level of that variable. For the efficacy RCT, we presented the effect of CHX cleansing of the umbilical cord stump on the risk of neonatal death separately by gender, place of birth and timing of first contact (Paper IV: Table 3). We did this to understand if differential effects in subgroups could have been responsible for an overall effect substantially different from what had been observed in the Asian studies or in the concomitantly undertaken trial in Zambia. The Asian studies predominantly enrolled babies born at home while approximately half of the babies in the two African trials were born in health facilities. There is a possibility that CHX cleansing reduced the risk of death differentially by place of birth and that could explain the disparity in the findings of the two Asian[75,76] and the African trials[4,87]. However, we observed identical efficacies of CHX among babies born at home and in health facilities in our study suggesting that having more facility-born babies in our trial was an unlikely explanation for the differences. Though the 95% CI around the stratum-specific effect estimates were wider than for the overall effect, the relative reduction in risk of death was virtually identical in

home- and facility-born babies. The findings of the trial in Nepal[75] suggest that there may be an additional benefit when CHX is applied within 24-hours of birth but our trial in Pemba could not substantiate this finding.

Summary of potential methodological limitations

Qualitative studies

One major issue in qualitative studies is "reflexivity", i.e. the viewpoint of researcher(s) influence the research question, methods of data collection, analysis and interpretation of study findings. We did not provide information about the background of the research team in the manuscript[1], therefore the reader may not have been able to understand how our team's opinions and views could have biased study findings. So I use the opportunity this mantle gives me to clarify that our study team did not have any prior position regarding the success or failure of the intervention and the interviewers were unaware of the study hypothesis.

Pembans communicate in Swahili and all the IDIs and FGDs were held in this language. I have a rather limited Swahili vocabulary. Therefore, we chose interviewers who not only spoke Swahili fluently but also spoke and understood English well. I held all the training sessions in English. I instructed the interviewers to note down all facial expressions in the transcripts and went thoroughly through translated transcripts. Not adequately knowing the language may have limited my understanding of what the participants had said and meant, though it is unlikely that it would have affected the study findings, because the Pemban study team members were proficient bilinguals and part of the local culture.

Randomized control trials

We used three letter codes for each of the intervention and control arms for allocation concealment in the pilot study. Separate randomization lists were used for the hospital and the community. Permuted block length of 18 probably lead to slight imbalance in the babies randomized in the three arms but this imbalance did not undermine our study results; if anything the estimated effect of CHX on bacterial colonization was conservative.

We followed a birth cohort for 28 days to estimate neonatal mortality. In the efficacy study, the impact of the CHX intervention was estimated based on the babies we were able to contact, but it is possible that a few babies might have died before the study team could establish contact with the family. Therefore, our estimates may not be fully reflective of the total neonatal mortality. If the study had enrolled all neonates, also those whom we could not enroll because they died on day 1, our effect measure may have been slightly lower. However, the estimates are realistic because most day 1 deaths are not likely to be preventable by applying CHX on the umbilical cord stump.

In summary, the four studies contributing to this thesis took elaborate measures during design, conduct and analysis to minimize bias and enhance representativeness. Therefore, the results and interpretation of our findings have high internal and external validity; and our findings contribute to understanding the role CHX cord care may play in reducing the risk of omphalitis and neonatal death.

Current situational analysis

WHO considered formulation of CHX cord care guidelines in 2013 based on available data from 3 South Asian trials[75-77] in populations with high NMR (i.e. 30 or more neonatal deaths/1,000 live births) and among children predominantly (~90%) born at home. Given the limited evidence-base, the guidelines restricted use of CHX to settings with high NMR and only in babies born at home. The guidelines were justified when they were formulated, as many countries in Asia and Africa at that time had high NMRs and most women in these countries gave birth at home. However, the situation has changed since then, and the guidelines should be reviewed in context of the present global health scenario. The current situational analysis needs to consider the following:

i) There has been a substantial decline in NMR in the last decade; only 17 of the 195 countries reported annual NMR of 30 or more /1,000 live births in 2017[5]. Of these countries, 15 are in sub-Saharan Africa.

ii) There has been a substantial increase in the proportion of babies born in health facilities.

Given this decline in NMR and increase in the proportion of babies born in health facilities, there would be a small number of newborns in 17 countries who would receive CHX cord care as per current WHO guidelines.

iii) Since its formulation, no country has implemented the WHO guidelines as stated. Advocacy groups have been pushing for global use of CHX, advising countries to use CHX cord care in both home- and facility-born babies, also in countries with NMR less than 30/1,000 live births[134]. Therefore, several countries in Asia and sub-Saharan Africa, also those with low NMR, have been implementing CHX cord care for babies born not only at home but also in facilities.

iv) WHO guidelines stated that the recommendations would be reviewed once additional data become available from sub-Saharan Africa[79]. Hence, the Department of Maternal, Newborn, Child and Adolescent Health at the WHO undertook a meta-analysis[135], which also included our trial in Pemba and the similarly large trial in Zambia.

The results of meta-analysis have been shared with the investigators of the CHX trials and need to be presented to the WHO guidelines review committee for its assessment and consideration to appropriately update cord care recommendations.

Ethical Considerations

The institutional review board (IRB) of Johns Hopkins School of Public Health, USA and Zanzibar Medical Research and Ethics Committee, Tanzania provided oversight and ethical approval for Study I, II and IV. For Study III, ethical approval and oversight was administered by Annamalai University, Tamil Nadu, India and Lady Hardinge Medical College & Kalawati Saran Children's Hospital, New Delhi, India. All study protocols, data collection tools, consent forms and SOPs were reviewed and approved by the respective IRBs and Ethics committees. Study investigators were certified in Basics Human Subjects Research and Conflict of interest training courses before starting field activities. We submitted six monthly/yearly progress reports to respective ethics committees to keep them apprised of the trial activities. Before the start of the project, a DSMB and Technical advisory group was established to provide safety monitoring and technical advice for the two CHX trials in Pemba and Zambia. DSMB and Technical advisory group had representatives from both countries, WHO, Gates foundation and a statistician. DSMB members finalized stopping rules for safety, efficacy and futility during their first meeting, prior to the start of CHX trials in Pemba and Zambia. They met at regular intervals and carried out two interim analyses to assess safety, efficacy and futility.

According to IRB guidelines and approved consent processes, study procedures, benefits and risks had been explained to the potential participants in their local language. Participants were enrolled into the trial only after obtaining verbal informed consent in the presence of a witness and all the participants were apprised of their participation being voluntary and possibility of withdrawal anytime without having any effect on their standard of care.

A hardcopy of the study related forms was stored in a locked cabinet in the storage room under supervision of the local PI and only approved study personnel had access to this information. After completion of the study, all data files were stripped of the identifier information and only the study IDs was used during analysis. All participant information had been kept confidential and had not been shared during data dissemination and in the published manuscripts.

Conclusions

Based on the findings of the studies presented in this mantle and a review of other available evidence, the summary conclusions are:

- Umbilical cord stump cleansing with CHX solution is a feasible intervention and is well accepted by mothers. This intervention can be easily implemented in diverse communities.
- Along with the concomitant trial in Zambia, our large randomized controlled trial in Pemba, Tanzania (the Pemba CHX RCT), did not confirm the earlier reported substantial reduction in the risk of neonatal death with CHX cleansing.
- Daily cleansing of the umbilical cord stump with CHX solution until 3 days after cord stump detachment substantially reduces the risk of omphalitis.
- There is no evidence of differences in any effect of CHX cleansing on neonatal mortality between babies born at home and in health facilities.
- CHX cleansing once daily until 3 days after cord separation increases the time to such separation. This is a concern for parents and therefore there is a need for parental counseling if such an intervention were to be implemented.
- The lack of a sufficiently large effect on the risk of neonatal death, and no difference in any effect between home- and facility-born babies in our Pemba CHX RCT support the current WHO guidelines to not apply CHX in settings with NMR below 30/1,000 in both home- and facility-born babies. The observed reduction in the risk of omphalitis may have clinical implications but this reduction does not necessitate a change in current policy.
- There is a need for newer studies with appropriate methods to better describe the bacteria that cause umbilical cord stump infections and neonatal sepsis. If CHX is to be used, such studies should explore whether it not only reduces the prevalence of colonization and infection with pathogens but also induces a shift in their antibiotic susceptibility.
- There is a need to investigate the effect of a single application of CHX on the day the baby is born on the time till cord separation, risk of omphalitis and neonatal sepsis, which remains an important cause of neonatal death.

Recommendations

Our study findings are consistent with WHO's recommendation of not using CHX for cleansing of the umbilical cord stump in babies born at home or in facilities in settings where NMR is less than 30/1,000 live births. Evidence from the two trials in sub-Saharan Africa does not merit change in current cord care guidelines. However, in consideration of the i) contextual, methodological and analytic issues with earlier Asian trials, ii) current situational analysis, iii) misinterpretation of WHO guidelines by advocacy groups and implementation of CHX cord care in both home- and facility-born babies in low NMR settings, and iv) an independent meta-analysis of the 5 CHX trials undertaken by the WHO, the WHO should review all available data and update and clarify the CHX cord care policy relevant to the present day scenario, such that the guidelines cannot be misinterpreted by the advocacy groups. This will enable policy makers to implement a uniform and evidence-based cord care policy.

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Paper I

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RESEARCH ARTICLE Open Access

Delivery, immediate newborn and cord care practices in Pemba Tanzania: a qualitative study of community, hospital staff and community level care providers for knowledge, attitudes, belief systems and practices

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Abstract

Background: Deaths during the neonatal period account for almost two-thirds of all deaths in the first year of life and 40 percent of deaths before the age of five. Most of these deaths could be prevented through proven cost-effective interventions. Although there are some recent data from sub-Saharan Africa, but there is paucity of qualitative data from Zanzibar and cord care practices data from most of East Africa. We undertook a qualitative study in Pemba Island as a pilot to explore the attitudes, beliefs and practices of the community and health workers related to delivery, newborn and cord care with the potential to inform the main chlorhexidine (CHX) trial.

Methods: 80 in-depth interviews (IDI) and 11 focus group discussions (FGD) involving mothers, grandmothers, fathers, traditional birth attendants and other health service providers from the community were undertaken. All IDIs and FGDs were audio taped, transcribed and analyzed using ATLAS ti 6.2.

Results: Poor transportation, cost of delivery at hospitals, overcrowding and ill treatment by hospital staff are some of the obstacles for achieving higher institutional delivery. TBAs and health professionals understand the need of using sterilized equipments to reduce risk of infection to both mothers and their babies during delivery. Despite this knowledge, use of gloves during delivery and hand washing before delivery were seldom reported. Early initiation of breastfeeding and feeding colostrum was almost universal. Hospital personnel and trained TBAs understood the importance of keeping babies warm after birth and delayed baby's first bath. The importance of cord care was well recognized in the community. Nearly all TBAs counseled the mothers to protect the cord from dust, flies and mosquitoes or any other kind of infections by covering it with cloth. There was consensus among respondents that CHX liquid cord cleansing could be successfully implemented in the community with appropriate education and awareness.

Conclusion: The willingness of community in accepting a CHX cord care practice was very high; the only requirement was that a MCH worker needs to do and demonstrate the use to the mother.

Trial registration: ClinicalTrials.gov: NCT01528852

Keywords: Newborn health, Cord care, Community, Delivery, Breastfeeding, Traditional practices

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Background

With forty-one deaths per 1000 live births, the risk of neonatal death is highest in sub-Saharan Africa. Each year, 51,000 newborns die in Tanzania, placing it among the top five countries in terms of newborn deaths in Africa [1]. Tanzania's newborn deaths represent 29 percent of all child deaths in Tanzania. Although, Tanzania has made great strides in reducing child mortality, it has demonstrated slower progress in reducing neonatal deaths [2]. In order to meet Millennium Development Goal (MDG) 4 for child survival, newborn deaths must be reduced.

Eighty five percent of these newborn deaths in Tanzania are due to three main causes: severe infections, primarily sepsis and pneumonia (28 percent); birth asphyxia (26 percent); and complications of preterm birth (27 percent) [1]. The rest of neonatal deaths stem from factors like poor maternal health, inadequate care during pregnancy, inappropriate management of complications during delivery, and lack of newborn care. In addition, many traditional practices, such as application of substances to the stump cord, letting the baby stay wet and cold, poor hygiene during delivery and the first hours after birth, discarding colostrum and feeding other foods, can also lead to serious infections.

Many of these neonatal deaths could be averted with simple preventive measures, such as hygienic care at birth and during the postnatal period. Since the umbilical stump blood vessels are exposed for the first few days after birth, they are a common portal of entry for invasive bacteria that cause systemic infections (sepsis) in newborn babies [3], which may lead to death. In order to reduce the risk of sepsis originating from the cord stump, World Health Organization (WHO) currently recommends keeping the cord clean and dry. They additionally recommend use of topical antiseptics to the cord stump in settings where risk of infection is high. Of the numerous potential topical products (e.g., ethanol, silver sulfadiazine, triple dye, gentian violet, chlorhexidine, povidine iodine), chlorhexidine is a broad spectrum antiseptic agent that has been used extensively in hospital and other clinical settings for many decades. Recent community level randomized controlled trials in Nepal, Pakistan, and Bangladesh have shown that applying a 4% chlorhexidine product (7.1% chlorhexidine digluconate) to the umbilical cord saves lives [4-7].

All of these studies have been conducted in populations from South East Asia but evidence of cord cleansing with chlorhexidine from large randomized controlled studies from sub-Saharan Africa is lacking. In view of this we proposed to carry out a double blind controlled trial in Pemba, Zanzibar to evaluate the efficacy of application of chlorhexidine on umbilical cord of neonates during first 10 days of life. There has been paucity of data regarding community attitudes, beliefs and behaviors during delivery and newborn care from the region. We felt the need to carry out an initial formative/qualitative phase to explore the current knowledge, attitudes and practices with regard to delivery, newborn and cord care before implementation of the randomized controlled trial. This formative phase was aimed 1) To collect information on delivery practices and to understand the existing neonatal and umbilical cord care practices in the community; 2) To get feedback, perceptions and suggestions from traditional birth attendants, community members and health professionals regarding liquid cleansing solution as an umbilical cord care practice; 3) To evaluate the acceptance and barriers for the use of the proposed chlorhexidine cleansing solution; and 4) To develop communication messages, study procedures and the framework for implementing a cord care intervention based on the information gathered. The present paper is presenting the findings from this formative research. We believe that the insight into current delivery, newborn and cord care practices prevalent in Pemba, Zanzibar will help in making policy decisions which can have impact in improving newborn survival and in achieving MDG4.

Methods

Study setting

The study was conducted in four districts of Pemba Island, the smaller of the two islands of the Zanzibar archipelago situated 50 kilometres east of mainland Tanzania. The island has a population of about 350 000, most of whom are Afro-Shiraji Muslims, and has a tropical climate. A baseline census, including a birth history for women of reproductive age, indicated an infant mortality rate of 89 per 1000 live births [8].

Study design

The study utilized a mixed methods approach to better understand the beliefs and practices of the community in relation to delivery, newborn care and umbilical cord care. Participants in the FGDs and IDIs were selected using a combination of purposive and random sampling. As part of qualitative research method, 80 in-depth interviews (IDIs) were conducted. In addition, 11 focus group discussions (FGDs) were held to triangulate findings from the indepth interviews. A total of 180 individuals participated in the study either as IDI participants (80) or FGD participants (100). The reason for adopting a mixed approach of using IDIs and FGDs was to ensure that triangulated responses actually represent the reality and not be affected by interviewee bias.

Sampling and sample size

This study was based on principles of qualitative research. Final sample size was based on the principle of achieving data saturation. Data collection continued until data

saturation was reached. We identified 6 significant key players contributing to maternal and newborn care (Hospital staff, PHU staff, registered TBA, unregistered TBA, mothers, grandmothers). There are 4 districts on the island and to account for variation in each of these strata by district, participants were selected from each strata from all 4 districts. A minimum of 8 respondents were selected for each strata to account for data saturation.

Sampling strategy

To identify women of reproductive age, two villages from each district were randomly selected. From the census data available with the local institution, five women of child bearing age who had given birth to at least one child in the year preceding survey were chosen randomly from each of these villages. In all, 80 in-depth interviews were conducted including 40 women of child bearing age, 20 elderly women (grandmothers), 20 untrained and trained Traditional birth Attendants (TBAs) (Figure 1). Trained TBAs have been part of the earlier Govt. program and were provided training and delivery kits to conduct safe delivery while untrained TBAs are community based workers undertaking deliveries as part of the community service.

A total of 11 FGDs were organized, with each group having 8 participants. FGDs included hospital staff (FGD1), Maternal Child Health (MCH) staff/registered TBAs (FGD2), mothers (FGD3-FGD6), grandmothers (who were also TBAs, FGD7-FGD10) and fathers (FGD11).

Data collection

Five health workers who had been involved in an earlier Global Burden of Disease Ethnography study were contacted and trained to do in-depth interviews and focus group discussions using the guidelines prepared by research team (Additional File 1: Focus Group Discussion and In-depth Interview Guidelines). The study instruments were pre-tested by health workers among themselves to check their appropriateness and to modify them so as to make them understandable by respondents. All the final forms and guidelines were translated into Swahili, the local language and each trained health worker conducted 2 mock interviews at Public health Laboratory (PHL) in the presence of the trainers.

In-depth interview process

In-depth interviews consisted of open ended questions, free listing and structured questions. The IDIs were one-on-one interviews held in local language (Swahili) between a trained interviewer and study participant. A prior appointment was sought from the participant. On the day of appointment the interviewer visited the selected respondent's household at the time preferred by the respondent. Interview lasted for 1.5 hours and was audio taped. The interviewer noted down key points of the interview in her diary. Interviewers were also asked to record interesting verbal and non verbal expressions of the participants and keep a note of local terms used.

Focus group discussion process

Focus Group Discussions were moderated by two health workers: one acting as a Focus Group Leader (FGL) who introduced the topics of discussion in a programmed order and another one as a note keeper who was taking notes of all key points of the discussion in her diary. FGL posed the questions to the group and then asked each participant one by one if h/she would like to express her view on the topic. FGDs typically lasted for 90 minutes and were conducted in Swahili. All the focus group discussions were audio taped.

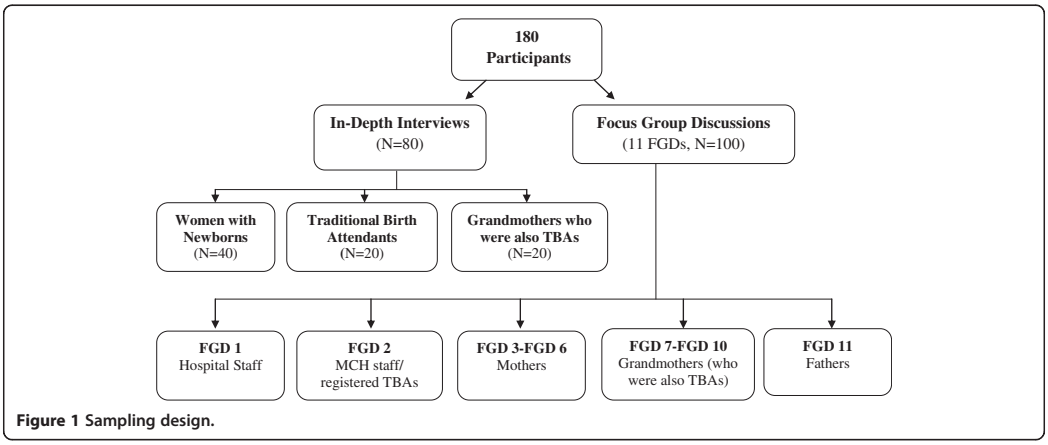

Figure 1 Sampling design.

Data analysis

Members of the field team went through the notes and audio tapes in the evening to transcribe the discussions and interviews in Swahili which were then translated into English. A thematic approach was used to analyze the transcripts [9]. It is one of a cluster of methods that focus on identifying patterned meaning across a dataset. Interviews and FGDs were read by research team and based on the texts, codes were developed and coded along thematic issues. Coded segments were examined to identify significant broad themes. New codes were developed to refine the broad themes into more specific themes. Two investigators independently applied codes to the interviews using ATLAS ti 6.2 qualitative data analysis software [10] and all final codes and themes were mutually agreed upon. The investigator team collectively selected the themes and representative quotes that were considered relevant to this publication.

Ethical approval

The study was approved by the Johns Hopkins Institutional Review Board and the Zanzibar Research Council. Verbal Informed consent was taken from all the participants.

Results

Delivery practices: delivery place

Both mothers and TBAs considered hospital deliveries to be safer and better for handling of complications that might arise during delivery. Serious complications like bleeding before delivery, delayed placenta, infectious disease (like AIDS), etc. can only be handled at the hospitals. Some of the mothers were concerned about the skills of TBAs. All TBAs interviewed said that they always recommend the women to deliver at hospital but they have to deliver the women at home in case of emergency or some women insist to deliver at home.

> *"There are a lot of problem appeared nowadays. That's why many women prefer to deliver at hospital. Also the TBA has not enough skills or knowledge for conducting delivery. Even little problems TBA cannot solve. For instance, the TBA might be faced with cord bleeding, and will then become frustrated as she may not know what to do." -(FGD with mothers)*

However, there were regional differences in regard to preference for birth place. In urban areas, the hospital was the place of choice while in rural areas, majority of women preferred to deliver at home either because of poverty or because of poor accessibility to health care services.

Several mothers explained that they delivered at home because of habit or tradition. Among the major reasons cited for choosing home as their preferred delivery place were: problems in transportation, past experience of safe delivery at home, lack of time to reach hospitals and excessive expenditure incurred at hospitals. Rude treatment and unavailability of beds at hospitals were cited as the prime reason for not delivering at hospitals by women.

> *"Some women do not prefer to go to hospital to avoid bad words from the doctors. Doctors use abusive language to their patients. Also our infrastructure is very poor. We have no road; poor transport will lead most of women to deliver at home. If you want to go to hospital, husband of the pregnant mother will hire a car which costs about 30,000/=and this is very expensive. That's why it seems to mothers that it's better to take delivery at home."- (FGD with mothers)*
> *"It is true that the delivering mothers are insulted beyond words at the hospital."- (FGD with fathers)*

Pre-delivery preparations

Women in the community reported certain things that they keep ready with them which might be useful at the time of delivery. These include gloves, plastic sheet, plain cloth, thread and razor which they boil and store in an alcohol bottle. Coconut oil, soap and Kanga (new cloth for wrapping baby) were kept for post delivery care of newborn. They also kept some food items that they eat after delivery such as ginger for making tea, rice, sugar and flour to make porridge.

Things to hasten delivery process

Pregnant women in the community are given specific food items, felt to aid in the delivery process. These include tea, porridge, honey, *Kombe* (a kind of holy water prepared by 'Sheikh" i.e. Muslim leader), *Mpatakuva* (kind of herb used for abdomen treatment), traditional medicine such as roots of rose tree, henna tree and jasmine tree, bush herbs. Some other things that were used to induce labor included *Tonga,* a kind of wild fruit obtained at sea shore. Some of them also mentioned bathing and drinking cold water as strategies to hasten the delivery process. Women were advised to walk around so that the baby can easily come down into the birth passage.

> *"Mostly we gave them porridge and tea so that the mother can have strength, and also I tell them to walk around to make the baby delivered very easily."-(IDI with TBA)*

Hand washing/glove wearing

There was a clear consensus for washing of hands after delivery, but the practice of washing hands before delivery and glove usage was not universally followed. Many

women who had their past deliveries at home informed that majority of the TBAs used gloves for conducting delivery but expressed skepticism about the practice of hand washing before delivery.

"After finishing (the delivery), the TBA must wash her hands. She washes them by using water and soap, and sometimes should wash/bath the whole of her body and change her clothes."- (FGD with mothers)

"I don't know whether she washes her hands or not, but I think she only wears gloves and start to massage me."-(IDI with mother)

These findings were confirmed during in-depth interviews with TBAs. *'I wear gloves, I boil thread and blade, and after delivery, I take off gloves and wash my hands and clean the mother and baby."-(IDI with TBA)*

One of the mother informed, delay in passing the message regarding labor pain initiation to the TBA as one of the reasons for not washing hands before delivery. She described that when TBAs reach their home, the pregnant women is already in the process to deliver because of which they do not get enough time to wash their hands.

"Sometimes TBA do not wash their hands. More often TBA just wear gloves or sometimes do not wear even gloves, she just make hurry to conduct delivery. In my view, I think pregnant mother themselves delays to inform and to call the TBA. When they come the baby have already started to come out i.e the head start to come out. So, the TBA make hurry to conduct delivery and not to wear gloves."-(FGD with mothers)

Delivery surface

When asked about the surface of delivery, women reported giving birth on a variety of surfaces including beds with rubber sheets at the hospital, on a mattress or on the floor at home. Most women including mothers, TBAs and grandmothers reported bed as the most frequently used place for delivery at home. Some of them used to cover the bed with mackintosh just as in hospital emphasizing the need for a clean delivery surface as a means of preventing infection to their babies; however those who found it expensive simply covered the bed with old cloth.

"I tell them to lay down on the bed as what they did at Hospital."-(IDI with TBAs)

Soon after delivery, baby was kept on bed where it was delivered until the delivery process gets completed: *"I put the baby on the bed, and I wrap her/him with a new Kanga."-(IDI with TBA),* other women and health care providers report that the baby is immediately placed on their mother's belly to provide him warmth from mother's body. *"After delivery, I put the baby on the mother's belly, then I take scissors, I tie the cord on two sides, the mother's side, and the baby's side, then I cut the cord."-(IDI with TBA)*

Rituals performed at birth

Observance of certain kind of rituals at the time of birth was quite common in the community. For instance, women who had delayed pregnancy or miscarriage in the past used to make a vow (known as *Nadhiri*) for conducting certain ceremony when they will deliver. So, they perform "Maulid Ceremony" to fulfill their vow, or distribute cooked food to the community.

"Sometimes we perform Maulid but not for every child, mostly it's performed when the mother have complication and delivered safely."-(IDI with mother)

One of the TBAs also reported a special ritual on the 7th day after delivery.

"In old days, we used to make special ritual on the 7^{th} day after delivery whereby we used to take the baby out. We used to put the baby in the winnowing basket, then we throw water on the roof, after that, we take the baby outside."-(IDI with TBA)

Newborn care practices: bathing and cleaning of newborn

Delaying the first bath of baby soon after birth is an important practice to prevent hypothermia. In Pemba, this practice was consistently performed among hospital deliveries mainly because hospital personnel discouraged immediate bathing. For deliveries taking place at home, TBAs were primarily responsible for giving first bath to newborns, however the study revealed differences in this practice performed by the TBAs trained by the government programs and that by untrained TBAs. Untrained TBAs reported that after delivery they clean the babies and then give them bath immediately with warm water, sometimes with soap also. The trained TBAs instead of giving bath immediately after birth, only wiped the babies. Some of them also reported to practice kangaroo care. However even among them, bath was delayed by barely 6-12 hours.

"Immediately after delivery the TBA cut the cord then she wiped the baby with dry clothes and she gave her bath."-(IDI with mother delivered by untrained TBA)

"I didn't bath the baby at that time because we were told at the Hospital not to bath the baby immediately

after delivery, they advise us we just have to wipe the baby then after some hours, the baby might be bathed."-(IDI with trained TBAs)

Triangulation in the Focus Group Discussions further clarified that in case of hospital deliveries, although the baby was not given bath at the hospital but they bathed the baby as soon as they reached home. In case of home deliveries, the baby was bathed immediately after birth.

"If we have not stayed at the hospital for a long time. We bathe the child when we get back to home"-(FGD with mother)

Subsequently the newborn was dressed up with new clothes (*Kanga*). Following this, newborn babies were bathed regularly during the first week of birth, usually two times a day i.e. morning and evening. Warm water, soap and basin were normally used for bathing. Some of them also reported use of *Jimbo*, a mixture of various traditional herbs. However, they didn't tell the names of those herbs as they were supposed to be kept confidential. After bath, babies were warmed using charcoal and oil massage. Majority of them used coconut oil although some of them also used olive oil and petroleum jelly. One of the TBAs also reported use of coconut oil mixed with the herbs of a tree named *Mchocha* to prevent the child from a fever called *Babu*.

"The care that was given to the baby within one week of delivery was that I used to bath him twice a day, in morning and evening. I warmed him with coconut oil and fire steam for 40 days."-(IDI with mother)

Breastfeeding and colostrum

In Pemba, breastfeeding is a common practice and most of the women breastfed their babies within 24 hrs of birth.

"I started to breast feed him after being cleaned and given a call for prayer "ADHANA" which was almost 2 hours after delivery."-(IDI with mother)

TBAs and women in the community were well aware of the nutritive value of breast milk. Women in the present study widely knew about the colostrum and its health benefits. Some of the TBAs were trained by the Ministry of Health where they were taught about the nutritive value of colostrum and they encourage the mothers to feed first milk to the babies.

"On baby's side, I tell them to just breastfeed them, they have to give nothing else to the baby even water because the breast milk is full in nutrition for the baby."-(IDI with TBA)

Mother's who were unable to secrete milk were generally advised to eat octopus which in their belief, helps in production of milk. Some of them did not breastfeed their babies if they suffered from HIV, had severe abdominal pain or had infection like fungus on their breast.

Cord care practices

The umbilical cord was cut soon after delivery and mostly it was TBAs who cut the cord in case of home deliveries. The importance of a clean cord cut was commonly understood and all TBAs generally used sterile scissors or a new razor to cut the cord. Almost all TBAs informed that they disinfected these delivery instruments (razors, scissor and thread) by boiling them in water.

"After the woman delivers, I boil the blade and roll the thread, then I tie the baby's cord by that thread and cut the cord by using the boiled blade. After cutting the cord we clean the baby and wrap in to the clean clothes"- (IDI with TBA)

TBAs further informed that razors were used only once for cutting and were buried after use.

"If we use the blades, we burry it after using, if we use the scissor we clean it by using hot water." -(IDI with TBA)

After cutting, umbilical cord was usually tied with normal tailoring thread; few of them also reported use of special thread provided by the hospital reiterating the importance of clean cord care. Respondents described the importance of tying the cord to stop bleeding as well as to prevent infection. Some of them also reported use of Utembo, a kind of thread obtained from a palm tree called Muale.

Cord length

There was mixed perception about the length at which cord should be cut, some of them gave no importance to cord length, according to them cord could be cut at any size while others reported that it should be cut at particular length mentioning it as an important part of delivery process.

"It doesn't matter because everyone cut it in her own way, on my side I cut it compared with the length of my mid finger (you know one type of rice, but various type of cooking)."-(IDI with TBA)
"Yes it is important; if you cut it too short, once it is untied how you can tie it again."-(IDI with mother)

Cord care

The importance of cord care was well recognized in the community. Nearly all TBAs counseled the mothers to

protect the cord from dust, flies and mosquitoes or any other kind of infections by covering it with cloth. The same was further confirmed by mothers during in-depth interview. Some mothers in the community informed that in case of male babies, they cover the surrounding area of the cord with cloth so as to prevent it from falling on the genital parts as they believe that if the cord falls on babies' private parts, he will get impotent.

"They say that for a boy, to protect him, to avoid the cord to fall on his "secret parts". They put a cloth around it. They say that if it falls on his secret part (sexual part) he may become impotent."-(IDI with TBA)

The interviews revealed that while various newborn care programs running in the area have talked about the cord, no program mentions about the raw area left after the cord falls off. So as soon as the cord falls off, a void is formed and all sorts of traditional beliefs and practices thrust in especially if the wound is wet. In-depth interviews as well as triangulation from focus group discussions conveyed that the practice of dry cord care had percolated down to the grass root level. Following cord cutting, most of the mothers reported either using nothing or application of array of substances including: Saliva (*Mate*), dirty door powder from old door (*Ganda La Popoo*), hot knife, Charcoal powder, Shells (*Gamba La Koa*), also PPF powder, burning wood (*Kijinga*), banana steam (*Tojo*), fish bone etc. During bathing, cord was mildly cleaned, dried immediately and was covered with a cloth to protect it from flies and fiddling by older siblings.

"We don't put anything, just apply oil and the baby powder only, we don't put baby powder to the cord, until the cord is fallen off itself."- (FGD with mothers)

The normal expected time for cord falling was reported to be 3-10 days. However they do not intervene if the cord takes longer to fall off but simply refer to hospital, which reflect the positive aspect of the community behavior towards the cord care. Once the cord fall-off, TBAs reported either application of door dust, charcoal powder, baby powder 444, spirit or nothing on the left out area, mainly to prevent it from infection. Cord was normally buried and some even reported to plant a coconut tree on it.

"I don't advise them to use anything nor charcoal powder nor dirty door powder as other do, but in my area, I have educated them so they do not apply anything."-(IDI with TBA)

Cord care in case of infection

Development of early symptoms of cord infection is thought to be an important proximal event in the pathway to sepsis and death in newborns. The common symptoms for cord infection reported are swelling, pus formation, redness or bleeding. Mothers informed that upon recognition of any of these danger signs, they contact TBAs who usually provide home remedies, such as application of hot knife, door dust, charcoal powder, sandalwood powder, ground sea shell, PPF or talcum powder and fire steam. Some of the respondents had the perception that cord infection is caused when the mother ate certain kind of fish or other food items. In such cases, they find out the food item causing that infection, burn it and put it on the cord. Failing these home remedies or traditional care, families opt for formal medical care at a nearby public facility or by a private doctor.

"Some time the cord might be affected by "WICHO" or "KILIMI" if that happens I ask them to find something that cause that infection "WICHO", burn it and put on the cord. It can be banana, or sea shells "KORONGONJO'. Recently there was one case happened, the infant's cord was having infection, the baby was taken to hospital but the baby's cord didn't cure, I told them to burn the stump of banana and put on the baby's cord and it recovered"- (IDI with TBAs)

Perceptions and suggestions about implementation of liquid cord cleaning

There was a clear consensus among the health professionals, TBAs and community members in regard to the successful implementation of liquid cord cleaning. There was an emphasis on creating awareness in the community about usefulness of CHX in cord cleaning; educating the community before implementing CHX cord care. While some of the mothers and TBAs had a view that CHX should be applied by health professionals, others were confident that if mothers are properly trained they can apply the CHX on baby's cord themselves.

"If people are educated on the use of this antiseptic solution they will be willing to use it. – (IDI with mother)
"My side I think it should be used and mother can apply it herself"- (IDI with TBAs)
"Liquid based cleansing solution is a good idea, but the people should be educated and there is no problem for mothers to apply it if they get instructions"- (FGD with mothers)
"This thing is new to us. I myself have never heard about this medicine. In the past they used to apply dust from the door on the cord. Now that is out of

date. Mothers use modern medicines. Hence if there is such a medicine and researches have been made to see that it is safe to use it we ask the Ministry to allow us to use it and we will be willing to use it" – (FGD with MCH)

Barriers/constraints to liquid cord cleaning

As part of the FGDs and IDIs respondents were specifically asked about the possible constraints in implementation of CHX as liquid cord care. Almost all the respondents were of the opinion that there will not be any constraints while very few respondents cited few concerns. Lack of understanding, cost of CHX solution and religious beliefs were cited as some of the potential issues in the community.

"No constraints" - (IDI with mother)
"There will not be any problem" - (FGD with TBAs)
"Some people may have constraints in introducing this cord care practice on ground that they may think it is against their custom" – (IDI with mother)

Findings from structured component of interviews

As part of the structured questionnaire relating to cord care, application of liquid antiseptic solution to cord, the perceptions of mothers were; 88% of the women did not know about the use of antiseptic solution for cord care and yet 99% were willing to use the liquid cord care solution. 93% of respondents were of the opinion that mothers can apply the CHX solution themselves insisting that they should be trained by MCH workers. The only constraints mentioned were cost of CHX if people have to buy it themselves (11.3%), lack of understanding (7%) and religious customs (2.8%). 69% of the respondents felt that there will not be any impediment to CHX liquid cord care.

(The detailed data pertaining to structured interview component are presented as Additional file 2: Supplementary Tables).

Discussion

Appropriate antenatal, intra-partum and post-natal care play a crucial role in preventing mortality and in achieving optimal health outcomes for infants and young children. The current study is one of the very few qualitative studies on selected delivery and newborn care practices conducted in East Africa (The only one conducted in Zanzibar). The study in addition to focusing on issues around delivery, newborn care practices complementing the data from other recent studies in Sub Saharan Africa [11,12] and Tanzania mainland [13] added a new dimension of cord care practices and perceptions regarding use of CHX as antiseptic cord care. Zanzibar has been under Omani influence for more than two centuries and therefore perceptions and practices might be different between Tanzania main land and Zanzibar.

The level of perinatal mortality rate (PNMR) in a community is said to be associated with the proportion of births that take place at a health facility and the coverage of skilled attendance at birth. Countries where skilled attendance and institutional delivery rates are low usually have a high PNMR [14]. Hospital deliveries accounted for majority (64.3%) of the births in this study. TBAs in the community were generally aware of the problems of delivering at home which can be easily handled at hospitals. Similar findings have been reported in another study conducted in Pemba [15].

The finding of the present study suggests that although there is improvement in the proportion of those delivering at hospitals in Pemba, they should be further encouraged to deliver at hospitals in efforts to reduce child mortality. Poor transportation facilities were found as one of the major hurdles in this direction which forced many families in the community to hire cars to reach hospitals which is generally very expensive. Bang *et al.* [16] have proven the feasibility of a low-cost approach of delivering primary neonatal care at home using intensive support by locally trained community health workers in India. Similar approach can be replicated in other poor resource countries like sub-Saharan Africa. Another hurdle observed in the present study was cost of delivery at hospitals. Increasing healthcare utilization among these communities would require eliminating this unnecessary cost, further, health care providers should be trained in proper patients dealing so as to make hospitals 'mother-child friendly'. Lack of knowledge about antenatal care is another hurdle in newborn and maternal health as finding of the study suggests that majority of the mothers who had not gone for antenatal visit were actually not aware about it.

Studies from rural areas have underscored the role of family members during delivery and care of newborn [17,18]. Such a practice highlights the importance of providing training to family members regarding delivery process which would better equip them in dealing with delivery complications.

According to the World Health Organization, the risk of umbilical cord infection increases when unclean materials are used to cut the cord [3]. In the present study, almost all TBAs reported using a sterilized blade, which is different than other studies conducted in rural area of Bangladesh [19] and Nepal [17]. Similarly, use of sterilized thread to tie the cord was also very common in the community. They were generally aware that unsterilized equipment was a major risk factor for cord infection. Thus, the public health recommendations to use sterilized equipment for delivery purpose fits well with the local cultural understanding.

The time immediately following delivery is a vulnerable period for both the woman and the baby. WHO has recommended thermal control of newborn in the essential newborn care [20]. In the present study, the practice of bathing newborns immediately after birth or simply wiping them off with a warm towel varied according to the place of delivery and training of the TBAs. TBAs who were trained regarding neonatal care generally avoided bath immediately after birth as compared to untrained TBAs. Bathing was also delayed when babies were born at hospitals. However, even in case of trained TBAs, delay was not more than 24 hrs as more than 70% of the newborns were given bath within 24 hrs after delivery. Further, there is a practice of regular bathing of newborns during the first few days after delivery, usually twice daily, which increases the risk of hypothermia. This practice is similar to those in other countries in the South Asian region [17,18].

On the positive side, mothers and TBAs in Pemba were concerned about the protection of newborns from cold and took great measures to keep them warm in the days following delivery. Immediately after birth, newborns are wrapped in new clean cloth (*Kanga*) and given bath with warm water and occasionally with some traditional herbs (*Jimbo*) to protect the baby from fever and cold. After bath, baby was kept warm with charcoal and coconut oil. Some traditional herbs were also added to the oil for protection from fever. However, use of traditional treatments such as exposing the newborns to charcoal fumes for warming should be avoided as exposing newborns to smoke could put them at risk of respiratory problems.

The present study showed that Pemba is one of the places where an optimal dry cord care is practiced. There are very few deviations which include application of dirty door powder from old door (*Ganda La Popoo*), hot knife, Charcoal powder, Shells (*Gamba La Koa*), also PPF powder, burning wood (*Kijinga*), banana steam (*Tojo*), fish bone etc. The main lacuna seems to be after falling of the cord where the health messages are not clear. Women reported a range of substances that were applied to umbilical stump. However, application of such harmful therapies in the management of babies exacerbates the probability for the development of infections like tetanus, omphalitis, fever, septicemia, etc. Earlier studies from Nepal and Pakistan also reported use of similar materials on the newborn's umbilical cord highlighting the prevalence of this practice at a wider geographical range [17,21,22].

Exclusive breastfeeding is an important behavior that should be initiated within the first hour after birth and maintained until the newborn is six months old. In the present study, breastfeeding practices seem to be adequate and healthy. The rate of initiation and exclusive breastfeeding seems to be healthy and encourageous, which is higher than the percentages reported in other studies [23]. We did not discover any negative perceptions about feeding colostrum except for one TBA. This finding has positive implications for infant nutrition. The only instance when mothers disagree to breastfeed was when they have some problem like severe abdominal pain, infection etc. Thus, the finding that breastmilk is highly nutritious and should be given immediately after birth, is clearly in accordance with the current global health recommendations.

What we learnt from the study to inform the main CHX application trial

After analyzing the data collected from this phase we realized that although most of the community was unaware of CHX antiseptic solution they were positive about its use as a cord care solution emphasizing that community should be educated about its usefulness. Community members believed that first CHX application should be done by hospital staff/MCH worker rather than the community health worker. MCH workers should demonstrate/instruct mothers as to the application of CHX solution on the cord and then the mothers can do it themselves. This was an important finding which lead us to re strategize the implementation of the main trial. We had initially planned to use community workers for application of CHX for the main trial but based on these findings engaged the MCH workers for CHX application on day 0, 1, 4 and 10. They taught the mothers on CHX application and mothers applied it on the rest of the days.

Limitation of the study

The current study was based on reported practices and not on actual observation and hence was subject to recall and response bias. Hence, the interpretation and generalizability of our findings may be limited. It is possible that some of the health functionaries reported what they were expected to practice in comparison to what they actually did.

Implications of the study

Despite the above-mentioned limitations, our study has obtained important information about delivery and newborn care practices. This information will assist in planning public health interventions to change these behaviors. Some of the practices reported in this study benefit the mother and the newborns and hence should be encouraged. This includes use of clean delivery instruments, TBAs' emotional and physical support to mothers, newborn warming and immediate breastfeeding. Unfortunately, some behaviors inadvertently undermine newborn survival like jeopardizing mother from antenatal care, immediate

bathing of newborn risking hypothermia and removal of protective vernix, cleanliness of TBAs (unclean hands), exposing the newborn to smoke for warming, application of some traditional substances on cord etc. and therefore should be discouraged.

Our study suggests that sheer availability of maternity services may not be enough to ensure the use of such services. Lack of utilization may be influenced by income, poor infrastructure in the form of transportation, hospital staff's behavior and traditional beliefs. These issues need to be addressed in order to promote hospital based deliveries which would in turn assist in reducing newborn mortality.

Conclusion

In Pemba the awareness among the community regarding importance of institutional deliveries seems to be very high. However impediments as regards to transportation and care at the facilities seemed to be a reason for this positive knowledge not resulting in appropriate practice with women choosing to deliver at home. The willingness of community in accepting a CHX cord care practice was very high; the only requirement was that a MCH worker needs to do and demonstrate the use to the mother. The current study highlighted very subtle and important issues that would help improve success of currently ongoing programs and also helped modifications in the design of main CHX efficacy trial which was hence successfully implemented with completion due in next 6 months.

Additional files

Additional file 1: Focus Group Discussion and In-depth Interview Guidelines.

Additional file 2: Supplementary Tables.

Abbreviations

CHX: Chlorhexidine; IDI: In-depth interviews; FGD: Focus group discussions; WHO: World health organization; MGD: Millennium development goal; TBA: Traditional birth attendants; MCH: Maternal child health; PHL: Public health laboratory; FGL: Focus group leader; PHU: Primary health unit; AIDS: Acquired immunodeficiency syndrome; HIV: Human immunodeficiency virus; PNMR: Perinatal mortality rate.

Competing interests

Authors declared no competing interest.

Authors' contributions

UD was involved in the development of the study questionnaires, guidelines, training of interviewers, coding and analysis of data, and the preparation of manuscript. JG helped with the development of study questionnaires, guidelines, analysis and preparation of manuscript. AMS was involved in primary data collection as well as the transcription and translation of data. SMS was involved in primary data collection as well as the transcription and translation of data. AD helped with the development of study questionnaires, guidelines and field management. SMA helped with the Pemba administrative system, community mobilization and provided leadership and supervision to team members. SG helped with the analysis and manuscript writing. REB was involved in the conceptualization of the research and the preparation of the manuscript. SS was involved in the conceptualization of research, development of study instrument, development of study protocol, coding and analysis of data, and the preparation of manuscript. All authors read and approved the final manuscript.

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Paper II

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Trial of improved practices approach to explore the acceptability and feasibility of different modes of chlorhexidine application for neonatal cord care in Pemba, Tanzania

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Abstract

Background: Infections are responsible for 30–40 % of 4 million neonatal deaths annually. Use of chlorhexidine (CHX), a broad-spectrum topical antiseptic with strong residual activity, for umbilical cord cleansing has been shown to reduce infections during the neonatal period. However, the challenge remains with regard to selection of best mode of CHX delivery. As a part of formative research, we undertook a qualitative study in Pemba Island as a pilot to explore the attitudes; beliefs and practices of the community and health workers related to delivery, newborn and cord care. During the second phase of formative research, we used Trials of Improved Practices (TIPs) methodology to explore the acceptance and impediments, for the three possible modes of chlorhexidine application- 100 ml bottle with cotton swab, 10 ml single use dropper bottle and 3 g single application squeeze tube containing gel, as an umbilical cord care intervention.

Methods: In this pilot study, 204 mother-newborn pairs were enrolled from hospital and community setting in Pemba, Tanzania using a randomized three period crossover design. Mothers/guardians, Trained Birth Attendants (TBA)/ medical staff and community health workers (CHWs) were requested to try three different modes of CHX application for cord cleaning. All participants were demonstrated the method of cord cleaning using all three modes of delivery; each delivery mode was used for 3 days and an interview was conducted on day 10 to collect summary of their experience. Acceptance and preference scores were calculated based on feedback from the participants.

Results: Of 204 mother-newborn pairs, 27 were lost to follow up. 177 mothers performed the intervention and applied CHX to the newborn cord for all 9 days. Mothers rated 10 ml dropper bottle (49.7 %) as most convenient in terms of ease and application. They selected 10 ml dropper bottle (44.6 %) as their first choice; gel tube (33.9 %) and 100 ml bottle (21.5 %) as their second and third choice. TBAs, medical staff and CHWs also preferred 10 ml dropper bottle (43.3 %) over 100 ml bottle (12.9 %) and gel (38.8 %).

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Conclusions: Overall acceptability of CHX application for cord cleansing was high. 10 ml single use dropper bottle was given highest preference for CHX application. An understanding of the attitudes, beliefs and cultural practices in the community and selection of the most acceptable mode of CHX delivery is essential to the design and implementation of the intervention trials examining the efficacy of CHX cord care in reducing neonatal mortality and subsequent implementation in the programs.

Trial registration: ClinicalTrials.gov NCT01528852 Registered February 3, 2012

Keywords: Trials of Improved Practices (TIPs), Chlorhexidine, Cord care

Background

Neonatal mortality accounts for 70 % of deaths in the first year and 40 % of total under-five mortality [1–3]. Each year nearly 4 million children die before 4 weeks of age globally, of which over 1.1 million neonatal deaths (28 %) occur in sub-Saharan Africa. Infections are responsible for 30–40 % of 4 million neonatal deaths annually [1, 4–6]. Omphalitis, an infection of the umbilical stump, resulting from colonization of the stump with bacteria from the maternal genital tract and the environment poses a significant risk of infection and death during the first 28 days of life. Effective interventions that can be carried out at the household level are critically needed to reduce neonatal infections and mortality.

WHO recommends clean and dry cord care for newborns born in health facilities, and at home in areas with low neonatal mortality rates (<30 per thousand). However, they also propagate application of topical antiseptics to the cord stump during the first week of life - for home deliveries in areas where the risk of bacterial infection appears high (30 or more neonatal deaths per 1000 live births) [7]. Chlorhexidine is a broad-spectrum topical antiseptic with strong residual activity. CHX has shown a potential to reduce infections during the neonatal period [8, 9]. Being inexpensive, along with a strong safety profile, CHX seems to be an ideal antiseptic for cord care in low-resource communities [10, 11]. In 2013, WHO added 7.1 % chlorhexidine digluconate (delivering 4 % chlorhexidine) to its list of essential medicines for children [12]. Community level randomized controlled trials in Nepal, Pakistan, and Bangladesh have shown that applying a 4 % chlorhexidine product (7.1 % chlorhexidine digluconate) to the umbilical cord has the potential to save lives [13–15]. Two studies in Nepal tested both aqueous and gel chlorhexidine formulation and observed that gel formulation was more acceptable and preferred than liquid solution [16, 17]. Use of 4 % chlorhexidine umbilical cord wash as a low-cost intervention can easily be scaled up and incorporated into preventive health care in sub-Saharan Africa, impacting a part of the 1.1 million neonatal deaths and 27 million years of life lost every year in sub-Saharan Africa [18–21].

Given the promising results from recent chlorhexidine research and an understanding of the existing practices and beliefs related to newborn care, feasibility of implementing a liquid cleansing solution and selection of the most acceptable mode of delivery of intervention are essential to the design and implementation of intervention trials examining the efficacy of use of chlorhexidine to clean umbilical cord of neonates in sub-Saharan Africa and also for implementation of programs if found efficacious.

We carried out a formative research phase before starting of the main efficacy trial. In phase 1 of formative research, Focus group discussions (FGD's) and in-depth interviews were held to understand the neonatal care and umbilical cord care perceptions and practices in the community; and evaluate the acceptance and barriers regarding the use of proposed chlorhexidine cleansing solution. In the second phase, Trial of Improved Practices (TIPs) methodology was used to ascertain the acceptability and preference for various possible modes of chlorhexidine delivery for cord care among the mothers/caretakers and health professionals. TIPs is developed by Manoff group, and is a formative research method that engages potential participants in the design of program strategies and activities focused on behavior change, wherein participants try new practices as part of their routine over a trial period; and then provide feedback at the end of the trial period [22]. The results of phase-1 have already been published elsewhere [23] and in this paper we are reporting the findings of the Phase-2 of the formative research. The findings from this phase would be useful in the design and implementation of a culturally acceptable intervention for a large double-blind community-based randomized controlled trial evaluating the impact of chlorhexidine cord cleansing in first 10 days for reduction in omphalitis and neonatal mortality in Pemba, Tanzania where the signs of omphalitis appear frequently and predominantly in the first week of life among newborns [24]. In Asia, Alam et al. [25] adopted a similar strategy and carried out a formative research study in Sylhet, Bangladesh to assess the umbilical and skin care knowledge and practices for neonates

in preparation for a cluster-randomized trial of the impact of topical chlorhexidine cord cleansing on neonatal mortality and omphalitis. Our pilot study will contribute to the design of programs intending to implement chlorhexidine cord care interventions in Africa and elsewhere.

Methods

Study area and subjects

The study was carried out in Pemba Island, Tanzania, the smaller of the two islands of the Zanzibar archipelago. All births occurring in October and November 2010 at five major hospitals (four district hospitals and one cottage hospital) in the island and in the community were included in the study till the desired sample size was achieved.

Ethical approval

Ethical approval for the study was obtained locally from the Zanzibar Medical Research and Ethics Committee (ZAMREC) and from the Johns Hopkins Bloomberg School of Public Health Committee on Human Research. Informed verbal consent was obtained from all study participants in local language.

Study procedures

From the community and maternity wards of the hospitals, 204 mother-newborn pairs were enrolled to pretest three possible modes of intervention i.e. 100 ml bottle with cotton swab (A), 10 ml single use dropper bottle (B) and 3 gm single application gel tube (C) using Trials of Improved Practices (TIPs) methodology. A communication network was established with all the trained birth attendants (TBAs), maternal and child health (MCH) workers, hospital staff and health professionals working on the island. Each personnel was provided with a cell phone and a 24-hour study call center was set up at the central office to ensure immediate and regular communication.

Data collection tools

The Research Scientists in consultation with the Principal Investigator designed the working protocol, methods for data collection, standard operating procedures (SOPs) and consent forms for study implementation. Detailed questionnaires were prepared for collection of socio-demographic information, pregnancy history, birth characteristics, and newborn care practices at enrollment, compliance information at follow up visits and participant feedback at the evaluation visit. Log sheets were designed to help mothers record CHX application on daily basis by simply putting a tick mark.

Training and reliability

Training sessions were organized to train the MCH staff, TBAs, hospital staff and health workers on application of all the three modes of intervention. This was followed by practice session wherein each one of them practiced CHX application on a dummy. A dry run was conducted to ensure reliability and effective implementation of study protocol. On scheduled visits, mothers were demonstrated and instructed to apply chlorhexidine to the tip (over the cut surface) of the cord, the stump and around the base of the stump.

TIPs intervention

First phase of formative research (ethnography) helped us to understand the barriers and facilitators to the introduction of chlorhexidine as a cord care regimen, develop communication messages, study procedures and the framework for implementing a cord care intervention based on the information gathered. TIPs phase involved initial enrollment visit, two follow up visits and a final assessment visit. Study team demonstrated the use of different containers for CHX application to the mother at the enrollment and follow up visits. Essential newborn and cord care messages were given to the mothers at each visitation. Mother's feedback about different containers was recorded in a standard questionnaire.

Randomization

Two separate randomization schedules were generated for hospitals and community births. There were 6 possible sequences of allocating the enrolled mother-newborn pair to one of the 3-intervention modes-100 ml bottle (A), 10 ml dropper bottle (B) or gel tube (C). A mother-newborn pair randomized to sequence 1 (A B C) would use 100 ml bottle for first 3 days, 10 ml single use dropper bottle for next 3 days and get tube for the last 3 days. Other Possible sequences were (A C B), (B A C), (B C A), (C A B) or (C B A). Each delivery mode was used for the same period (three consecutive days). Randomization was done using permuted block randomization method (block length of 12) which generated a list of randomly allocated intervention sequence against a serial number. This ensured uniform distribution of the intervention sequence (three application methods) to one of the six possible delivery sequences. Envelopes were prepared with serial number written on them and the assigned intervention sequence sealed inside the envelope. Upon enrollment, the supervisor opened the next envelope from the sequence and allocated the enrolled mother-newborn pair to the intervention sequence/pack printed inside it and applied the first application mentioned in the slip to the child. Until the opening of the seal of the envelope, both the supervisor/

researcher and the mothers were kept blind to the allocation of the intervention sequences.

Enrollment in hospital

A surveillance system was established in the maternity ward of all five major hospitals in the island. Female hospital supervisors worked in shifts at the maternity ward to cover all deliveries occurring from 7 AM till 8 PM at night. Deliveries occurring after 8 PM were enrolled the next morning. After birth the study team, comprised of hospital staff and study supervisor, screened the newborn for eligibility to participate in the study. If the newborn was found eligible (not very sick, did not need hospitalization and ICU care and without any congenital malformation eliminating the possibility of CHX intervention), the study procedure and purpose was explained to the mother once she was stable. In case the mother was deemed not fit, it was explained to the nearest kin and their consent to participate was sought. If the consent was obtained, the mother-newborn pair was enrolled in the study. The hospital supervisor then opened the envelope for the enrolled pair which contained information about the intervention sequence and the pack. The supervisor took the intervention pack out and handed it over to the hospital staff to apply on the cord of the baby. The hospital supervisor/hospital staff applied CHX on the cord as per the first method mentioned in the sequence and also demonstrated the application to the mother/caretaker and gave the supply for the next 2 days. On discharge, the hospital supervisor completed discharge slip with detailed information from mother about the place where she will be moving after discharge. The case was then handed over to the respective district in charge for follow-up visits in the community.

Enrollment in community

MCH/TBA informed the central information system (CIS) for any new births occurring in the community. CIS after getting new birth information organized a conference call between the District In charge, Field Supervisor and MCH staff responsible for that area to plan immediate visit to that household. Field supervisor with MCH staff visited the household of the newborn and took consent from mother. Supervisor then opened the envelope containing the intervention sequence and container for that newborn. MCH staff applied the cleansing solution to the tip, base and stump of newborn's umbilical cord and Supervisor demonstrated the application to the mother/caretaker and gave the supply for the next 2 days. At enrollment, information was collected on SES (socio-economic status) features, pregnancy history, problems during delivery, birth characteristics, and newborn care practices.

Follow-up visits

Follow-up home visits were conducted by the MCH staff/study supervisor on day 4, 7 and 10. During the follow-up, mother was asked to put a tick on the log sheet on the days she applied the allocated mode of intervention. On the visit day-4 and −7, MCH staff/study supervisor applied CHX using second and third type of container, respectively (as per the sequence allocated). They also demonstrated the application method for cleaning the cord to the mother and left the containers to be used for next 2 days with the mother. On these visits, data on the reported use of solution by the mother was recorded by checking the log sheet and counting the number of used containers. In case the mother had not applied the CHX, the study team member asked the mother of the reason for not applying the intervention and recorded it in the questionnaire.

Assessment visit

The household was visited on day 10 for final assessment. The mother was asked about her experience of using different delivery modes for cleaning the cord in terms of convenience and preference for the choice of the container i.e. how easy or difficult it was to use them and her preferred container. The staff also recorded number of days mother used the cleaning solution/gel from the log sheet.

TIPs for MCH and hospital staff conducting deliveries

TBA, MCH and hospital staff undertaking the deliveries and involved in the TIPs component of the study were also interviewed regarding their experience and feedback on the three different delivery methods used.

Chlorhexidine preparations

CHX solution was prepared by Galentic Pharma (India) Pvt. Ltd. It contained chlorhexidine gluconate 20 % *w/v* solution BP, polyoxyl 40 hydrogenated castor oil NF (RH 40), carmoisine, purified water BP, and isopropyl alcohol BP. Chlorhexidine gel contained chlorhexidine gluconate, hydroxyl-propyl methyl cellulose, glycerin, methyl-paraben, propyl-paraben and purified water.

Statistical analysis

Descriptive statistics (frequencies, percentages, means and standard deviation) were calculated, excluding missing data from the analysis. Convenience and preference scores were calculated based on mothers, MCH's and hospital staff's feedback. A container was assigned convenience score of '2' if it was selected as most convenient to use, a score of '1' if it was selected as convenient and a score of '0' if it was difficult to use. Preference scores were assigned based on the preference/choice of container. A score of "2" for first preference; "1" for

second preference, and the non-preferred container received a score of '0'. Data were analyzed using SPSS Statistical Program Version 18.0 (SPSS, Chicago, IL).

Results

Among the 204 mother-newborn pairs, 27 were lost to follow up primarily due to families moving out of the area, leaving 177 pairs (87 %) who completed the 10 days follow-up period. 17 % of the pregnant women in this study were over 35 years of age and 24 % were illiterate. More than half of the enrolled women were housewives (Table 1).

TIPs for different modes of CHX delivery

Allocation of the intervention sequence (three application methods; A-100 ml bottle, B-10 ml dropper bottle and C-gel) to one of the six possible delivery sequences i.e. (A B C) or (A C B) or (B A C) or (B C A) or (C A B) or (C B A) was uniformly distributed. TIPs revealed that in 81 % of the cases, first application of CHX occurred within 12 h of birth and in 72 % cases within 8 h, irrespective of the mode.

The compliance was high; 97 % of mothers used all the three modes of intervention. No adverse event due to any mode of intervention was reported during the course of study. Mothers reported little difficulty in using three application methods (100 ml bottle – 83.1 % reported no difficulties, 10 ml dropper bottle – 89.3 %, Gel – 71.8 %). It was observed that an additional effort was required to apply the gel (15.8 %); 100 ml bottle (10.7 %) and 10 ml dropper bottle (5.1 %). Gel preparation took more time to dry (7.3 %) than the other two application methods.

Most of the mothers felt that the 10 ml dropper bottle was most convenient to apply (49.7 %) compared to 100 ml container (19.8 %) or gel tube (32.2 %). From the mothers' perspective, even though cotton ball made the application easier, single use dropper bottle was more convenient to use than single use gel. Even when convenience scores were calculated, 10 ml single use dropper bottle was found to be more convenient by the mothers/caretakers than 100 ml container or gel tube (Mean convenience score for 10 ml bottle 1.4, in comparison to 0.8 and 0.9 for 100 ml and gel respectively). Mothers/families selected 10 ml dropper bottle (44.6 %) as their most preferred choice over the 100 ml bottle (20.9 %) or gel tube (33.9 %) for cleansing the umbilical cord of the newborn. When the different application methods were compared, the preference score was highest for 10 ml single use dropper bottle (Mean preference score 1.4 as compared to 0.8 and 0.9 for 100 ml and gel respectively- Table 2). Mothers preferring 10 ml single use bottle or gel tube also seem to be well aware of good newborn care practices (Table 3). Based on the preference score, TBAs, medical staff and CHWs preferred 10 ml dropper bottle (43.3 %) over 100 ml bottle (12.9 %) and gel (38.8 %). Delivery sequence did not change the preference for mode of delivery (Fig. 1).

Table 1 Study participant characteristics ($N = 204$)

Characteristics	N (%)
Age of mother	
≤18 years	8 (3.9)
19–35 years	161 (78.9)
>35 years	35 (17.2)
Literacy	
Mother (Illiterate)	49 (24.0)
Father (Illiterate)	37 (18.1)
Occupation	
Mother (Housewife)	105 (51.5)
Father (Fishing/ Farming)	86 (42.2)
Income	
Mother (None)	123 (60.3)
Father (None/ < 50,000 shilling)	72 (35.3)
Parity	
Primiparous	34 (16.7)
2–3	61 (29.9)
4–8	91 (44.6)
>8	18 (8.8)

Discussion

Chlorhexidine is an inexpensive, safe and effective cord care intervention for reducing neonatal morbidity and mortality in low-resource settings [13–15]. The present study evaluated the acceptance and impediments to using chlorhexidine comparing three different modes (3 different packaging: 100 ml bottle with cotton swab, 10 ml single use dropper bottle and 3 gm single application gel tube) of delivery for cord cleaning in terms of acceptance, ease of use and effectiveness in covering the target area using TIPs (Trials of Improved Practices) methodology. The overall acceptability in terms of convenience and preference was high for 10 ml single use dropper bottle; which was liked by most of the mothers, TBA/MCH and hospital staff over 100 ml bottle and gel tube. Despite chlorhexidine (in liquid form) being spread over the abdomen through its use, 10 ml single use dropper bottle was the preferred choice. Mothers did not find much difficulty in applying the solution. The advantage of using a crossover design was that every mother had an experience of testing all the three modes of delivery of chlorhexidine to apply on the umbilical cord and could therefore perceive the risks/benefits associated with each.

Table 2 Preference and convenience scores

Preference Scores	100 ml	10 ml	Gel
Mothers			
Most preferred (Score 2)	37 (20.9)	79 (44.6)	60 (33.9)
Less preferred (Score 1)	60 (33.9)	69 (39.0)	48 (27.1)
Not preferred (Score 0)	80 (45.2)	29 (16.4)	60 (39.0)
Mean scores for preference ± (SD)	0.8 (0.7)	1.4 (0.7)	0.9 (0.8)
MCH Workers			
Most preferred (Score 2)	12 (17.9)	26 (38.8)	29 (43.3)
Less preferred (Score 1)	11 (16.4)	30 (44.8)	26 (38.8)
Not preferred (Score 0)	44 (65.7)	11 (16.4)	12 (17.9)
Mean scores for preference ± (SD)	0.5 (0.8)	1.2 (0.7)	1.2 (0.8)
Convenience Scores			
Mothers			
Most preferred (Score 2)	35 (19.8)	88 (49.7)	57 (32.2)
Less preferred (Score 1)	77 (43.5)	66 (37.3)	53 (29.9)
Not preferred (Score 0)	65 (36.7)	23 (13.0)	67 (37.9)
Mean scores for convenience ± (SD)	0.8 (0.7)	1.4 (0.7)	0.9 (0.8)
MCH Workers			
Most preferred (Score 2)	10 (14.9)	31 (46.3)	32 (47.8)
Less preferred (Score 1)	40 (59.7)	35 (55.2)	25 (37.3)
Not preferred (Score 0)	17 (25.4)	1 (1.5)	10 (14.9)
Mean scores for convenience ± (SD)	0.9 (0.6)	1.4 (0.5)	1.3 (0.7)

All figures shown are N(proportion) unless otherwise indicated

There were no apparent side-effects and no serious adverse events related to any of the interventions used during the course of study. The concentration of chlorhexidine used was equivalent to that used in prior trials [13–15, 17, 26, 27]. All the three modes had the same concentration of chlorhexidine (4 %) and the gel formulation was thickened using hydroxy propyl methyl cellulose. Both the gel and liquid formulations were produced by Galentic Pharma (India) Pvt. Ltd. and made available at a low cost of ~ USD 0.02 per application.

Two studies conducted in Nepal which evaluated the acceptability and ease of use of gel and liquid chlorhexidine indicated that gel formulation was more acceptable and a preferred approach by families over liquid formulation [16, 17]. However, no information on the choice of delivery container (100 ml bottle with cotton swab or 10 ml dropper bottle) was provided for chlorhexidine liquid solution application. There can also be a possibility in those studies that the participants failed to express negative concerns about the intervention, anticipating better care. In previously conducted trials in Nepal, Bangladesh and Pakistan, chlorhexidine was applied using wipes, cotton balls or syringes [15, 17, 28, 29].

Information collected through TIPs helped in selection and implementation of a culturally acceptable intervention for the main trial 'evaluating the efficacy of use of chlorhexidine to clean umbilical cord of neonates in first

Table 3 Newborn care practices and mode of Chlorhexidine application preference

Practices	100 ml	10 ml	Gel
Thermal care provided ($N = 108$)	20 (18.6)	44 (40.7)	44 (40.7)
Skin to skin contact ($N = 158$)	32 (20.3)	73 (46.2)	53 (33.5)
Child wrapped in clean cloth ($N = 174$)	38 (21.8)	76 (43.7)	60 (34.5)
Did not bath baby immediately after birth ($N = 152$)	31 (20.4)	73 (48.0)	48 (31.6)
Fed on colostrum ($N = 153$)	36 (23.5)	67 (43.8)	50 (32.7)

Figures shown are N(%)
N indicates the number of mothers/caregivers providing newborn care practice

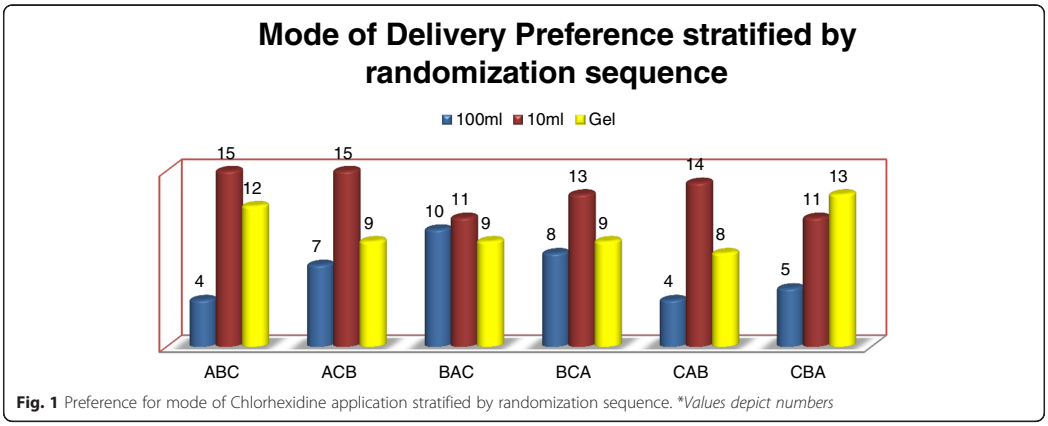

Fig. 1 Preference for mode of Chlorhexidine application stratified by randomization sequence. **Values depict numbers*

10 days for reduction in neonatal mortality and omphalitis (Clinical Trial Number: ClinicalTrials.gov NCT01528852)'. Based on the choice of mothers, hospital staff and TBA/MCH, the 10 ml single use dropper bottle was selected for the RCT.

Conclusion

It is the first trial of its kind reporting mothers/caretakers and health professionals' acceptability and preference for various possible modes of chlorhexidine delivery for cord care. 10 ml single use dropper bottle was given highest preference for delivery of intervention. In wake of current effort to scale up chlorhexidine cord care interventions in various countries, with appropriate changes in WHO recommendation for cord care, our pilot study has lot of relevance for the programs intending to implement chlorhexidine interventions for reduction in omphalitis and neonatal mortality. Selection of the most acceptable method of intervention delivery is essential to the design and implementation of the intervention efficacy trials as well as successful implementation of programs.

Abbreviations

CHX: Chlorhexidine; TIPs: Trials of Improved Practices; TBA: Trained birth attendants; MCH: Maternal and child health; SES: Socio-economic status; SPSS: Statistical Package for the Social Sciences.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

UD, SS and REB were involved in the conceptualization of research, development of study protocol and questionnaires, and the preparation of manuscript. PD helped with the development of study questionnaires, interpretation of data and manuscript writing. AD and SD assisted with development of study tools, field management, data analyses, interpretation of results, and contributed to the text of the manuscript. Both SMAs helped with adaptation of methods, Pemba study administration, community mobilization and provided leadership and supervision to field team members. AMS helped with data collection and supervision in the field and reviewed the manuscript. All authors read and approved the final manuscript.

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Paper III

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Effect of 4 % chlorhexidine on cord colonization among hospital and community births in India: a randomized controlled study

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Abstract

Background: Infections are the single most important cause of neonatal mortality in developing countries. Results from trials in Asia evaluating the effect of chlorhexidine on neonatal mortality have been encouraging but limited data are available on the impact of cord cleansing on bacterial colonization. Further, no data from facility deliveries and impact with time is available. This pilot study was aimed to evaluate the impact of 4 % commercially prepared chlorhexidine on cord colonization and density of colonization among newborns in India.

Methods: Three hundred twenty-six newborns (hospital-247; community-79) were enrolled within 24 h of birth and randomly assigned to one of three groups: chlorhexidine, placebo or dry cord care. Umbilical swabs were collected at baseline, 2- and 48- hours after intervention application.

Results: At baseline, growth positivity (any bacterial growth) was 20 % (50 of 247 swabs) and 81 % (64 of 79 swabs) among hospital and community born neonates, respectively. In both settings, chlorhexidine compared to placebo and dry cord care, reduced colonization following 2- and 48-hour post application. Chlorhexidine significantly reduced 48-hour post application colony counts in comparison to placebo [Hospital: mean difference = −1.01; 95 % CI: −1.72, −0.30 Community: mean difference = −1.76; 95 % CI: −2.60, −0.93] and dry cord care [Hospital: mean difference = −1.16; 95 % CI: −1.93, −0.39 Community: mean difference = −2.23; 95 % CI: −3.18, −1.29]. Differences were similar for gram-positive and gram-negative bacteria.

Conclusions: Cord cleansing with 4 % chlorhexidine soon after birth reduced colonization as well as density of colonization significantly; however this pilot study does not address the impact of chlorhexidine on mortality. The control preparation neither increased or decreased colonization.

Trial registration: Clinical Trial Registration: clinicaltrials.gov: NCT01528852, Registered February 7, 2012.

Keywords: Neonates, Newborns, Chlorhexidine, Colonization, Bacterial count, Cord cleaning, Umbilical cord, India

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Background

Neonatal deaths account for 40 % of global under-five mortality [1–3] and are a major health concern in the developing countries, particularly in the south Asian and sub-Saharan countries [4, 5]. Each year serious infections account for nearly 13 % of the 3 million neonatal deaths, this proportion is closer to 50 % in settings with high mortality risk [2]. Five countries namely India, Nigeria, Democratic Republic of the Congo, Pakistan and China alone account for half (2.440 million) of the global deaths from infections and 53.3 % (1.636 million) of neonatal deaths [5]. Community based studies from developing world suggest that infections are responsible for 8 to 80 % of all neonatal deaths and as many as 42 % deaths in first week of life [5, 6].

In neonates, umbilicus acts as a bacterial reservoir and a potential entry point for the infection, especially in first few days of life, when umbilical vein is patent. This may lead to sepsis with or without omphalitis [7, 8]. The responsible organisms most likely originate in maternal genital tract and are acquired during labor and delivery [9]. In low income countries, many neonatal infections are environmentally acquired because of higher number of home deliveries, unsafe traditional practices, untrained birth attendants and unclean living conditions, all of which pose an increased risk of umbilical cord infection [10]. The local signs of umbilical cord infection include pus, redness, swelling, warmth, tenderness and foul odour [11] and seem to be associated with increased risk of mortality [12]. Infectious organisms may get directly transmitted from patent umbilical cord to the blood stream without evident signs of local cord infection [13].

Recommendations for umbilical cord care, particularly in regard to prevention of umbilical cord infection, are controversial. In developing countries, World Health Organization (WHO) [10, 14] promotes dry cord care. These recommendations were based on lack of evidence for alternative approaches [14]. Based on currently available evidence, the WHO recommends 7.1 % chlorhexidine digluconate solution or gel, (delivering 4 % chlorhexidine) among home deliveries in settings with neonatal mortality rate more than 30 [15]. This interim recommendation awaits review of further data especially the results of two large trials in Africa.

Of topical antiseptics (eg, ethanol, silver sulfadiazine, triple dye, gentian violet, chlorhexidine, povidine iodine), chlorhexidine with strong residual activity has shown potential as an effective cord care agent during the neonatal period against both gram-positive and gram-negative organisms [16–18]. It has an excellent safety profile, is rarely associated with bacterial resistance, is easy to administer and costs few cents per application [9, 19, 20]. Available high-quality evidence from the recently conducted Cochrane review indicates that cord cleansing with 4 % chlorhexidine reduces the risk of neonatal mortality by 12 % and sepsis (omphalitis)/infections by 50 % in low-resource community settings including Nepal, Bangladesh, and Pakistan [21]. However, in hospital settings, chlorhexidine cord cleansing reduces the risk of omphalitis/infections by 52 % and may lead to no difference in neonatal mortality as compared to dry cord care. Despite all these data, limited evidence is available on the mechanism of this protective effect [22–24] and only one study [25] published recently suggested an impact on bacterial colonization of the cord and provided information on early neonatal colonization dynamics in community setting. There are no data available evaluating impact of chlorhexidine on colonization among facility births, and spectrum of chlorhexidine overtime. In all the earlier chlorhexidine trials evaluating impact on mortality and/or cord infections, 4.0 % free chlorhexidine were prepared by diluting 20 % chlorhexidine digluconate to the appropriate concentration with purified water at the study sites. As there was no data available from Africa, two large trials in Africa (Pemba, Tanzania and Zambia) were funded by Bill and Melinda gates Foundation to evaluate the mortality impact of the intervention. In the present pilot study, we tested the antimicrobial activity of the commercially prepared 4 % chlorhexidine solution as a cord-cleansing agent, and evaluated its impact on bacterial colonization and colony counts after 2- and 48-hour post-application in hospital- and community born neonates in comparison to placebo (same solution without active ingredients) and dry cord care. The aim of this pilot study was to ensure and document the efficacy of preparation before evaluating mortality and sepsis impact in large trials (more than 60,000 newborns in African setting). In addition, the pilot study provided data on potential cord colonizing pathogens that could be responsible for sepsis, apart from regular skin flora.

Methods

Study design

This pilot study was a prospective, randomized, controlled trial conducted in New Delhi, India. There could be significant differences in environmental cleanliness and bacteriologic profile in the community and hospital settings; study subjects from both the settings were included in order to have a representative sample.

Study population

The hospital component of the pilot study was conducted at Kalawati Saran Children's Hospital, New Delhi while the community component was undertaken at Sangam Vihar, New Delhi.

Kalawati Saran Children's Hospital is a government hospital catering to patients from the lower socio-economic

strata. Approximately 40–60 deliveries take place daily in this hospital.

Sangam Vihar is a resettlement colony mainly comprising of migrants from rural areas of the country. Its population is predominantly from the low socio-economic strata of the society. Almost 60 % of the births in Sangam Vihar take place at home (20–25 deliveries daily).

Training and reliability

Training sessions were organized for the field workers, supervisors and hospital staff who were apprised of the study protocol and the various forms that need to be filled at different time points. Swab sample collection from the umbilical cord as well as cleaning of cord using the assigned intervention method was demonstrated using a dummy doll. This was followed by practice and return demonstration by trainees on the dummy. Subsequently, dry run sessions were undertaken by the staff to ensure reliability and effective implementation of study protocol.

Recruitment and enrollment

Enrollment in hospital

Study team was stationed outside the maternity ward from 8.00 am to 5.30 pm. After birth, the study team comprising of hospital staff and study supervisor visited the mother baby duo and screened the newborn for eligibility to participate in the study. If the newborn was found eligible (normal delivery, full term healthy newborns of both sexes with birth weight >2500 g, first contact ≤48 h), the study purpose and procedure were explained to the mother once she was stable or else to the nearest kin and the consent for their newborn's participation in the study was sought. The newborn was enrolled in the study after the consent was obtained from the parents. Neonates requiring resuscitation and admission to NICU and also those with major congenital malformation were excluded.

Enrollment in community

A survey was carried out by trained birth attendants (TBAs) to identify the pregnant women in the six blocks of Sangam Vihar. A record of all identified pregnancies with their tentative due delivery dates was prepared. The family members and TBAs were instructed to contact the study supervisor at the time of delivery or immediately thereafter. The study supervisor and fieldworker along with the TBA visited the newborn within 48 h of delivery. They screened the newborn for eligibility (vaginally delivered, term healthy newborns, first contact ≤48 h) and if found eligible, the study purpose and procedure were explained to the mother and the consent for their newborn's participation in the study was sought.

Sample size estimations

Based on Nepal hospital data (Hodgins et al [26]) the sample size was estimated to be 80 in each intervention group for the hospital setting, with an alpha of 0.05 and power of 80 %, to detect a reduction in bacterial colonization from 29 to 11 % in the chlorhexidine group. Based on Bangladesh study (Mullany et al, personal communication), the sample size was estimated to be 44 in each intervention group in the community setting, with an alpha of 0.05 and power of 80 %, to detect a reduction in bacterial colonization from 93 to 69 % in the chlorhexidine group.

Masking and randomization

All enrolled neonates were randomly allocated to 1 of the 3 intervention groups: (a) 4 % chlorhexidine or (b) placebo (mild soap water) or (c) dry cord care group. Both the chlorhexidine and the placebo groups were blinded and the preparations were identical in packaging, appearance, colour, consistency and odour. It was not possible to blind the allocation to the dry cord care group. Each intervention group was identified by a letter code. The manager in the manufacturing unit decided the codes to be used for the solutions and kept the information secret till the data analysis was complete. Thus, neither the mothers of the neonates nor the hospital/field staff knew which intervention was being used. Two separate randomization lists were prepared; one for the hospital and other for the community births. For the hospital, a randomization list containing running serial number and randomly allocated code (A through I) was generated. These letter codes were used to identify three intervention groups. In order to ensure equalization of groups, six letter codes for the solution (3 codes for chlorhexidine and 3 for placebo groups) and three letter codes for the dry cord care were used. In-house computer software generated a random sequence of group codes with permuted block length of 18. Neonates were allocated to one of the groups in the order in which they got enrolled. For the community births, similar randomization procedure was used.

Intervention description

Intervention was prepared by Galentic Pharma (India) Pvt. Ltd, Mumbai, India. Table 1 shows the composition of the chlorhexidine and placebo solution. For the intervention stability, potency, colour, odour and consistency, the preparations were tested at various stages.

Study procedures

Baseline (0 h) swab collection

For hospital births, soon after delivery, hospital supervisor checked the hospital records to record birth related information. Before beginning the procedures, the resident

Table 1 Composition of chlorhexidine and chlorhexidine placebo

Ingredients	Chlorhexidine 4 % solution (% *v/v*)	Placebo solution (% *v/v*)
Chlorhexidine gluconate 20 % *w/v* solution BP	35.70	-
Polyoxyl 40 hydrogenated castor oil NF (RH 40)	0.80	0.80
Carmoisine	0.0005	0.0005
Purified water BP	Q.S to 100.00	Q.S to 100.00
Isopropyl alcohol BP	4.00	-
Mild soap [Sodium lauryl sulphate[a] (STEROCARE SLS (L))]	-	0.40
Silicon antifoaming agent[a]	-	0.001

Note: [a]sodium lauryl sulfate is used to match the foaming which is seen in chlorhexidine 4 % solution and silicon antifoaming agent is used to avoid the excess foam which forms in the placebo

doctor washed his/her hands and donned sterile gloves. The resident doctor on duty collected the swab using HIMEDIA HiCulture™ transport swabs with amies medium with charcoal (Cat no: MS 651) from the tip, stump and base of the umbilicus and peri-umbilical region (2.5 cm radius around umbilicus) and the hospital supervisor immediately put the bar-coded sticker on the swab tube and pasted the corresponding duplicate sticker on the form. The date and time of the collection of each swab was recorded on a form. Within 4 h of collection, the swabs were transferred to the laboratory for culture analysis. Field/study worker used the same procedure to collect swabs among the community births.

Application of intervention (immediately following the collection of swab)

After the collection of swab for culture analysis, hospital staff/fieldworker applied the assigned intervention immediately. She/he opened the designated intervention pack and applied the intervention to cleanse the tip, stump and base of the umbilicus and peri-umbilical region. The intervention was applied twice: first after the collection of baseline swab and second 24 h after first application. In dry cord group, no intervention was applied and the standard WHO guidelines for cord care were followed [10].

Swab collection after 2-hours and 48-hours of application of intervention

In both settings, 2-hours and 48-hours post application, the study staff collected the umbilical swab, following the same procedure used for pre-application swab collection. In case of dry cord, the sample collection and timing was matched i.e. it was taken 2- and 48- h after the first sample.

Culture analysis

The culture analysis for umbilical cord swabs was performed at Dr Dang's Medical Diagnostic Center, Hauz Khas, New Delhi, India. Umbilical swabs collected using HiCulture™ Transport Swabs (Cat No. MS651) were transported to laboratory for aerobic culture. All samples (i.e. 10 μL of the neat specimen) were inoculated in 3 media plates i.e. McConkey Agar, Blood Agar and Chocolate Agar. The plates were incubated as per recommended time and temperature and were checked for absence or presence of bacterial isolates. Smear was prepared for identifying gram-negative and gram-positive bacteria. Based on the morphology and colour of the stain taken by the bacteria, they were classified into gram-positive or gram-negative organisms. Samples were further tested for species identification using standard manual methods and Vitek 2 compact system from BioMerieux France. For colony counts, the samples were put in 1 ml of normal saline for 3 log dilution and sub-cultured using standard subculture technique [27]. Presence of bacterial growth, identification to gram-positive/-negative bacterial organisms and semi-quantitative colony count was estimated for all samples. Laboratory staff members were blinded to the formulation used; the swabs and the accompanying forms were labeled with bar-coded stickers. In addition, various quality control measures were followed using standard American Type Culture Collection strains during each step and inter/intra observer reliability tests in microscopy.

Statistical analysis

We used Visual Basic 6.0/ASP.net and Oracle 8i to manage the data, with stringent range, consistency, and logical checks. To ensure data quality and accuracy, real time data entry was done using netbook. Data were analyzed separately for the hospital and community births using Statistical Package of Social Sciences (SPSS Inc., Chicago, Illinois, USA, SPSS, Version 19.0 for Windows) and Stata (Stata Corp., College Station, Texas, USA, Intercooled Stata 12.0 Version), $P \leq 0.05$ was considered statistically significant for all analyses. Descriptive statistics (frequencies, percentages, means and standard deviation) were calculated. We examined the characteristics of newborns and mothers across the allocated groups on a range of variables to determine the degree of balance achieved by the randomization. We analyzed colonization positivity data by intervention groups and follow-up time. Among those

that were positive, we further estimated distribution of colony counts by intervention groups and follow-up time. A paired analysis comparing the baseline positivity/colony count of the child with his 2- and 48-hours post intervention positivity/counts was performed to study any group differences as well as changes over follow-up time within group. The chi square, *t*- test and OR with 95 % CI were used to estimate statistics and significance.

Outcomes

The primary outcome was culture positivity at 2-hours and 48-hours post application and the secondary outcome was bacterial colony count from umbilical and peri-umbilical region to estimate the concentration of viable bacteria. The reduction in bacterial colonization was assessed as proportion of neonates with positive culture (from umbilical and peri-umbilical area) 2-hours and 48-hours after application of 4 % chlorhexidine solution as compared to dry cord care and control groups. The reduction in density of bacterial colonization (limited to samples with growth) after 2-hours and 48-hours of application of 4 % chlorhexidine solution as compared to dry cord care and control groups was assessed in terms of mean reduction in bacterial colony counts. The risk of colonization stratified by gram-positive and gram-negative organisms was estimated by intervention groups.

Results

Participants

Between November 6, 2010 and December 10, 2010 and then from January 31, 2011 to February 9, 2011, a total of 326 newborns both from the hospital (n-247) and community (n-79) were enrolled in the study. In both the settings, newborns were randomly assigned to chlorhexidine, placebo or dry cord care group and umbilical swabs were collected at baseline (before the application of intervention), 2-hour and 48-hour after the application of the assigned intervention (Fig. 1). Of the 247 neonates enrolled from the hospital, 48-hour swab sample could not be collected in 62 neonates (chlorhexidine-23, placebo-22, dry cord care-17) as the subjects took early discharge from the hospital and only contributed the first (baseline) and second (2-hour) swabs. The rate of discharge before the third swab did not differ between the 3 intervention groups. A total of 916 umbilical swabs (hospital 679, community 237) were collected. There was no reported adverse event in the intervention groups in both the settings.

Baseline characteristics

Hospital data

In hospital, among the 247 enrolled neonates, 86 were allocated to chlorhexidine, 86 to placebo, and 75 to dry cord care. Comparison of baseline characteristics is shown

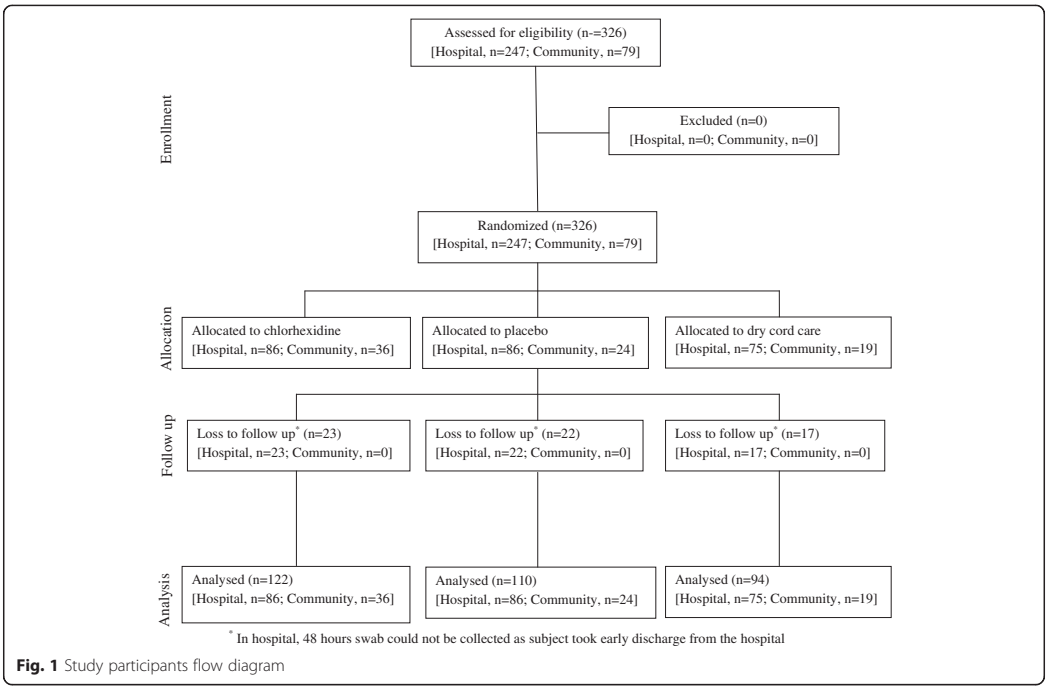

Fig. 1 Study participants flow diagram

in Table 2. All groups were comparable as regards the baseline characteristics such as age of the mother, mode of delivery, and birth weight of newborns etc. The proportion of males was higher in groups other than the chlorhexidine group. Risk factors for infection such as prolonged labor >24 h, leaking per vagina >24 h, meconium staining, and maternal urinary tract infection were present but the proportion was very low. The time since birth when the neonate got enrolled in the study was similar across the three groups.

Community data

Of the 79 neonates enrolled in the community; 36 were assigned to chlorhexidine, 24 to placebo and 19 to dry cord care groups. At baseline, no significant differences were seen in a variety of variables measured, such as age of the mother, cord cutting methods, hand cleaning before conducting delivery and babies receiving bath immediately after delivery. The proportion of males was lower in chlorhexidine group (Table 2).

Bacterial colonization and density of colonization among positive cultures

Hospital data

At baseline, proportion of positive swabs was 20 % (50 of 247 swabs) among hospital born neonates; it was little higher in chlorhexidine group (26.7 %) than dry cord care (18.7 %) and placebo (15.1 %) groups. Chlorhexidine group showed reduction in colonization in both 2-hours and 48-hours post application in comparison to baseline. However, there was an increase in growth positivity rates following 2-hour and 48-hour post application in dry cord (91.4 %) and placebo groups (90.6 %). Among those with positive culture results, colony counts were observed to assess if there was any intervention impact on density. In the chlorhexidine group, compared to placebo and dry cord care group, the mean colony count at the 2-hour and 48-hour follow-up was lower than the baseline (Table 3) indicating that chlorhexidine was effective in reducing the bacterial load.

Table 4 presents the paired comparison between the groups for bacterial colonization and colony counts. Comparison of change in growth positivity from baseline to 2-hours post application showed 80 % reduction with chlorhexidine application in comparison to placebo [OR = 0.20; $p = 0.001$] and 81 % reduction compared to dry cord care [OR = 0.19; $p < 0.001$]. There was a 98 % reduction in change in growth positivity between baseline and 48-hour post application [chlorhexidine vs. placebo: OR = 0.02; $p < 0.001$ and chlorhexidine vs. dry cord: OR = 0.02; $p < 0.001$]. Chlorhexidine showed significant reduction in change in colony counts from baseline to

Table 2 Baseline characteristics and risk factors for infection[a]

Variables	Chlorhexidine	Placebo	Dry cord
Hospital data	(*n* = 86)	(*n* = 86)	(*n* = 75)
Age of mother (years, mean ± SD)	23.87 ± 2.54	23.77 ± 2.86	25.12 ± 3.24
Vaginal delivery	100.0	100.0	100.0
Male births	48.8	64.0	58.7
Birth weight (grams, mean ± SD)	2728.3 ± 479.5	2684.3 ± 453.6	2794.8 ± 437.6
No. of pelvic examinations (mean ± SD)	2.37 ± 1.25	2.07 ± 1.00	2.00 ± 0.97
Problems during delivery			
Meconium staining	2.3	2.3	5.3
Prolonged labor	2.3	1.2	1.3
Maternal risk factors for infection			
LPV[b] > 24 h	4.7	2.3	2.7
Chorioamnionitis	0.0	0.0	0.0
Fever in mother	1.2	0	1.3
Maternal UTI	0.0	0.0	1.3
Time since birth (hrs:min, mean ± SD)	4:39 ± 2.31	4:35 ± 2:49	4:12 ± 2:37
Community data	(*n* = 36)	(*n* = 24)	(*n* = 19)
Age of mother (years, mean ± SD)	24.42 ± 3.95	24.62 ± 4.13	25.79 ± 3.91
Male births	38.9	50.0	52.6
Did the person clean hands before conducting delivery	97.2	100.0	100.0
New razor blade for cutting the cord	100.0	95.8	100.0
Baby given bath immediately after delivery	94.4	100.0	100.0

[a]Values are in percentages unless specified
[b]*LPV* leaking per vagina

Table 3 Bacterial colonization (proportion with positive culture) and colony counts by intervention group and time of swab collection

Variables	Baseline	2-hour post intervention	48-hour post intervention
Hospital data			
Bacterial colonization[a,b]			
Chlorhexidine (n-86, 86, 63)	23 (26.7)	8 (9.3)	12 (19.0)
Placebo (n-86, 86, 64)	13 (15.1)	23 (26.7)	58 (90.6)
Dry Cord (n-75, 75, 58)	14 (18.7)	24 (32.0)	53 (91.4)
Colony counts (Limited to samples with growth)[c,d]			
Chlorhexidine (n-23,8,12)	3.67 ± 1.15	3.01 ± 0.88	3.96 ± 1.69
Placebo (n-13,23,58)	3.71 ± 0.75	3.77 ± 1.06	4.97 ± 0.97
Dry Cord (n-14, 24, 53)	3.88 ± 0.91	3.96 ± 1.69	5.12 ± 1.07
Community Data			
Bacterial colonization[a,b]			
Chlorhexidine (n-36, 36, 36)	30 (83.3)	16 (44.4)	14 (38.9)
Placebo (n-24, 24, 24)	20 (83.3)	15 (62.5)	16 (80.0)
Dry Cord (n-19, 19, 19)	14 (73.7)	14 (73.7)	16 (84.2)
Colony Counts (Limited to samples with growth)[c,d]			
Chlorhexidine (n-30, 16, 14)	5.28 ± 1.00	3.85 ± 1.15	3.32 ± 1.36
Placebo (n-20, 15, 16)	5.25 ± 1.10	5.35 ± 0.77	5.08 ± 0.80
Dry Cord (n-14, 14, 16)	5.85 ± 1.31	5.76 ± 1.24	5.55 ± 0.93

[a]Bacterial colonization was defined as the growth of any organism from the sample; each swab was defined as positive or negative. The total proportion of neonates positive for any organism was estimated at specific time points i.e. at baseline, 2 and 48 h post intervention and was compared across 3 intervention groups
[b]Values are expressed in N (%)
[c]Colony counts were estimated among the positive culture swabs at baseline, 2-hour and 48- hour post intervention in three intervention groups to measure the density of bacterial colonization. The counts were measured in terms of colony-forming units (CFUs) per ml
[d]Values are expressed in mean ± SD

48-hour post application in comparison to placebo [difference in mean = −1.01; $p = 0.006$] and dry cord [difference in mean = −1.16; $p = 0.004$].

Community data

Among community births, baseline positivity was 81 % (64 of 79 swabs) (Table 3). Chlorhexidine showed a non-significant reduction of 53 % (OR = 0.47; $p = 0.17$) and a significant reduction of 86 % (OR = 0.14; $p = 0.02$) in change in bacterial colonization from baseline to 2 h sample as compared to placebo and dry cord care groups, respectively (Table 4). There was also a significant 83 % reduction in change in bacterial colonization from baseline to 48-hour post application in chlorhexidine group compared to placebo; 90 % compared to dry cord care. Change in mean colony counts from baseline to 48-hour post intervention among growth positives in chlorhexidine group were significantly reduced in

Table 4 Comparison between chlorhexidine vs. placebo/dry cord care for the bacterial colonization and colony counts

Variables	Chlorhexidine vs. placebo		Chlorhexidine vs. dry cord	
	Odds ratio (95 % CI)	*p* value	Odds ratio (95 % CI)	*p* value
Hospital				
Paired comparison for bacterial colonization between-				
Baseline and 2-hour post intervention	0.20 (0.08–0.52)	0.001	0.19 (0.08–0.47)	<0.001
Baseline and 48-hour post intervention	0.02 (0.008–0.07)	<0.001	0.02 (0.007–0.07)	<0.001
Difference in mean of colony counts (95 % CI) at 48 h	−1.01 (−1.72– −0.30)	0.006	−1.16 (−1.93– −0.39)	0.004
Community				
Paired comparison for bacterial colonization between-				
Baseline and 2-hour post intervention	0.47 (0.16–1.37)	0.17	0.14 (0.03–0.71)	0.02
Baseline and 48-hour post intervention	0.17 (0.05–0.63)	0.008	0.10 (0.02–0.51)	0.006
Difference in mean of colony counts (95 % CI) at 48 h	−1.76 (−2.60– −0.93)	<0.001	−2.23 (−3.18– −1.29)	<0.001

comparison to placebo [difference in mean = −1.76; $p < 0.001$] and dry cord care [difference in mean = −2.23; $p < 0.001$].

Specific organisms identified in the hospital and community setting

Among swabs with positive culture, the presence of specific organisms was assessed. In the hospital setting, *Acinetobacter* spp., *Citrobacter diversus*, *Citrobacter* spp., Coagulase-negative *Staphylococcus*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Pseudomonas* spp., *Staphylococcus aureus*, *Staphylococcus* spp. and *Viridans streptococci* were the most common organisms, constituting both gram-positive and gram-negative strains, identified on the umbilical stump, base and the peri-umbilical region. However, in the community, *Acinetobacter iwoffii.*, *Acinetobacter junii*, *Acinetobacter baumanii*, *Acinetobacter haemolyticus*, *Acinetobacter* spp., *Acinetobacter ursingii*, *Aeromonas hydrophilia*, *Aeromonas spp.*, *Cedecea davisae*, *Citrobacter diversus*, *Citrobacter* spp., Coagulase Negative *Staphylococcus*, *Escherichia Coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus lentus*, and *Staphylococcus sciuri* were the most common pathogens identified on the umbilical cord.

When colonization positivity data was analyzed by gram-positive/gram-negative strains, intervention groups and follow-up time, the reductions with chlorhexidine persisted. In hospital born neonates, the overall reduction in colonization for gram-positive as well as gram-negative organisms remained significant for the chlorhexidine group compared to placebo or dry cord care groups for 2-hours and 48-hours post application samples. For the community births, there was a significant reduction in the chlorhexidine group compared to placebo and dry cord for gram-positive bacteria. For the gram-negative bacteria, although there was a trend of reduction, the differences were significant only at 48-hour post application (Additional file 1).

Discussion

Chlorhexidine application over umbilical cord has been shown to reduce the risk of omphalitis and neonatal mortality in community settings in recent trials done in Nepal, Pakistan and Bangladesh [21, 28]. In this double blind randomized controlled trial with facility and community data, we evaluated the impact of 4 % chlorhexidine cord cleansing compared to placebo or dry cord care on cord colonization. Cord cleansing with 4 % chlorhexidine showed significant reduction in colonization and density of pathogens in both facility and community setting. As compared to dry cord care, the impact of 4 % chlorhexidine was greater in the first 48 h after birth suggesting that chlorhexidine may be a possible intervention for reducing omphalitis and sepsis arising from transmission of bacterial infections via the umbilical remnant. These findings are in line with observations reported in a study conducted in Bangladesh [29, 30].

The potential mechanisms for the chlorhexidine action in reducing the colonization rates could possibly be the result of the increased binding efficiency of the detergent form to the umbilical tissues, resulting in prolonged residual effect [30]. This bactericidal effect of chlorhexidine could be attributed to its chemical structure, which is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and enters the cell either through active or passive transport mechanism [31]. Interaction of the positive charge of the molecule with the negatively charged phosphate groups on microbial cell wall [32, 33] alters the cells' osmotic equilibrium, increasing the permeability of the cell wall thus allowing the chlorhexidine molecule to penetrate into the bacteria [34]. Damage to this delicate membrane is followed by leakage of intracellular constituents, particularly phosphate entities such as adenosine triphosphate and nucleic acids. As a consequence, the cytoplasm becomes congealed, with resultant reduction in leakage; thus, there is a biphasic effect on membrane permeability. These early changes are beneficial during the critical first few hours and days of life when most neonatal deaths occur in resource-poor settings [4]. Our data also suggests that mild soap and water solution (placebo) was not very effective in reducing bacterial colonization in comparison to dry cord care. This placebo solution only reduced the pathogens in immediate 2-hours post application swabs in the community setting and did not produce extended effect for 48 h. This could be a mere chance finding or cord cleansing process itself mechanically removed the organisms from the umbilical and peri-umbilical region, subsequently decreasing the bacterial load. This indicates that for residual and cumulative effects, antibacterial agents such as chlorhexidine are substantially more effective than non-antiseptic agents [20].

Bacteriologic colonization rates, profile, and dynamics in a community setting might differ substantially from that in the hospital setting [35]. In the present study, the observed colonization positivity rate was higher in the community-setting (81 %) than in the hospital (20 %) which could be due to unhygienic environment, unclean delivery practices at homes and immediate post-delivery traditional practices. Further, as compared to other studies, the overall pre-application bacterial growth was low in the hospital setting which could be due to differences in neonate handling and infection prevention practices [26, 35, 36]. In the present study, the reported impact of 4 %

chlorhexidine on the reduction of bacterial colonization was consistently high in both settings.

This study also describes the specific pathogens colonizing the umbilical cord. Trends in colonization positivity data were assessed by intervention groups, follow-up time and gram stain status of organisms and it was observed that chlorhexidine showed high bactericidal activity. The evidence presented here for the immediate and extended effect of 4 % chlorhexidine on large and sustained reductions in colonization by gram-negative and gram-positive organisms due to early application with chlorhexidine complements the Cochrane conclusion [14] that colonization is substantially lower among infants receiving topical antiseptics compared with those with no specific treatment (OR = 0.28; 95 % CI: 0.22–0.36).

There were few limitations to our study. The sample size limited our ability to definitively determine whether any of the study outcomes could be influenced by one intervention or the other. But the encouraging findings in our study were that most of the results were statistically significant. Our results provide evidence that the application of 4 % chlorhexidine solution on the umbilical cord provides protection to the baby from bacterial colonization and growth. The findings of our pilot study are important but need to be confirmed in a large community based trial with adequate statistical power as reduction in colonization and colony count in small number of subjects does not have adequate power to evaluate the impact on mortality or sepsis but does provide data on possible mechanisms.

The study cannot address an impact that these changes can have on the microbiome and its implications. From a comparison study in Pemba and Zambia (personal communication and in submission) it is clear that reduction in omphalitis may not essentially result in reduction in sepsis and mortality.

Conclusions

Our study has shown that cord cleaning with the first time commercially produced 4 % chlorhexidine soon after birth can significantly reduce colonization as well as density of colonization among newborns. The study also demonstrated no impact of the control preparation compared to dry cord care on colonization (increase or decrease). The study showed impact of chlorhexidine on colonization in both hospital and community settings, but does not speak to relationship between this reduction and occurrence of sepsis or mortality. The findings of our pilot study are important but need to be tested in large community based trials to establish linkage between reduction in bacterial colonization and sepsis and or mortality. If the link between reduced colonization and reduction in neonatal mortality is established in larger trials in Africa, this intervention could have impact on neonatal mortality in developing country settings where there is limited availability of resources, stringent customs and poor environmental hygiene.

Additional file

Additional file 1: Supplementary Tables (Supplementary Tables 1–3). (PDF 75 kb)

Abbreviations

CI, confidence interval; GN, gram-negative; GP, gram-positive; OR, odds ratio; TBA, trained birth attendant

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Availability of data and materials

Data will not be put in the public domain for another year as we are still working on another manuscript from this data set and will wait for that manuscript to be published before putting the data in the public domain.

Authors' contributions

SN conceptualized and designed the study, critically reviewed and revised the manuscript. PD coordinated and supervised data collection, involved in data analysis, interpreted the results and drafted the initial manuscript. UD and AD designed the data collection instruments, carried out the initial analysis, reviewed and revised the manuscript. VPM and REB were involved in the conceptualization of the research, development of study protocol, and preparation of manuscript. SS conceptualized and designed the study, provided oversight for implementation, directed the analysis, critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Ethical approval was obtained from the Annamalai University, Tamil Nadu, India and from the Lady Hardinge Medical College & Kalawati Saran Children's Hospital, New Delhi, India. Verbal informed consent was obtained in local language from the parents of the newborns prior to the enrolment in the study.

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Paper IV

Efficacy of chlorhexidine application to umbilical cord on neonatal mortality in Pemba, Tanzania: a community-based randomised controlled trial

Sunil Sazawal, Usha Dhingra, Said M Ali, Arup Dutta, Saikat Deb, Shaali M Ame, Mkasha H Mkasha, Ashish Yadav, Robert E Black

Summary

Background In low-income countries, including the east African region, a third of neonatal deaths are due to infections. A substantial proportion of these have been attributed to sepsis, which can result from umbilical cord infections. Evidence from Asia suggests that chlorhexidine application to the neonatal umbilical cord reduces mortality, but no data from Africa are available. We aimed to assess the effect of umbilical cord cleansing with 4% chlorhexidine solution on neonatal mortality and omphalitis in rural settings of sub-Saharan Africa.

Methods We did a community-based randomised controlled trial on Pemba Island, Zanzibar, Tanzania. All eligible babies (aged 1 h to 48 h, without congenital malformations) from hospital-based and community-based deliveries on Pemba Island were enrolled. Participants were randomly assigned to either 4% free chlorhexidine for cord care or to dry cord care using a computer-generated random sequence. For babies allocated to the chlorhexidine group, mothers or caretakers were advised to apply the solution to the cord every day until 3 days after the cord had dropped off. Cord stumps were examined for redness, pus, swelling, and foul odour on day 0, 1, 4, 10, and 28. The primary outcome for this study was mortality until day 28 on an intention-to-treat basis. The trial is registered with ClinicalTrials.gov, number NCT01528852.

Findings Between May 19, 2011, and Aug 31, 2014, 36 911 newborn babies were enrolled into the chlorhexidine (n=18 015) and dry cord care study (n=18 896) groups. 17 468 (96·9%) of 18 015 neonates in the chlorhexidine group were available for complete follow-up (28 days) compared with 18 384 (97·3%) of 18 896 neonates in the dry cord care group. Mortality rate in the chlorhexidine group (10·5 deaths per 1000 livebirths) was not significantly lower than that in the dry cord care group (11·7 per 1000 livebirths; relative risk 0·90, 0·74–1·09; p=0·27).

Interpretation Our findings do not support the use of chlorhexidine for reduction of neonatal mortality in this east African setting, which might not justify a change in the WHO policy. To inform global policy, a detailed meta-analysis and pooled analysis needs to be undertaken using data from both African and Asian settings.

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Introduction

Asia and sub-Saharan Africa have the highest neonatal mortality rates in the world (about 29 deaths per 1000 livebirths),[1] with about 50% of neonatal deaths occurring on the first day of life.[2] Although post-neonatal mortality rates have reduced substantially over the past 25 years, deaths in the neonatal period have decreased more slowly and now account for 47% of all deaths in children younger than 5 years.[1] The worldwide neonatal mortality rate fell from 36 deaths (95% CI 35–38) per 1000 livebirths in 1990 to 19 (95% CI 18–21) in 2015.[1] Infections are estimated to be responsible for 31·5% of these deaths between 2000 and 2015.[3,4] Neonatal sepsis affects six to 21 babies per 1000 livebirths, with a case-fatality rate of 27–56% leading to more than 336 357 deaths per year.[3] Percutaneous invasion of pathogens from umbilical cord infections has been postulated as a major cause of neonatal sepsis.[5,6]

Unsanitary conditions in delivery and care of newborn babies might contribute to the high rate of omphalitis and serious systemic infection.[7] Approaches such as hygiene promotion (including handwashing related to delivery and neonatal care), intrapartum vaginal and neonate skin cleansing with antiseptics such as chlorhexidine, and use of clean birth kits have been implemented to reduce the risk of neonatal infections.[8–10] Three clinical trials[7,8,11] have provided evidence about the effectiveness of chlorhexidine application to the neonatal umbilical cord in Asia, but no data from Africa exists. Current WHO guidelines[12] suggest application of chlorhexidine to the umbilical stump during the first week of life for babies born at home in settings with high neonatal mortality (≥30 neonatal deaths per 1000 livebirths) and to use dry cord care for newborn babies in settings with lower (<30 deaths per 1000 livebirths) neonatal mortality.

Chlorhexidine, a broad-spectrum topical antiseptic with strong residual activity, has been shown to be

Research in context

Evidence before this study

We searched PubMed and the Cochrane Library with search terms "chlorhexidine", "cord care", "mortality", and "omphalitis" for all articles published in English until November, 2015. Three trials from southeast Asia (Nepal, Bangladesh, and Pakistan) have reported the effects of chlorhexidine on neonatal mortality and omphalitis. They reported significant effects of chlorhexidine on reducing neonatal mortality, but some questions remain unanswered. The study in Bangladesh showed an effect of 1 day treatment of chlorhexidine, but no effect was observed with 7 day treatment; the study in Nepal was not statistically significant for overall effect; and the study in Pakistan presented a factorial analysis with a clinically important interaction (maternal handwashing and chlorhexidine application increased mortality by 17–18%). No data was available in an African setting for the effect of chlorhexidine cord care on neonatal mortality. Additionally, all the previous studies were done in settings with neonatal mortality rate of 40 deaths per 1000 livebirths or higher and almost all births took place in the community.

Added value of this study

Two studies (done in Pemba and Zambia) were designed to bridge the knowledge gap. This study, done in Pemba and combined with the mortality results from Zambia, is the first to our knowledge to provide data on the effect of chlorhexidine cord care on neonatal mortality and cord infections in an east African setting. Previous research did not address the issues raised by the WHO review about the effects of chlorhexidine in settings with hospital-based deliveries of more than 40% and with neonatal mortality rate of less than 30 deaths per 1000 livebirths. The study is unique in providing data on both omphalitis and neonatal mortality and showing a disconnect between a reduction in omphalitis and reduction in neonatal mortality, similar to the results of the Bangladesh study.

Implications of all the available evidence

All the available data (three Asian and two African studies) need to be reviewed using common methods and a meaningful meta-analysis needs to be done to guide the policy for use of chlorhexidine cord care in Asia and Africa. The data from African settings provided by this study combined with the results from Zambia might alter the global policy consensus about chlorhexidine cord care.

effective in reducing infections in the neonatal period.[13] Chlorhexidine is not expensive and, with a strong safety record, is a potential intervention in low-resource settings.[14,15] Chlorhexidine is included in the WHO model list of essential medicines for children.[16]

All trial sites in the studies in south Asia[7,8,11] had neonatal mortality rates of more than 35 deaths per 1000 livebirths, and nearly all births took place at home. A WHO expert panel discussed the possible role of chlorhexidine for cord care in low-resource community settings and emphasised the need for more studies from south Asia, as well as the need for similar trials in Africa.[17] In this community-based randomised trial, we aimed to investigate the effect of umbilical cord cleansing with 4% chlorhexidine solution on the rates of neonatal mortality and omphalitis in a rural setting in Tanzania. A similar trial was started at the same time in Zambia, the results of which are also published in *The Lancet Global Health*.[18]

Methods

Study design

We did a community-based randomised controlled trial on Pemba Island in the Zanzibar archipelago off the coast of east Africa, where the neonatal mortality rate was 25 deaths per 1000 livebirths and infant mortality rate was 89 deaths per 1000 livebirths.[19] More than 99% of the population are Shirazi Muslims and reside in an estimated 70 000 households on the island. The literacy rate of the population is 52%, and the birth rate is 8500–9000 births per year, about 40% of which take place at home (actual data collected during the study).[19]

The island has four district hospitals and a cottage hospital that provide outpatient and inpatient health services. At the community level, many government health centres provide basic maternal and child health (MCH) services. Other community-based health-care providers include trained or untrained traditional birth attendants (TBAs) at the village level and several over-the-counter drug outlets and traditional healers.

The study protocol was reviewed and approved by the institutional review board of the Johns Hopkins Bloomberg School of Public Health, USA, and the Zanzibar Medical Research and Ethics Committee, Tanzania. A joint technical advisory group (TAG) and data safety monitoring board (DSMB) for the Zambia and Pemba trials were established prior to study implementation.

Participants

All newborn babies from 1 h to 48 h of age born in hospital or the community (home based) on Pemba Island between May 19, 2011, and Aug 31, 2014, were eligible for inclusion in the study. The mother or father of the newborn baby must have been a permanent resident of the island, and the parents (or a literate witness if the parents were illiterate) must have given written consent to participate in the trial. Newborn babies with any congenital malformations that prevented application of intervention and babies

who needed to be admitted to hospital were excluded from the study.

As part of the pre-trial preparation (formative research phase), all the TBAs on the island and MCH staff were contacted and trained in the study procedures. Two study supervisors were stationed at each of the maternity wards of all the four major hospitals in Pemba (in two shifts) to cover deliveries occurring from 0700 h to 2000 h. Additionally, staff nurses were hired on a temporary basis. After delivery, the study team (consisting of hospital staff and a study supervisor) screened each newborn baby for eligibility to participate in the study. If the baby was eligible and clinically stable, the study procedure and purpose was explained to the mother. This information was explained to the nearest kin and their consent to participate was sought if the mother was not stable or was deceased. Neonates were enrolled in the study after consent was obtained. For enrolment in the community, all TBAs were provided with a mobile phone. Whenever a delivery took place, a conference call between the TBA, study supervisor of that area, and MCH staff was arranged to schedule a visit to the household. The study supervisor and MCH staff would then screen the newborn baby for eligibility and obtain consent accordingly.

Randomisation and masking

This study had two phases. During phase 1, participants were randomly assigned (1:1:1) to one of three intervention groups: treatment group using chlorhexidine, a control group using a placebo solution, and a control group with dry cord care. During phase 2 (Feb 20, 2013, onwards), the placebo solution was not used and the participants previously in that group were randomly assigned (1:1) to receive either chlorhexidine or dry cord care. This was done to facilitate combination of the findings with those from the Zambia trial (see Statistical analysis section). Randomisation was done using a computer-generated random sequence. The data for chlorhexidine and dry cord care groups from both phases of the study are presented in this Article. Other results from phase 1 will be presented in future papers.

For phase 1, chlorhexidine and placebo bottles were marked with 50 intervention codes (25 chlorhexidine and 25 placebo) by an independent member of the DSMB, assisted by a team of temporary workers. Each enrolled newborn baby was randomly assigned (1:2) to either dry cord or wet cord care. For babies allocated to wet cord care, a second stage of random assignment allocated the baby to one of the 50 intervention codes. Based on the distribution of villages and health-care centres, the study area was divided into 25 working areas with a study supervisor assigned to each area. Each supervisor was provided with two sets of envelopes. The first set contained the randomisation code to allocate a baby to the dry or wet group, and the second set of envelopes contained the randomisation codes to allocate the baby to one of the 50 intervention (chlorhexidine or placebo) codes. At the hospital, random assignment to wet or dry cord care was done by day of enrolment so that all babies born on a particular day were allocated to either the wet cord care or dry cord care. The second set of envelopes were opened on the wet cord day only to allocate the child to one of the 50 intervention codes. During phase 2, a new list was generated by the DSMB statistician to randomly assign babies (1:1) into dry and chlorhexidine cord care groups. Implementation was changed slightly, because the second envelope was not needed. Masking of allocation of intervention to workers and participants during phase 2 was not possible because of the nature of the interventions.

Procedures

The intervention used in the study was a solution of chlorhexidine gluconate (4% free chlorhexidine; Galantic Pharma, Mumbai, India). Stability, potency, colour, odour, and consistency of the solution were tested at three stages of the preparation and also tested twice at the field site. As per the findings of our initial study comparing different modes of administration of chlorhexidine solution for cord cleaning,[20] 10 mL opaque dropper bottles were used for administration.

All babies in the trial had an initial examination, and neonatal care messages were relayed to the mother or caretaker. For babies allocated to the chlorhexidine group and the placebo group (in phase 1), the MCH worker showed the mother or caretaker how to apply the solution to the baby, gave 3 days supply of the solution, and instructed the mother or caretaker to apply the solution to the cord every day including 3 days after the cord had dropped off. The mother or caretaker was informed that the MCH worker would visit again on day 4 and day 10 to apply the solution. On day 4, the worker delivered six more bottles to the household. The MCH staff provided a date chart and a pen for the mother to mark the days on which she applied the solution. The mothers were requested to keep the bottles after use and the empty bottles were collected on subsequent visits. For babies assigned to the dry cord care group, in addition to the initial examination, data collection, and neonatal care messages, TBAs and hospital staff instructed the mothers and caretakers not to cleanse the umbilical cord stump and to keep it dry.

Each family was visited on day 0 (day of enrolment), 1, 4, 10, and 28. At each visit, the study team (study supervisor and MCH staff) collected mortality and morbidity information on the baby from the mother or caretaker and examined the cord for redness, pus, swelling, and foul odour, and collected baseline data and demographic characteristics on the day of enrolment. Redness was categorised into four grades (none, mild, moderate, or severe). Mild redness was defined as restricted to the cord stump only; moderate as less than 2 cm extension onto the abdominal skin at

the base of the cord stump; and severe as spreading noticeably (>2 cm) outward from the base of the stump. If infection was suspected, the supervisor informed the district in charge, the district in charge then visited the household and examined the baby, took a photograph of the cord, and referred the mother to the nearest hospital if the cord was infected. MCH staff and the study supervisor revisited the household on day 28 to collect information regarding morbidity and hospital admission, and collected and reported the mortality information in cases of death. Cord infection signs recorded during multiple visits for all infants were combined and assessed for positive status according to the omphalitis definitions.

An electronic data capture system based on a netbook (small laptop) was used for collection of data. Each supervisor was provided with a netbook with the data collection software and pre-installed data uploading and backup plugin. Data from all netbook computers was backed up at the end of the day with universal serial bus (USB) drives, which were transported back to the central office (Public Health Laboratory Ivo de Carneri) the same evening and data downloaded to the central database server. The updated server database was uploaded to the same USB drive. The database in each netbook was replaced by the updated server database from the USB drive each morning before the start of work to ensure data consistency and integrity. We designed a robust data management system to help the teams plan their activities and quality control.

Outcomes

The primary outcome for this study was mortality until day 28. The secondary outcome was omphalitis occurring any time during first 10 days after birth. Any adverse events related to chlorhexidine use were noted.

Statistical analysis

During study design, the sample size was calculated on the assumption that the neonatal mortality rate in Pemba was 31 per 1000 livebirths and with a target of a 25% reduction in mortality. With 90% power and 5% two-sided type I error, and accounting for a 20% loss of deaths (due to death occurring before the intervention was started, loss to follow-up, or refusals), the sample size required was 11 990 per group. The DSMB did two interim analyses in June, 2012, with a third of all enrolled newborn babies, and in June, 2014, with two-thirds of all enrolled babies. Because actual mortality rates were substantially lower than our original assumption, the DSMB recommended (in November, 2012) that the required sample size for the primary outcome of mortality with 80% power be 30 000 per group. Based on the DSMB's recommendation and after approvals from institutional review boards, the placebo group of wet cord care was discontinued from Feb 20, 2013, onwards, to allow data from Pemba to be combined with that in Zambia,[18] which had chlorhexidine and dry cord care groups only. This decision was taken to provide a sample of reasonable power to assess the effect of chlorhexidine on neonatal mortality in sub-Saharan Africa. The study strategy did not change between phase 1 and phase 2.

To assess the effect of the intervention on mortality and omphalitis, intention-to-treat analyses included all enrolled babies. For babies whose families had moved out of the area or withdrawn from the study, data up until the date of censorship were included. We analysed survival of newborn babies in the first 28 days with Poisson regression models with the chlorhexidine group as the independent variable, using Stata (version 13). We used Cox survival regression models to reconfirm these results. Mortality was expressed as deaths per 1000 enrolled livebirths. Mortality outcomes were estimated overall, and stratified by place of birth (community or hospital), sex, first contact within 12 h, and birthweight. For births in hospital, day of enrolment was considered as a cluster; Poisson regression, with day of enrolment as the cluster variable, was done to adjust for the clustering effect. We assessed the estimated difference in treatment effect between hospital and community births by Mantel-Cox comparison between subgroups, estimating χ^2 for unequal relative rates (effect modification) and its p value (STMC procedure in Stata). Additionally, we built a regression model with death as the dependent variable and intervention group, place of birth, and interaction term (intervention × place of birth) as independent variables.

Risk estimates from both Pemba and Zambia were combined using the metan command in Stata. Primary data from both the studies were pooled and analysed using a Poisson regression model adjusting for the clustering effect (cluster-id as cluster variable in Zambia; day of enrolment for hospital births and study-id for community births in Pemba).

The trial is registered with ClinicalTrials.gov, number NCT01528852.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between May 19, 2011, and Aug 31, 2014, 47 545 births were identified in the study area, from which 44 232 newborn babies were enrolled into the study (figure). 36 911 newborn babies were assigned to the chlorhexidine and dry cord care groups in phases 1 and 2. Of the 22 097 babies enrolled during phase 1, 7484 (34%) were assigned to the dry cord care group and 7292 (33%) were assigned to the chlorhexidine group, with the remainder assigned to the placebo group, which was not

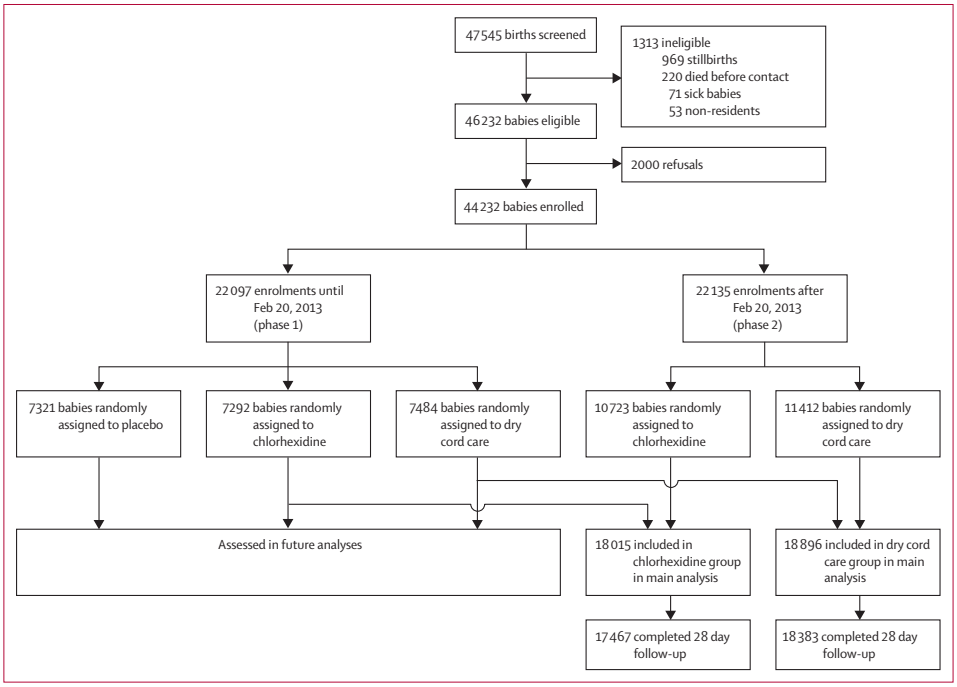

Figure: **Trial profile**

analysed further in this main outcomes study. Of the 22 135 babies enrolled during phase 2, 11 412 (52%) were in the dry cord care group and 10 723 (48%) were in the chlorhexidine group. There were no baseline differences in neonatal or parental demographic characteristics between the two groups (table 1). 19 426 (52·6%) of all 36 911 births took place in a hospital (table 1). 16 896 (93·8%) of 18 015 babies in the chlorhexidine group and 17 577 (93·0%) of 18 896 in the dry cord care group were contacted within 24 h of birth (table 2); 17 468 (97·0%) of 18 015 neonates in the chlorhexidine group were available for complete follow-up (28 days) compared with 18 384 (97·3%) of 18 896 neonates in the dry cord care group. 36 162 (98·0%) of babies attended all the planned visits (table 2).

Overall, neonatal deaths were reported in 221 (1·2%) babies in the dry cord care group compared with 189 (1·0%) babies in the chlorhexidine group. The risk of mortality after enrolment was not significantly different between the two groups (table 3). The risk of mortality did not differ by place of birth (relative risk [RR] 0·90, 95% CI 0·74–1·10; p=0·32; both treatment groups combined). Additionally, the model using intervention group, place of birth, and an interaction term between intervention and place of birth as independent variables calculated an RR of 0·97 (95% CI 0·65–1·45) for the interaction term and RR of 0·92 (0·67–1·27) for the intervention group. The sex of the baby and time of first application did not have a differential effect on mortality. There were no significant differences in the effect of chlorhexidine on early (first 7 days) or late (8–27 days) neonatal mortality (early mortality occurred in 140 [0·8%] of 18 015 babies in the chlorhexidine group *vs* 172 [0·9%] of 18 896 babies in the dry cord care group; late mortality occurred in 49 [0·3%] of 18 015 *vs* 49 [0·3%] of 18 896 babies).

Combination of the results from Pemba and Zambia (meta-analysis using random effects model) gave an RR of 0·99 (95% CI 0·80–1·23) for overall mortality risk by day 28 between chlorhexidine and dry cord care, whereas the combination of individual patient data (pooled analysis) gave an RR of 1·02 (0·86–1·20).

Babies in the chlorhexidine group had a lower risk of omphalitis with severe redness and pus (94% lower, 95% CI 74–98%) and of any redness or pus (35% lower, 30–39%) than did those in the dry cord care group (table 4).

Discussion

Within the constraints of reduced power caused by lower rates of mortality than expected in both treatment groups, we did not observe a significant reduction in neonatal mortality in the chlorhexidine group compared with the

	Chlorhexidine (n=18 015)	Dry cord care (n=18 896)
Parental occupation		
Father: fishing or farming	6440 (35·7%)	6728 (35·7%)
Mother: housewife	12 422 (69·0%)	13 019 (68·9%)
Parental literacy		
Paternal illiteracy	3472 (19·3%)	3487 (18·5%)
Maternal illiteracy	4916 (27·3%)	5012 (26·5%)
Household ownership	15 692 (87·1%)	16 383 (86·7%)
Maternal parity		
First or second child	3963 (22·0%)	4365 (23·1%)
Third or fourth child	5260 (29·2%)	5537 (29·3%)
Fifth or higher	8791 (48·8%)	8994 (47·6%)
Single or multiple birth		
Single	17 384 (96·5%)	18 178 (96·2%)
Twins	631 (3·5%)	718 (3·8%)
Sex of neonate		
Male	9201 (51·1%)	9589 (50·7%)
Female	8814 (48·9%)	9307 (49·3%)
Birthplace of neonate		
Hospital	9272 (51·5%)	10 154 (53·7%)
Community	8742 (48·5%)	8743 (46·3%)

Data are n (%).

***Table 1*: Baseline demographic neonatal, maternal, and household characteristics**

	Chlorhexidine (n=18 015)	Dry cord care (n=18 896)
Day 0 visit	18 015 (100%)	18 896 (100%)
Day 1 visit	17 820 (98·9%)	18 742 (99·2%)
Day 4 visit	17 702 (98·3%)	18 611 (98·5%)
Day 10 visit	17 620 (97·8%)	18 542 (98·1%)
All planned visits made	17 620 (97·8%)	18 542 (98·1%)
Excluding deaths	17 580 (98·6%)	18 504 (99·1%)
Timing of cord intervention		
Within 12 h	9721 (54·0%)	10 008 (53·4%)
13–24 h	7175 (39·8%)	7569 (40·1%)
>24 h	1119 (6·2%)	1239 (6·5%)

Data are n (%).

***Table 2*: Coverage and timing of intervention or visit**

dry cord care group, or when we combined these results from Pemba, Tanzania, with those from Zambia. Additionally, the 10% lower mortality rate reported in the chlorhexidine group was much smaller than that hypothesised based on the findings of the Asian trials.[11]

However, our findings did show that application of chlorhexidine significantly reduced the prevalence of omphalitis when compared with dry cord care. The risk of omphalitis was reduced by 24–39% for most grades of infection and was reduced by 94% for omphalitis when defined as severe redness with pus. These findings are consistent with those from earlier trials in Nepal,[7] Pakistan,[8] and Bangladesh.[11] Similar to our findings, the risk of cord infection in Nepal was reduced by 32–75% for different grades of infection in the chlorhexidine cleansing group, and in Bangladesh, newborn babies who had 7 days of chlorhexidine application were at a lower risk of any redness in the umbilical stump or pus than babies who had 1 day of chlorhexidine application. Results from the trial in Pakistan also showed a lower risk of omphalitis of any grade in babies in the chlorhexidine group than babies in the dry cord care group.

The umbilical cord stump of a newborn baby is a prime site of bacterial colonisation. Infection of the stump—omphalitis—poses a serious threat to the newborn baby and has been suggested to play an important role in systemic infection and mortality risk. The patency of the umbilical blood vessels in the first few days of life is known to provide access for pathogens to enter the blood stream even in the absence of omphalitis.[6,21,22] Hence, one possible explanation for the disparity in the effects of chlorhexidine between omphalitis and mortality could be the occurrence of sepsis without omphalitis. Sepsis in the absence of omphalitis can occur when the host immunity does not limit the infection to the umbilical stump, causing direct systemic infection without eliciting localised signs of omphalitis. Application of chlorhexidine on the cord stump might reduce the risk of local cord infection, but not prevent the pathogens from entering into the bloodstream through patent umbilical vessels, leading to sepsis and death.[23] Application of chlorhexidine is known to substantially delay cord separation. If the delay in cord separation perpetuates the risk of exposing the vessels to bacterial contamination,[24] it might have been responsible for the differences between 1 day versus 7 days of chlorhexidine application in the Bangladesh study and the inconsistency between omphalitis and mortality results in our study. Any risk related to delayed cord separation needs further investigation.

Before the start of the study, the neonatal mortality rate in sub-Saharan Africa was reported to be higher (31 deaths per 1000 livebirths) than in Pakistan (29 per 1000 livebirths),[8] Bangladesh (24 per 1000 livebirths), and Nepal (23 per 1000 livebirths).[4] However, mortality rates in Africa have changed substantially in the past decade. The observed per-protocol mortality rates in the chlorhexidine and dry cord care groups in our study (10·5 and 11·7 per 1000 livebirths, respectively), were much lower than we estimated. These rates were also lower than reported in studies in Bangladesh (22·5 and 28·3 deaths per 1000 livebirths, respectively) and Nepal (14·6 and 19·3 per 1000 livebirths, respectively). The low overall mortality rates in our study could be attributed to a general time trend as indicated by decline of neonatal mortality rate from 31 to 25 per 1000 livebirths in 2010[19] and 19 per 1000 livebirths in 2015.[1] The mortality rates recorded in this study could also have been affected by increased sensitisation of the mothers and other family members of cord care hygiene during the formative phase of the study,

and the prevalent cord care practices in this region. A study[25] done during the formative phase of this trial revealed that dry cord care is a well understood and practised concept in Pemba. Therefore, dry cord care might already have been better practised in this setting than in the Asian study populations. However, the effects on omphalitis would suggest that there was still a substantial risk of contamination of the cord stump in Pemba and any practice related to cord care would be unlikely to affect neonatal mortality. Even though the results from three trials of chlorhexidine for cord care show reduction of neonatal infections and deaths, these studies have not established the ideal timing and duration of chlorhexidine application.[26] With the conflicting results of 1 day and 7 day application of chlorhexidine in the trial in Bangladesh, further investigation is needed to understand the effects in Asia and sub-Saharan Africa.

By contrast with the previous trials in south Asia,[26] which predominantly enrolled home births (>90%) and were cluster randomised trials with potential for baseline differences, the strengths of our study are that this trial is an individually randomised controlled trial with a large sample size; we enrolled children both from community births (47%) and hospital births (53%); the proportion of babies contacted within 24 h was higher in our study, with 93% of first contacts made within 24 h in both groups, than in Bangladesh (86–88%) and in Nepal (62–64%), which should have improved the impact of chlorhexidine in our trial; and more than 95% of the screened and eligible newborn babies were included in the final analysis, thereby reducing selection bias.

A weakness of our study is that the independent power of the study is low because the observed mortality rates were lower than expected. However, along with a concomitant trial in Zambia,[18] we present important first evidence that chlorhexidine application on the umbilical cord has no effect on mortality in low-resource settings in sub-Saharan Africa. The study results and combined analysis suggest that chlorhexidine does not have a substantial impact on mortality in both settings.

The study area is comparable to other African countries in terms of limited resources, low rates of skilled attendance at birth, and unhygienic cord care practices.[7,8,11,18] In this study, we did not observe any differences in the effect of chlorhexidine on omphalitis or mortality between hospital births and community births. This argues against a lack of impact compared with Asian studies being due to higher hospital births in our setting.

The findings in our study suggest that use of chlorhexidine for the reduction of omphalitis is justified, but in an African setting there is insufficient evidence to promote this intervention to reduce neonatal mortality. In most African settings, mortality estimates are much lower than those reported by Demographic and Health Surveys, especially where prospective data have been collected. The low neonatal mortality rate observed in this trial (17 per 1000 livebirths [including deaths before randomisation] compared with 25 per 1000 livebirths reported by the Tanzania Demographic Health Survey[19]) is consistent with the situation in most African settings. A pooled analysis of the two African trials combined with the three Asia trials needs to be undertaken to inform policy. Such an analysis will require careful description of the different contexts (eg, community *vs* hospital delivery, low *vs* high neonatal mortality rate, and different cultural practices) and attention to weighting of the studies in the meta-analysis, quality scoring, and other related statistical details. This meta-analysis should be done under the coordination of WHO with the involvement of independent consultants in addition to the investigators.

	Chlorhexidine (n=18 015)		Dry cord care (n=18 896)		Relative risk (95% CI)	p value
	Deaths	Deaths per 1000 livebirths	Deaths	Deaths per 1000 livebirths		
Overall	189	10·5	221	11·7	0·90 (0·74–1·09)	0·27
Place of birth						
Hospital	116	12·5	142	14·0	0·89 0·70–1·14)	0·37
Community	73	8·4	79	9·0	0·92 (0·67–1·27)	0·63
Sex						
Male	107	11·6	123	12·8	0·91 (0·71–1·11)	0·46
Female	82	9·3	98	10·5	0·88 (0·66–1·18)	0·41
First contact						
≤12 h	111	11·4	123	12·2	0·90 (0·69–1·16)	0·42
≥12 h	78	9·4	97	11·0	0·84 (0·62–1·13)	0·24
Birthweight						
Low birthweight	29	66·7	32	67·1	0·99 (0·59–1·67)	0·98
Normal birthweight	56	8·1	54	7·2	1·14 (0·78–1·65)	0·50

***Table 3*: Neonatal mortality in the intention-to-treat population**

	Chlorhexidine (n=18 015)		Dry cord care (n=18 896)		Relative risk (95% CI)	p value
	N	n per 1000 livebirths	N	n per 1000 livebirths		
Any redness or pus	1413	78·4	2183	115·5	0·65 (0·61–0·70)	<0·0001
Any redness without pus	1051	58·4	1427	75·5	0·76 (0·70–0·82)	<0·0001
Moderate redness with pus or severe redness	166	9·2	286	15·1	0·61 (0·50–0·73)	<0·0001
Severe redness with pus	2	0·1	33	1·8	0·06 (0·02–0·26)	0·0001

***Table 4*: Omphalitis in the intention-to-treat population**

Contributors

All authors participated in the research and intervention design. SS, UD, and REB were involved in the conceptualisation of research, development of study protocol, analysis of data, and the preparation of manuscript. AD and UD were responsible for data management, field implementation, and preliminary analysis. SD was responsible for training and quality control for omphalitis, and contributed to manuscript writing and editing. SMAl and SMAm helped with the Pemba administrative system, community mobilisation, and provided leadership and supervision to team members. MHM provided administrative support. AY helped with the analysis and manuscript writing. All authors reviewed the paper and approved the final version.

Declaration of interests
We declare no competing interests.

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